MEASUREMENT AND ANALYSIS OF EXTRACELLULAR CARDIAC POTENTIALS TO GUIDE RADIOFREQUENCY ABLATION THERAPY FOR FIBRILLATION

Sevan Mark Abashian

A thesis submitted to the faculty of the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Master of Science in the Department of Biomedical Engineering.

Chapel Hill
2009

Approved By:

Stephen Knisley, Ph.D.

Oleg Favorov, Ph.D.

Stephen Quint, Ph.D.

Timothy Johnson, Ph.D.

ABSTRACT

SEVAN MARK ABASHIAN: Measurement and Analysis of Extracellular Cardiac Potentials to Guide Radiofrequency Ablation Therapy for Fibrillation
(Under the direction of Stephen B. Knisley, Ph.D.)

Metrics for completeness of cardiac antiarrhythmic ablation lesions are needed to guide ablation therapy. Extracellular bipolar cardiac potentials were measured on either side of the lesion in isolated rabbit hearts (N=25). Three analyses of the signals were examined as possible metrics. Variances in dominant frequency of fibrillatory recordings decreased after ablation by factors of 1.51 for the frequency-domain analysis using the Fast-Fourier Transform; and 1.45 for the time-domain analysis using intervals between super-threshold peaks. This suggests an increase in organization of fibrillation. Morphologies of the signals from different sides of the lesion examined with cross-correlation indicated no consistent change in morphology before vs. after ablation. Slow pacing to determine translesion stimulus-excitation delays (TED) showed that mean TED increased post-ablation, consistent with increased conduction path length. During fibrillation, no consistent change in TED was observed. Thus, certain metrics may be useful to distinguish lesion completeness.

ACKNOWLEDGEMENTS

I would like to graciously acknowledge the support, direction, and patience of my advisor, Dr. Stephen Knisley, who provided me with this research opportunity, guided my studies, and took a vested interest in my education. I would like to thank the members of my thesis committee for their advice, time commitment, and expertise in the field of signal processing and cardiac fibrillation. I am also grateful for the assistance of Jared A. Sims, John H. Dumas III, and Herman D. Himel, IV, who all lent me their expertise and time in the laboratory to make the experiments run smoothly. Finally, I would like to thank my loving mother and father for their unwavering support and for instilling a value for education in their children.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

AF – atrial fibrillation

DC – direct current

DF – dominant frequency

FFT – fast Fourier transform

RF – radiofrequency

TED – translesion stimulus excitation delay

TTC – 2,3,5-Triphenyltetrazolium chloride

VF – ventricular fibrillation

CHAPTER 1

INTRODUCTION

Fibrillation of the heart is a common and serious disorder in all age groups that can lead to stroke and other cardiopulmonary blockages[1]. Fibrillation occurs when the heart cannot correctly propagate electric signals through cardiac tissue; instead, the tissue beats ineffectively or "quivers" as the cardiac muscle contracts erratically. Fibrillation of the atria (Atrial Fibrillation, or AF) affects roughly 2.2 million Americans, and can appear at any age, although it develops more commonly with age. AF is known to be a factor in the formation of clotted blood that can produce strokes. Ventricular fibrillation (VF), the fibrillation of the ventricles, is more serious and often fatal. Without immediate treatment, persons experience VF will likely die in a matter of minutes due to the lack of blood flow caused by fibrillation in these larger chambers of the heart[2].

Individuals with the predisposition to develop VF can be treated with the implantation of portable defibrillators, which provide a large pulse of electrical current to the heart when fibrillation is detected. Depending on the severity of AF, it is most often treated pharmaceutically. With more severe cases of AF, surgical options are considered. While pacemakers will treat many kinds of arrhythmia including lower severity AF, the use of radiofrequency (RF) ablation is becoming more common in disrupting the fibrillation pathways. RF ablation involves either surgical operation or catheter injection to deliver a high current to the surface of the heart with the hopes of creating a point or linear transmural lesion that will disrupt the fibrillatory circuits. Catheter ablation is much less invasive, and

new innovation has made the placement and efficiency of such catheters greater than ever. While the open-heart surgery allows the physician to confirm the quality of lesions, catheter ablation has proven more difficult to analyze for quality. If the quality of these lesions can be determined by analyzing epicardial signals near the ablation by using electrodes on the catheter, then physicians will have a powerful tool to ensure that a complete lesion has been created. The goal of this project was to explore signal processing methods to assess the quality of a lesion while measuring cardiac signals during fibrillation. Previous studies have shown that physiological changes in heart tissue can be measured by analyzing cardiac signals in normal sinus rhythm taken before and after ablation[3],[4], but frequently patients are still experiencing fibrillation when catheter ablation is being performed. We hoped to determine a metric of completeness by comparing fibrillatory signals before and after ablation.

Because of its electrophysiological similarity to the human heart, a rabbit heart was used as a model as in other studies performed by members of this laboratory. From January to May 2007, 15 rabbit hearts were used in a study of dominant frequency of VF signals as a metric for completeness of lesion. MATLAB mathematical software was used to find the dominant frequency of segments of epicardial signals on either side of a 3 cm linear lesion on the left ventricle before and after ablation. We hypothesized that after the lesion, the dominant frequency of the signal would be less varied due to the disruption of re-entrant circuits.

From January to May 2008, 10 additional rabbit hearts were used to study the pacing of the heart during fibrillation. As shown by Himel et al.[3], slow paced stimulation of the heart could be used to determine completeness of lesion by examining translesion stimulus

excitation delay (TED). This was performed while the heart was stable in sinus rhythm. This study aimed to build upon those findings by examining TED during fibrillatory episodes. Based on findings of prior studies, we hypothesized that creation of a complete lesion would significantly increase the TED due to an increased conduction path length.

In addition to studying TEDs during VF, the same hearts were used to study the morphology of translesion signals. Using the correlation of signals as a metric, we hypothesized that by disrupting re-entrant circuits around the lesion, translesion signal correlation would decrease, and variability of correlation values would increase as a result of ablation.

In all, 25 rabbit hearts were used in the studies, over the course of two years of experiments. In one experiment (5/13/2008), the RF Generator malfunctioned and no lesion was created. The data from that experiment was not included in any analyses. The following table shows the inclusion of sets of hearts in specific analyses.

Analysis	**Jan.-May 2007 15 Hearts, 10 kHz, 10 sec**	**Jan.-May 2008 9 Hearts, 5 & 10 kHz, 10 & 60 sec**
Dominant Frequency	10 Hearts, Complete 5 Hearts, Gap & Complete	
TED		
Slow Pacing		4 Hearts, Complete
VF Pacing		4 Hearts, Complete
Morphology Comparison		
Sinus Rhythm		9 Hearts, Incomplete & Complete
VF		8 Hearts, Incomplete & Complete

Table 1.1 - Experiment Inclusion for Specific Analyses

CHAPTER 2

EXPERIMENTAL METHODOLOGY

Over the course of two years, hearts from 25 New Zealand White rabbits were isolated in accordance with the Institutional Animal Care and Use Committee of the University of North Carolina at Chapel Hill.

Surgical Removal

Animal subjects were sheltered at an animal care facility on the UNC-CH campus until time of use. While retrieving the rabbit from the animal facility, the mass of the rabbit was determined. Rabbits were euthanized via Euthasol (sodium pentobarbital) injection, with the dosage dependent upon the mass of the rabbit (120 mg/kg). The Euthasol was mixed with Heparin to thin the blood and speed the flow of the Euthasol. Upon confirmation of death by pinching the toe, confirming that all reaction to pain has been eliminated, the heart was removed by cutting through ribs from the xiphoid process diagonally up toward the shoulders. Underneath the ribs, the connective tissue around the heart was cut away until the heart was visible, at which time the vessels to and from the heart were cut away. The heart was removed and excess blood was washed away using Tyrode's solution (129 mM NaCl, 5.4 mM KCl,1.8 mM CaCl2, 1.1 mM MgCl2, 26 mM NaHCO3, 1 mM Na2HPO4, 11 mM dextrose and 0.6 μM bovine serum albumin) baths.

The heart was attached using suture thread to tie the aorta into a Langendorff-perfusion system that provided heated (37° C) oxygenated (95% O_2, 5% CO_2) Tyrode's

solution, a physiological solution prepared before the surgery to mimicked the chemical and physiological properties of blood. By perfusing this solution through the heart, the remaining blood cleared away and was discarded. In most cases, the heart would begin to beat again on its own. However, some hearts were manually pumped to induce beating. Using this solution, the hearts could be maintained outside the body for an adequate time to perform the experiment.

RF Ablation

RF Ablation is frequently used as a treatment for fibrillation to disrupt the propagation of re-entrant fibrillatory circuits. In conjunction with pharmaceutical therapies, ablation can treat many cases of atrial and ventricular fibrillation. Ablation may be used to either target the source of fibrillatory trigger, or interrupt the rotary character of fibrillation.

Ablation in this experiment was performed with a prototype device built by nContact Surgical, Inc. (Morrisville, NC) that contacted the epicardial surface by using suction provided by a vacuum pump. The lesion was created using a coiled length of metal pressed against the surface, through which a prescribed current was passed, depending upon the desired depth and continuity of the lesion. nContact provided the laboratory with an RF generator that regulated the power (15-40W) and time (30-35 seconds) of the burning of the lesion, while monitoring the resistance across the heart. The control device automatically adjusted the power if the resistance fluctuated from normal values. For one round of experiments, the ablation coil length was two centimeters; in the later round, the coil length was 3 centimeters. These lengths were chosen by representatives of nContact, Inc.

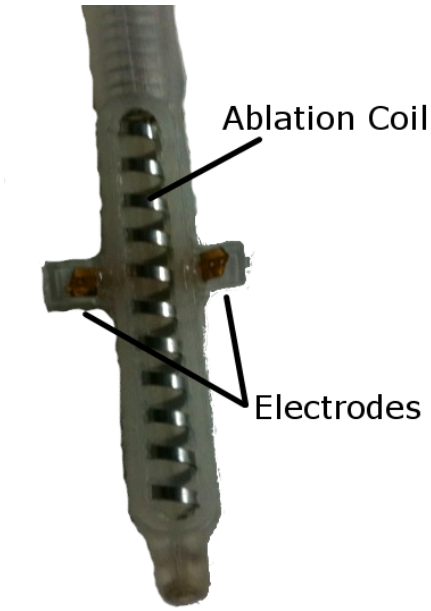


Figure 2.1 – Prototype 3 cm ablation probe. Two sets of bipolar electrodes protrude from the center of the ablation coil, trimmed for optimal contact with epicardial surface.

The power and burn time were adjusted for the length, as a longer coil needed more time and power to achieve similar physiological characteristics of the lesion. When using a 2 cm probe, the lesion was found to be continuous and complete when ablating for 30 seconds at 20 W. With the larger 3 cm probe, the best results were found by increasing the power to 40 W, and ablating for 30 seconds.

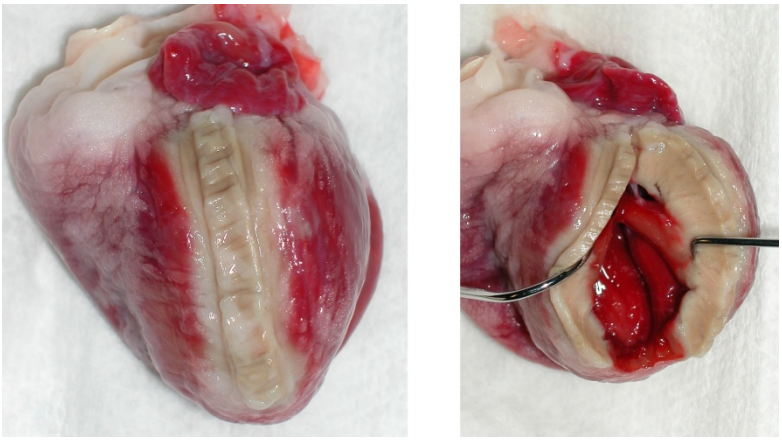


Figure 2.2 – Photographs of complete transmural lesion. After TTC staining, confirmation that ablated tissue extends the entire depth of the ventricular wall. Lesion Dimensions 31 mm Length, 7 mm Width, 5 mm Depth.

nContact also provided the lab with gapped probes, which had a small piece of silicone rubber molded over the center of the coil. This produced a lesion with a small gap, to simulate an incomplete lesion.

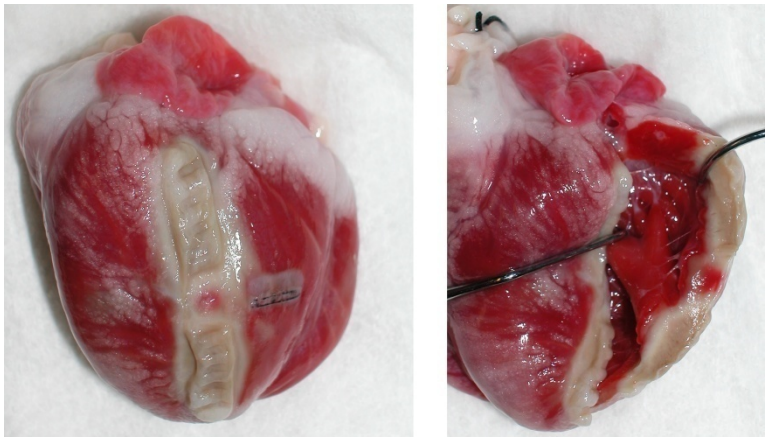


Figure 2.3 – Photographs of gap lesion. Silicone gel covered 5mm of the ablation coil at the center of the probe, causing a gap in the otherwise transmural lesion.

In addition, several experiments provided incomplete lesions due to inadequate ablation power or time. Figure 2.4 below shows a non-transmural lesion near the mitral valve caused by too little ablation power. In one heart, the subject of the May 13, 2008 experiment, the RF generator failed to provide adequate power to the ablation coil, and no lesion was formed. The data from that experiment was discarded as it could not compare any post-ablation state to the control recordings.

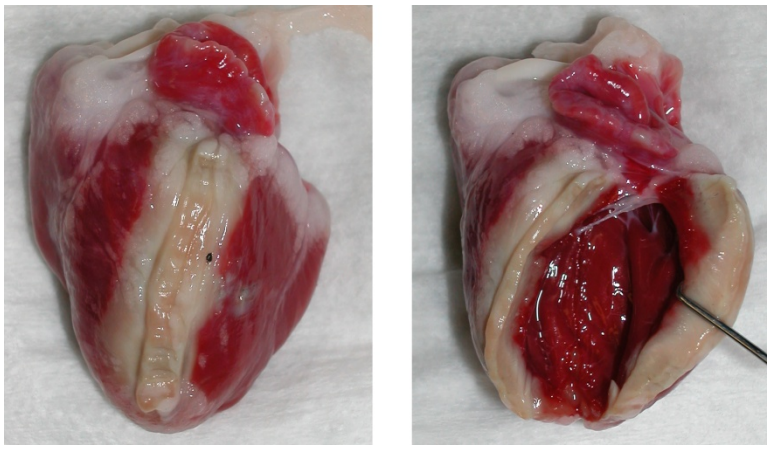


Figure 2.4 – Photographs of incomplete lesion. Non-adequate ablation time led to non-transmural lesion.

The probe was placed vertically on the left ventricle, between the left mitral valve and the apex of the heart. Being the thickest section of the rabbit cardiac tissue, with an average depth of 5.0 mm, it would give the most accurate model for the needs of ablating human tissue.

Signal Recording

General Methods

The probes provided by nContact Inc. had two sets of bipolar electrodes, one on each side of the ablation coil. In the first round of experiments, performed in the spring of 2007, the two electrodes on either side were oriented perpendicular to the axis of the ablation coil. However, in the second round of experiments, performed in 2008, the electrodes were parallel to that axis. These orientations were determined by nContact design engineers. Both orientations allowed us to take recordings on either side of the lesion. The electrodes were not part of the vacuum system that held the ablation coil to the surface of the heart, but were oriented so that they would remain in contact with the surface as long as the probe was being held onto the heart by vacuum.

From the electrodes, the signals were conditioned through an operational-amplifier circuit, designed with a gain of 100x, and based around an isolation amplifier, model AD210AN (See Appendix A – Signal Conditioning Circuit Diagram). The resulting signal was digitized by a National Instruments DAQPad-6070E data acquisition board, and recorded using a custom-built LabVIEW program. Data was stored on the laboratory computer until the conclusion of the experiment, at which time the data was transferred to an office computer for analysis.

In order to determine the effect of the ablation on the epicardial signals, data was collected before and after ablation. After allowing the heart to stabilize in sinus rhythm, several data segments were collected.

After collecting sinus rhythm and pacing data, the heart was induced into ventricular fibrillation. The ability to induce fibrillation varied from rabbit to rabbit. Only VF recordings that showed a Type III level of complexity were retained for analysis[5]. Any recording that was suspect of being Type I or II VF, or tachycardia, was discarded.

Dominant Frequency Recordings

Recordings taken during the 2007 round of experiments were used in the analysis of Dominant Frequency. In each heart, multiple VF recordings were taken both before and after ablation. 10-second recordings of VF signals were taken with a scan rate of 10 kHz. In some cases, recordings were taken during the same fibrillation episode; while in many others, fibrillation would be re-induced to take additional recordings. To induce fibrillation, hearts were paced with a gradually increasing frequency. A separate pacing electrode was put in contact with the heart. The heart was paced starting with a period of 500 ms, which was

decreased over 5 minutes to less than 100 ms. The induction of fibrillation would occur as the frequency increased. Before collecting data, the pacing stimulus would be removed.

Signal Morphology Recordings

In the 2008 round of experiments, 60-second recordings were taken during both VF and sinus rhythm to assess the degree of signal morphology before and after ablation at a scan rate of 5 kHz. After taking several sinus rhythm recordings, the heart would be induced into fibrillation. In some rabbits, fibrillation could be induced by touching the left ventricular surface with a 9V battery. The short burst of current would trigger a fibrillation episode. A pacing regimen used when collecting data was used as well to induce fibrillation, when the battery method was unsuccessful. While pacing on one set of the bipolar electrodes at increasing frequency, the heart could be induced into fibrillation. Once the heart was able to sustain a fibrillatory episode without pacing, the electrodes would be switched to collect data again.

TED Pacing Recordings

Recordings during pacing were collected in the 2008 round of experiments. To pace, one set of electrodes was attached to a stimulator, whose rate was controlled by a function generator with a user-determined frequency. Epicardial signal data was collected from the opposite set of electrodes, as well as the square wave which showed the times when pacing occurred. These 60-second recordings were taken with a scan rate of 5 kHz. For slow pacing not in VF, the pacing frequency was set to 3.333 Hz. From this set of data, it was thought that the Stimulus-Excitation delay could be determined.

The study of pacing during fibrillation began during the 2008 round of experiments. Early in the experiments, the procedure for pacing was not well defined, and consequently the amount of data collected varied between experiments. In the January 25, 2008 experiment, the heart was paced at 9.5, 10, and 10.5 Hz while in VF. This was thought to be centered on a dominant frequency of 10 Hz. In the next experiment, on 1/30/08, the VF signals were analyzed first, using an oscilloscope, to find a dominant frequency. The hearts were then paced at 95, 100, and 105 percents of that frequency. Because of the variant nature of VF, this method proved to be inconsistent. For the next experiment, on 2/11/08, VF pacing data was taken at one hertz increments from 6 to 12 Hz, along with a pacing sweep from 7 to 13 Hz. This sweep method was available on the function generator, and allowed for the 7 to 13 Hz sweep over 60 seconds. In an effort to examine whether pacing pulses were being captured by ventricular tissue, high-frequency pacing was also performed, at 20, 30 and 40 Hz. This procedure was repeated after ablation as well. In the final experiment that included a pacing procedure, pacing was performed both before and after ablation at 0.5 increments from 7.5 to 10.5 Hz, as well as a sweep over one minute from 6 to 12 Hz. The sweeping method was performed in an attempt to gain many different pacing frequency responses in a short amount of time.

Visual Inspection and Staining

Upon the completion of data collection, the heart was removed from the perfusion system and stained for physical inspection of the lesion. Staining was performed using a 0.94 mM concentration of 2,3,5-Triphenyltetrazolium chloride (TTC) in saline solution. The heart was attached to a saline bag in which the TTC solution had been added. The solution was drawn through the heart by gravity. TTC stains live tissue dark red, while not staining

any dead or ablated tissue. After staining the heart with approximately 150mL of TTC solution, the heart was removed and dissected to examine the completeness of the lesion. Digital photographs were taken along with the visual inspection to verify the desired transmural depth and/or continuity of the lesion. The lesion was dissected along its long axis, and the depth was measured. Figures 2.2-4 show the post-experiment stained hearts.

CHAPTER 3

COMPUTER ANALYSIS

Dominant Frequency Analysis

Background

The use of frequency analysis has become common in the study of fibrillation. Due to the complex nature of the atrial fibrillation signal, using frequency analysis on a segment of data is often quicker and easier to perform than examining the activation complexes individually and the variations therein[6]. For the purposes of this experiment, frequency spectra from opposite sides of the lesion were compared in an attempt to determine the completeness. The dominant frequency (DF), defined for this experiment as the frequency of the highest peak or power, was examined in and compared between pre- and post-ablation in order to determine a statistical change caused by the completeness of the lesion. By examining prior research into using frequency analysis, we included the proper signal processing to highlight the dominant frequency. A summary of this analysis was published in *Computers in Cardiology*, vol. 34, pg. 781-783, titled "Effect of Ablation on Local Activation Intervals and Dominant Frequencies of Fibrillation."[7]

Algorithm

The fifteen rabbit hearts from the 2007 group of experiments were the subject of this analysis. Candidate recordings that showed user-verified fibrillation episodes were combined into one folder, and labeled according to control (pre-ablation), gap lesion, and

complete lesion. Data files were opened and formatted using a program written by John H. Dumas III and Herman Himel, as the files were saved in binary format. The user selected a range of files to analyze, in most cases the entire set of control or post-ablation files. For each file, each channel recording was split into two second segments, as that time produced multiple DF values over the course of the data file. Additionally, we felt that with the constantly changing nature of fibrillation excitation patterns, using a small segment would let us find a more exact dominant frequency at each segment.

Filtering the Recordings

Prior to Fast Fourier Transform (FFT) analysis, the data was run through a filter based on Ng et al, which was shown to emphasize the dominant frequency in cardiac recordings[8]. This "Ng-filter" consisted of a 3rd order Butterworth band-pass filter from 40-250 Hz, followed by rectification, followed by a 3rd order Butterworth low-pass filter with a cutoff frequency of 20 Hz. Both filters were "zero-phase" filters to negate any phase alterations caused by the filters. This is shown in the figure below:

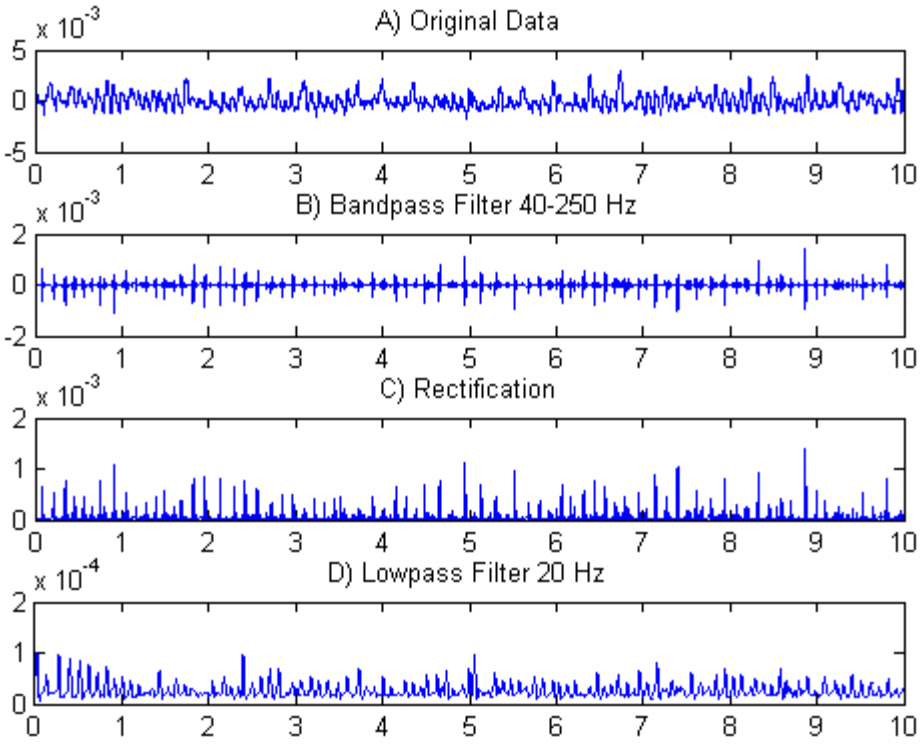


Figure 3.1 – Example of raw VF data filtering steps. A) Original Data with only offset subtracted. B) Signal after bandpass filter from 40-250 Hz. C) Resulting signal after rectification. D) Lowpass filtered signal, cutoff frequency 20Hz.

Fast Fourier Transform

Each two-second segment was multiplied by a Hann window to reduce edge-effects. The two filtered signals were then transformed using the Discrete Fast Fourier Transform (FFT). To get the power spectrum, the resulting array was multiplied by conjugate and divided by the length of the array. The phase of the FFT signal was found using the unwrap(angle()) functions. The complex FFT signal was now separated into phase and power, or magnitude, signals.

The frequencies from DC to 1 Hz were removed from the frequency, power spectra, and phase spectra data arrays. The DF in each power spectra (channel 1 and 2) was found by searching the array for the point with the highest magnitude. The peak heights of each power spectrum at each of the DFs were stored and compared, as well as the phase of

each FFT at each dominant frequency. The difference in DF was stored, as well as the difference in phase at each of the DF. A custom subprogram was created to reduce phase values to a range of [-pi, pi]. This process was repeated for each segment of each data file, building a cumulative array of phases and dominant frequencies, along with the differences thereof.

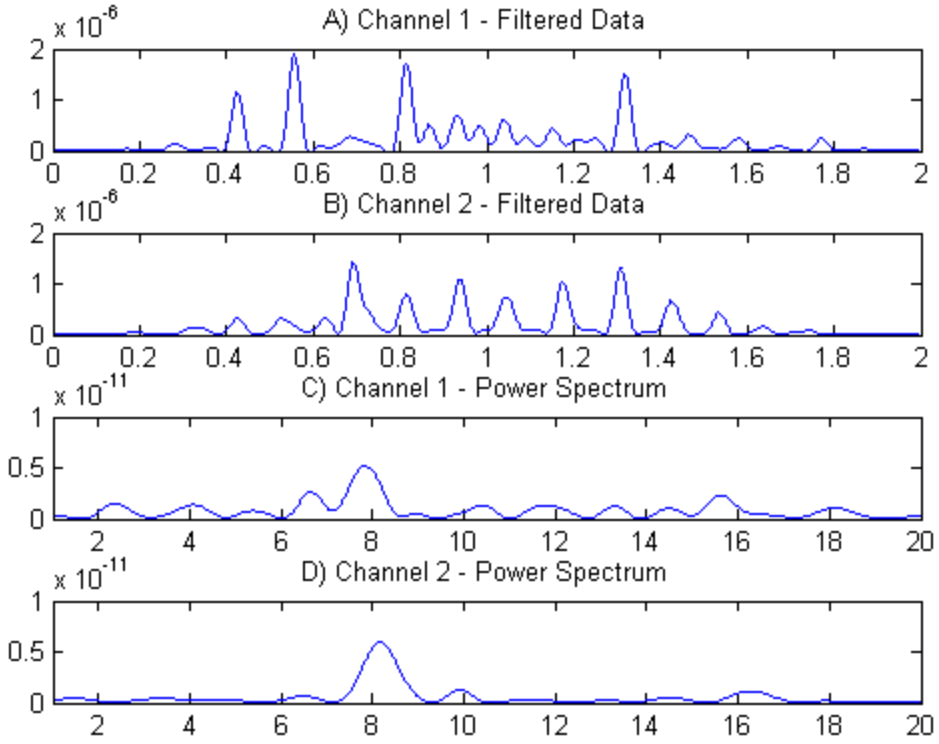


Figure 3.2 – FFT example. A) and B) Filtered data with Hann Window applied. C) and D) Corresponding FFT limited to range 1-20Hz. FFT Maxima can be seen at 7.8 and 8.2 Hz, respectively.

Time Domain Analysis

The time interval between sampling points was calculated for the same Ng-filtered data of the cardiac recordings, with added drift removal to remove any low frequency drift and move the baseline to zero. Thresholds, located at one-quarter of the maximum voltage of each of the channels, were calculated. This threshold was decided upon by

examining various recordings to determine whether the desired peaks were included. Thresholds of 0.75*max, 0.50*max, and 0.375*max were investigated as well. All points above the threshold were found. This preliminary array would be used to find the specific peaks to be used in this analysis. The array contained all indices of points above their channel's threshold. For a specific peak, there might be many points in the array that represent that peak, especially for a large peak above the threshold. The number of these points, or the time interval corresponding to the number of points, was referred to as peak width. Also, in many recordings, bit fluctuations would lead to false peaks with very small peak widths. The first processing step to the array created by the threshold was to remove any peak with a width less than 1 ms. For each channel, the intervals between consecutive peaks were found, as well as the center index of those intervals, by taking the average of the two peak indices.

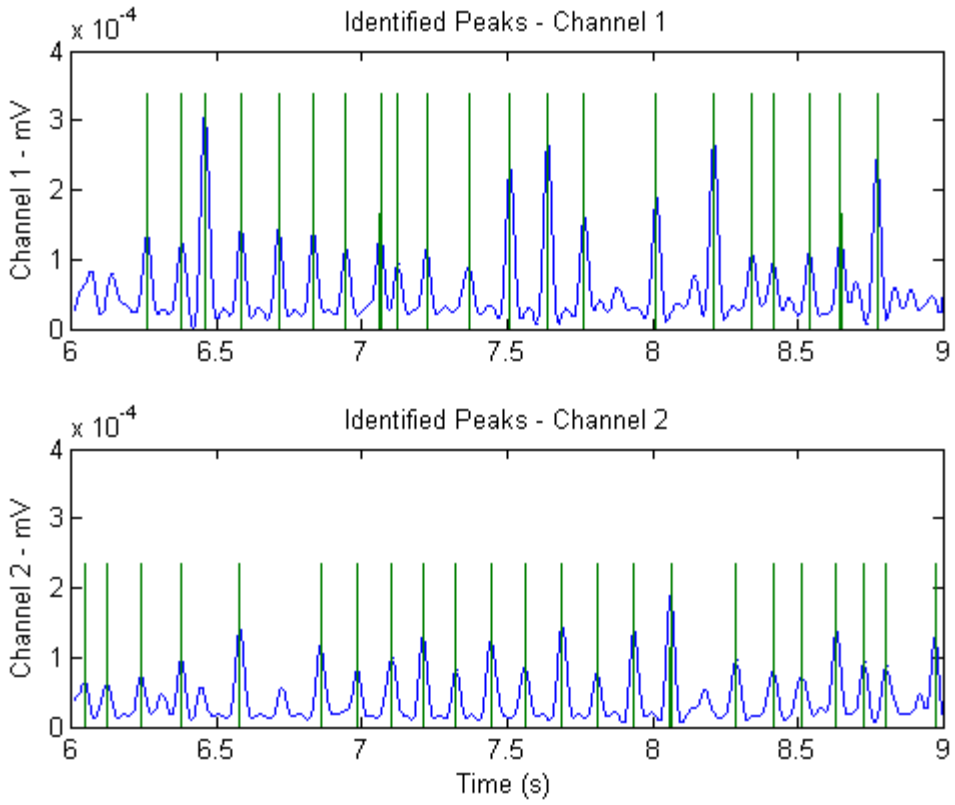


Figure 3.3 – Time-domain peak selection. Peaks identified by crossing threshold of one quarter of the maximum magnitude and finding local maxima. Height of the green lines indicates maximum peak height for respective channel.

These interval centers were used in matching beats between channels. For each center in channel 1, the closest center in channel 2 was calculated. The difference, in time and radians, is calculated between the two matched centers. Radians were calculated by dividing the time difference by the average of the two intervals and multiplied by 2π. The difference in frequency was found by taking the difference of the inverses of the two intervals. These differences in frequency and phase are compiled for each set of data run.

Signal Morphology

Background

Signal wave-morphology of fibrillatory signals is a relatively unstudied branch of AF research. While wave-morphology has been used as a metric for the classification of

complexity of AF^5, its use as a metric for quality of lesion is unique to this study. In recording high-complexity VF epicardial signals, we hoped to find that wave propagation across the sets of electrodes would lead to similar morphological signals delayed by the conduction velocity.

Correlation, the comparison of signal morphologies, is common in signal processing comparison. Cross-correlation is the process of time-shifting one signal across another at discrete points. The sum of the products the magnitudes of aligning points at each shift forms the cross-correlation. Using this process, we examined time delays in signals and their corresponding correlations.

Algorithm

Morphology analysis was performed on non-paced sinus and VF recordings. In order to more accurately compare the morphology of the two signals, it was necessary to perform filtering and other signal processing. In some recordings, signals had slowly drifted from a zero-voltage baseline, either positive or negative. This added DC component would skew the correlation result, so a drift removal algorithm was developed. For each channel, left and right, five points were selected at 10, 30, 50, 70, and 90% of the total length. We felt as though this small number of points would be able to remove low-frequency drift while not distorting the morphology of the signal. At each of these center points, the mean of data points 5% of total length to either side of the center point was found. This mean gave us an estimation of the offset at each point.

After finding the five means and their locations along the signal, that data was used to form a splined curve, using the *pchip* (Piecewise Cubic Hermite Interpolating Polynomial) function of MATLAB. This polynomial splined curve was created to estimate the drift over

the entire signal, and was created to have the same number of data points as the signal itself. This splined curve was subtracted from the original signal, setting any offset to zero. To be sure any constant offset did not remain; the mean of the resulting signal was subtracted from the signal as well. This process was repeated for the other channel.

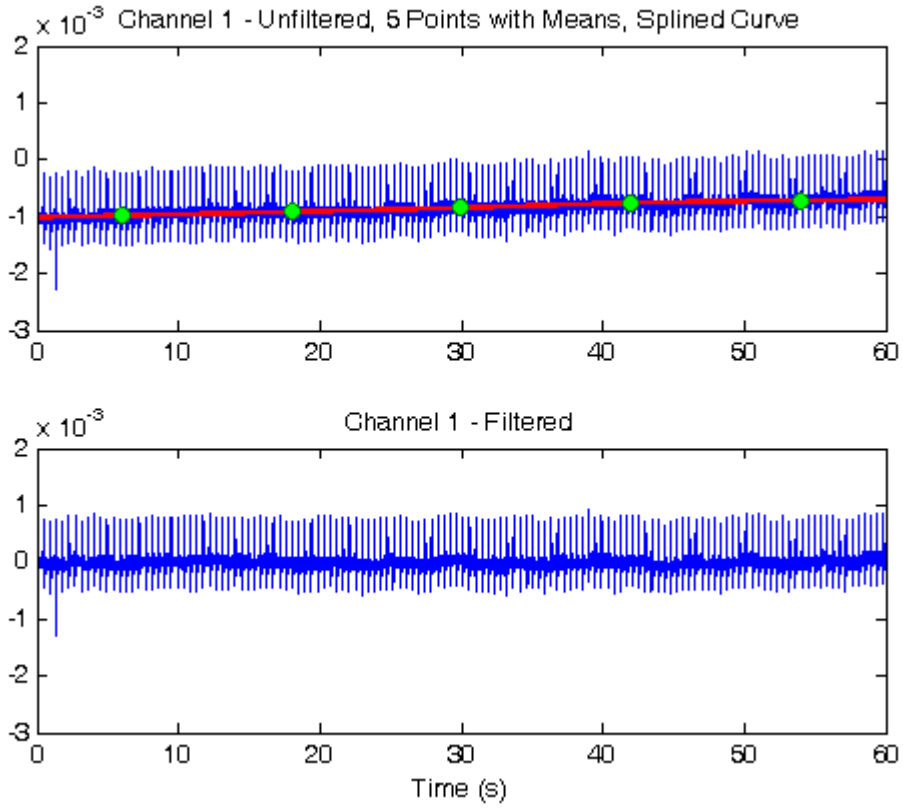


Figure 3.4 – Drift removal by splined means subtraction. Segments of data (in this case 12-second segments) are averaged together. Resulting means are polynomial splined together and subtracted away.

Additionally, in many recordings, there was a high-frequency (60Hz) noise component present, possibly due to electrical interference in the laboratory. To remove this, the data was passed through a 4th-order Butterworth low-pass filter with cutoff frequency of 50 Hz. This removed the vast majority of noise, as well as bit jitter that was an artifact from the data acquisition board. To negate any offset effect caused by this filtering, the means of the signals were once again subtracted away from the signals.

Users selected a time interval to compare the two channels. The data was split into segments with the user-selected lengths. For sinus rhythm recordings, segment length of one second was used, as this normally included one activation. For VF recordings, segment length was reduced to 0.2 s. This length would limit the amount of data being compared, while still allowing for a reasonable delay due to conduction velocity.

Segments from one channel were compared to simultaneous segments from the other channel. To find the offset in which the two segments had the best correlation, or signal morphology, the cross correlation was performed. The inner half of the resulting cross correlation, whose length was twice that of the segment, was calculated, and the absolute maximum of that window was found. For sinus rhythm, this window was reduced to the inner one quarter of the cross correlation array. To validate this window, 15 seconds of recordings of a rabbit heart were divided into 1-second segments. Cross-correlations of the recordings of left and right sides were produced using the *xcorr* function with '*coeff*' normalization. The objective was to find the shift that gave the best correlation. This shift may correspond to the conduction time across the lesion. However, we wanted to avoid the shift for non-related beats on the left and right. Therefore we limited the range for delays to a fraction of the interbeat interval. We made the interbeat interval +/- 0.5, 0.333, 0.25, 0.2, or 0.125 the length of the interval. A sensitivity analysis was performed for these search intervals. The delays were within 8 to 9 ms within all cases. This indicates that the delay was not sensitive to the choice of window. Thus we chose the window of 0.375-0.625, which corresponds to a maximum delay of +/- 250 ms. An advantage of that value is that does not allow for different sinus beats to be correlated, based on the interbeat interval in sinus rhythm observed.

This maximum value correlated to the delay that provided the maximum cross correlation, and was converted to seconds and stored. Figure 3.5 shows an example of the cross-correlation result. The delay calculated from this example is 0.146 seconds, used to calculate a correlation coefficient (*r*) of -0.6928 (absolute value stored).

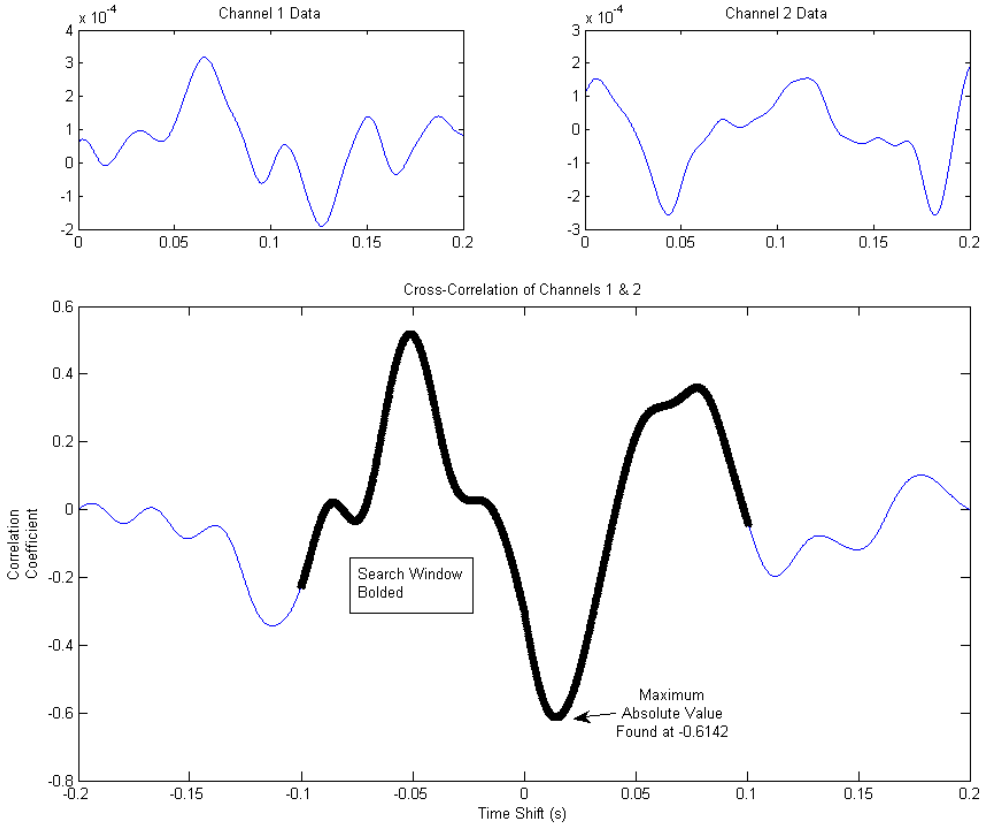


Figure 3.5 – Cross-correlation example. Two segments of data shown above, with resulting cross-correlation array. Search window for absolute maximum is bolded, with absolute maximum found at -0.6142, which corresponds to a positive shift.

Because the algorithm behind the *xcorr* function was unknown, it was determined that the *xcorr* function would be used to find the delay with the highest correlation value *r*. Using this delay, the functions were offset from each other, and the correlation taken using the corr2 function. This function found *r* of the shifted signals, allowing us to more accurately judge the similarity of morphology of the two signals. The absolute value of *r*

was taken, as it was decided that two signals with highly negative correlation (approaching $r = -1$) would have very similar morphology, only one signal would have been flipped in sign.

In order to properly align the signals, the signals were zero-padded to adjust the length. To do this, the sign of the delay was determined. If this delay was positive, meaning that the channel 2 signal was delayed after channel 1, then an appropriate number of zeros was added to the beginning of the channel 2 signal array. The number of zeros was found by taking the length of the delay (in seconds) multiplied by the scan rate. The same number of zeros was also added to the end of the channel 1 signal. By doing this, the two arrays would have the same length, which was a requirement to use any of the MATLAB correlation functions.

There are several issues with this method. By zero-padding the sides of these signals, we are introducing extra time segments to the two signals. While the means of both signals have been offset to zero, the addition of the extra zeros may lead to a lower correlation value as those zeros are being compared to part of the signal on the other channel.

It was determined, after visual inspection of various data segments, to truncate the signals where they did not align instead of zero-padding. By truncating, these extraneous segments are removed from the sides of the two signals, leaving two samples, equal in length, that correspond to the maximum cross-correlation value calculated. In cross-correlation, the portions of samples that do not overlap are multiplied by zero to not add into the sum correlation value, so this truncation correlation method agrees best with the cross-correlation algorithm. Figure 3.6 shows an example of zero-padding and truncating the VF signals after alignment, and lists correlation coefficients for the appropriate alignment methods.

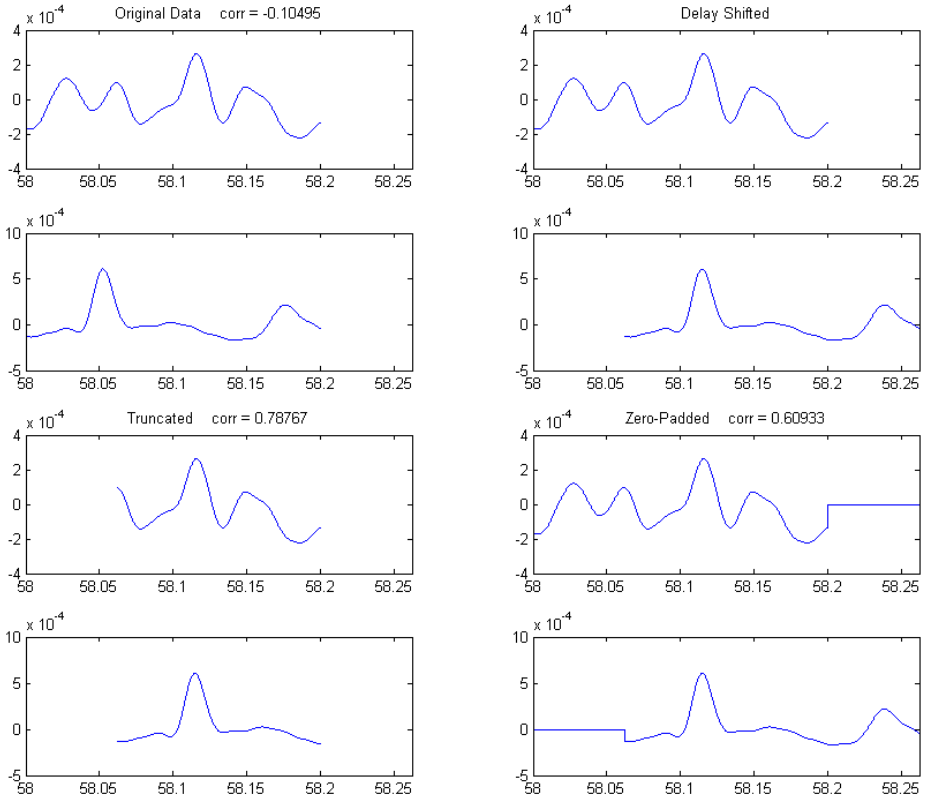


Figure 3.6 – Correlation of shifted signals. Channel 1 shown above channel 2. Top Left: Original Data Alignment, r = -0.105. Top Right: Original Data with Delay Shift. Bottom Left: Delay Aligned Signals after Truncation, r = 0.788. Bottom Right: Delay Aligned Signals with Zero-Padding, r = 0.609.

Translesion Stimulus Excitation Delay Pacing

Background

Based on previous research from H. Himel et al.[3], a pacing regimen was instituted during VF to study the change in peak delay associated with a complete lesion. While rapid pacing has been studied as a trigger for fibrillation[9], the use of pacing during AF or VF is relatively unknown. It was decided to study the use of translesion stimulus-excitation delay during fibrillation as a metric for the lesion completeness. As described in the Methodology section, pacing was accomplished by stimulating across one set of electrodes, on the left side

of the lesion, and recording the translesion response. Pacing was performed in both sinus rhythm and fibrillation episodes.

Algorithm

From the pacing data recordings, the trigger signal was isolated and the stimulus times were found by taking the signal's derivative. After each trigger, a sample from the opposite channel was taken from 30 ms past the trigger point to 2 ms before the next trigger. All samples were averaged together to attain a baseline for the entire recording. The omission of points close to the trigger was done to remove any immediate spikes caused by the trigger. This baseline was subtracted away from the signal sample in order to accurately calculate the peak delay.

For each trigger event, a sample of the other channel was taken from 30 ms past the trigger pulse, until 2 ms before the following trigger pulse. The length of this sample was dependent upon the pacing frequency, thus a constant sample length was not used. After the baseline calculated previously was subtracted away from the sample, the signal was decimated by a factor of 5. The differential, an approximation of the derivative, of this decimated signal was taken, and the maximum and minimum values were found within this differential. This value was the time at which the signal had the greatest positive or negative change over a short time, our estimate of the stimulus response. Figure 3.7 shows an example of responses found in slow pacing during sinus rhythm.

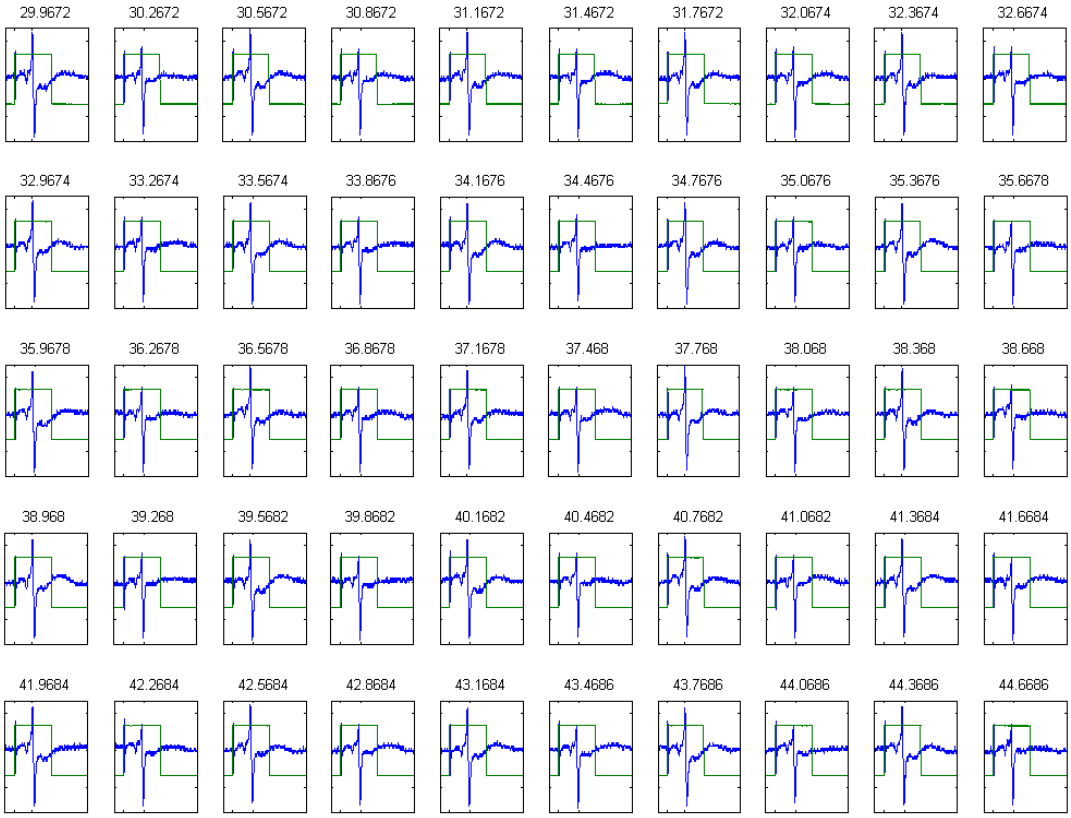


Figure 3.7 – Stimulus excitation response shows a consistent TED. Time of stimulation shown above each plot. In green, square wave upstroke shows time of stimulation pulse.

Its delay and sign of the signal at which the upstroke occurred were recorded. In order to study the similarity of stimulus responses, the correlation between the sample and the next sample, and the correlation between the sample and the (i+2) sample was taken, which may give a greater value in cases with alternans.

Analysis Discontinuation

It was decided that the investigation into pacing during fibrillation would be halted because capture did not occur frequently, so identifying the response peaks was not accurate. Because of the complex nature of VF, there was not a consistent response to the stimulus and made any comparison between pre- and post-ablation statistically weak. In sinus rhythm, the stimulus beats were captured with a high success rate, showing that the tissue was responsive

to the stimulus rate and strength. This consistency was not the case in fibrillation episodes. Priority was given to morphology analysis when the decision was made between experimenters and representatives of nContact, to abandon the pacing regimen during experiments.

CHAPTER 4

SUMMARY OF RESULTS

Dominant Frequency Analysis

As shown in Abashian et al., ablation was shown to alter the distribution of dominant frequencies measured using our algorithm[7]. In both time and frequency domains, the dominant frequencies of the left and right channels were statistically different when compared before vs. after ablation. Data was collected from all year 2007 experiments (n = 15 rabbits). For the CINC proceedings paper, the time- and frequency-domain analyses were compared in order to show the usefulness of these analyses. The following graphs show the control (pre-ablation) comparisons of frequency- and time-domain DF analyses.

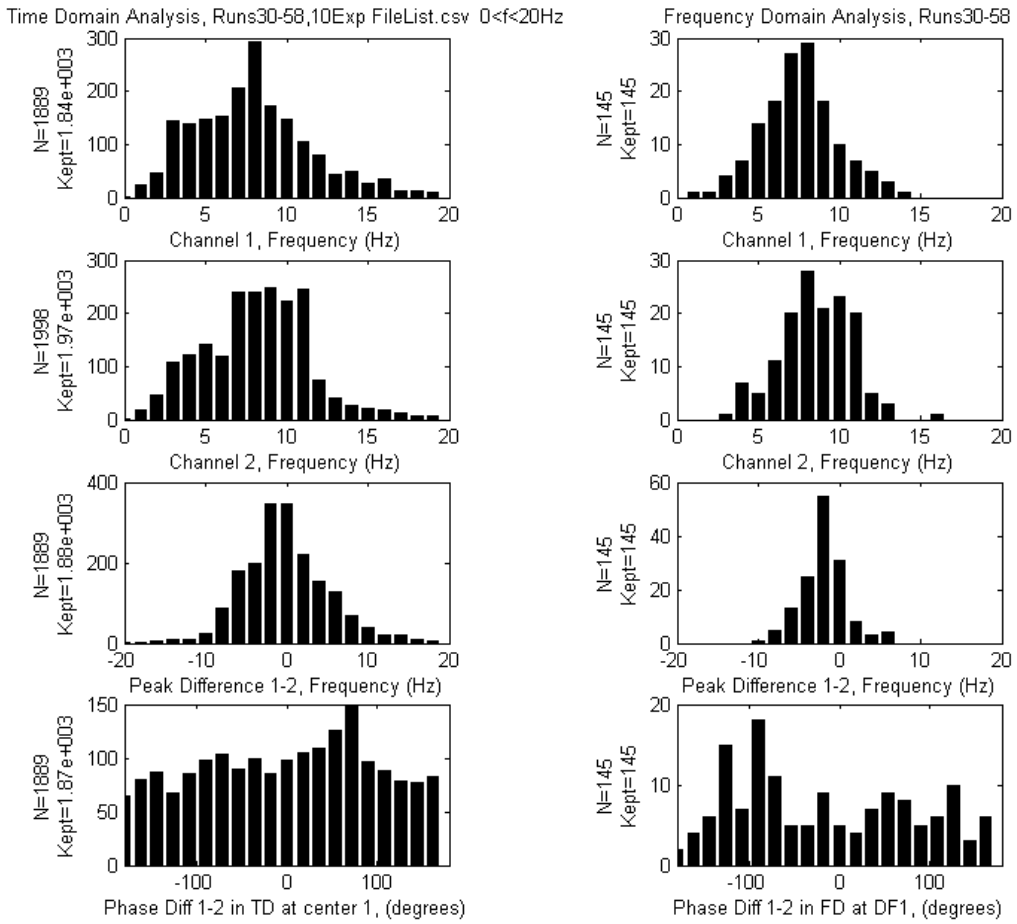


Figure 4.1 – Pre-ablation dominant frequency analysis. Time-domain and FFT results in left and right columns, respectively. In each column, dominant frequency histograms for Channel 1 and 2, followed by histogram of DF difference. The final two plots in each column are Phase differences and magnitudes relating to phase.

By visual inspection, the distribution of DFs found using the FFT is similar to the frequencies found in the time-domain analysis. In comparing DF means and standard deviations, the time-domain analysis was more widely dispersed, suggesting that the dominant frequency analysis, using 2-second segments, does not capture the variability of the beat-to-beat intervals[7]. The values to the left of each graph show the number of calculated frequencies that were found and included in the analysis. In the time-domain analysis, only intervals that corresponded to frequencies in the range of 0-20 Hz were used; the dominant frequency analysis had the same limitations as well.

In post ablation recordings, the following graphs were again created to show the similarity of the two analyses. As with the pre-ablation recordings, the two analysis algorithms were both visually and statistically similar.

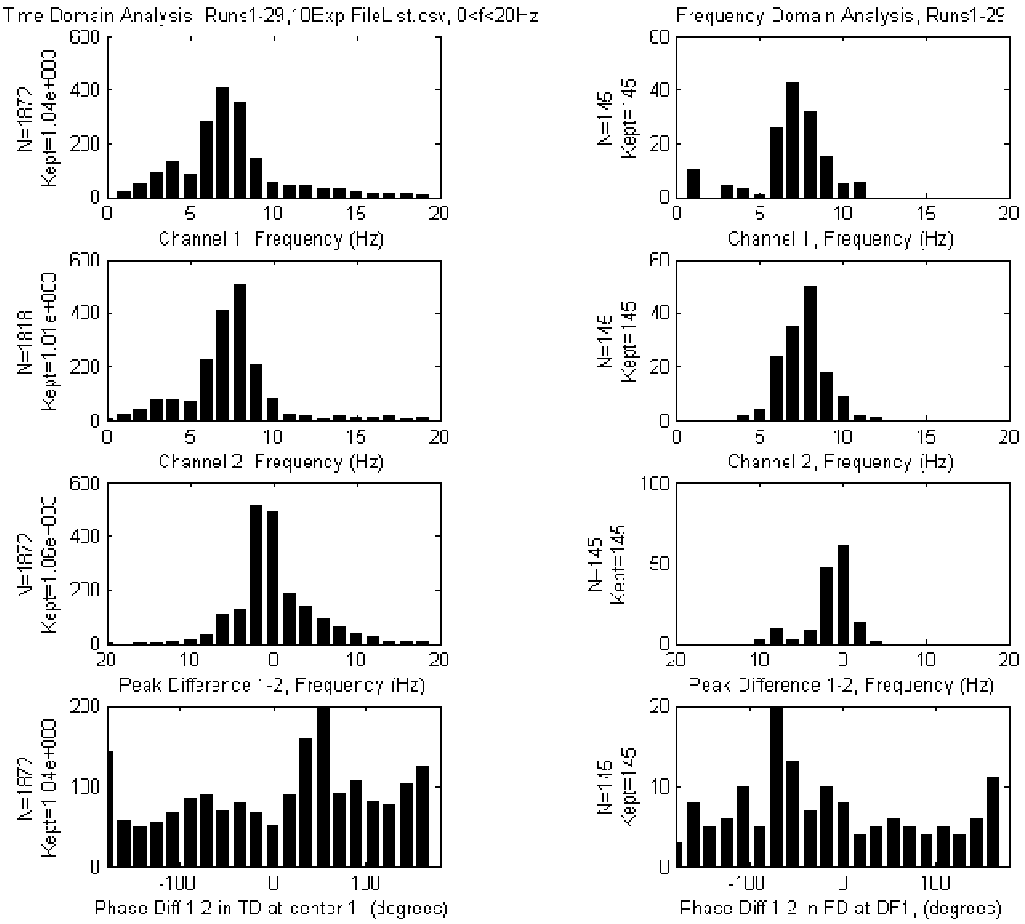


Figure 4.2 – Post-ablation dominant frequency analysis.
Time- and frequency-domain analyses results left and right, respectively.

For both pre- and post-ablation analyses, the phase calculations, wrapped from $-\pi$ to π, did not show a similarity between frequency- and time-domain analyses. Further analysis of phase relation was not pursued. Additionally, for the five final rabbit experiments of 2007, a gapped lesion was created, and data was similarly analyzed for both dominant frequency and peak-to-peak intervals.

Frequency-Domain Analysis

To compare pre- and post-ablation dominant frequencies, values from both sides of the lesion were pooled together. Means were calculated using the formula

$$\overline{x_{pooled}} = \frac{\overline{x_R} n_R + \overline{x_L} n_L}{n_R + n_L}$$

Pooled variance was calculated using

$$s^2{}_{pooled} = \frac{(n_R - 1)s_R^2 + (n_L - 1)s_L^2}{n_R + n_L - 1}$$

	Pre		Complete	
	Left	**Right**	**Left**	**Right**
N	145	145	145	145
Mean	8.077	9.069	7.416	8.082
Std Dev	2.336	2.199	2.240	1.336
Var	5.455	4.833	5.019	1.784
	Pooled		**Pooled**	
N	290		290	
Mean	8.573		7.749	
Variance	5.126		3.390	
Std Dev	2.264		1.841	
D.F.	289		289	

Table 4.1 – DF analysis frequency-domain results, experiments 1-10.

From the pooled data for Pre and Complete recordings, an F-test to compare variances found a value of 1.512, which corresponds to a *p*-value of 0.000232. This shows that the variances of the two sets of data are statistically different ($p<0.05$).

For the data that included the gapped recordings, the following table shows the summary of analysis.

	Pre		Gap		Complete	
	Left	**Right**	**Left**	**Right**	**Left**	**Right**
N	85	85	85	85	85	85
Mean	9.150	8.853	9.774	10.020	8.793	9.133
Std Dev	1.508	2.111	1.748	1.329	1.566	1.483
Var	2.275	4.458	3.055	1.767	2.451	2.198
	Pooled		**Pooled**		**Pooled**	
N	170		170		170	
Mean	9.002		9.897		8.963	
Variance	3.347		2.397		2.311	
Std Dev	1.829		1.548		1.520	

Table 4.2 – DF analysis frequency-domain results, gap-lesion experiments 11-15.

This data allowed for three comparisons of variances between the sets. For Pre vs. Gap, an F-stat value of 1.396 and *p*-value of 0.0153 were found. For Pre vs. Complete, an F-stat of 1.448 and a *p*-value of 0.00826 were found. For Gap vs. Complete, an F-stat of 1.037 was calculated which gave a *p*-value of 0.406. Thus, both Pre vs. Gap and Pre vs. Complete DF variances were statistically distinguishable, while variances in Gap vs. Complete dominant frequencies were not statistically different.

Time-Domain Analysis

Results of the time-domain analysis were collected and pooled in a similar way to the dominant frequency analysis. The following table shows the data from the 10 rabbits where only pre- and post-ablation recordings were taken.

	Pre		Complete	
	Left	**Right**	**Left**	**Right**
N	1842	1965	1841	1812
Mean	8.379	8.662	7.872	7.803
Std Dev	3.571	3.230	3.054	2.507
Var	12.752	10.431	9.329	6.283
	Pooled		**Pooled**	
N	3807		3653	
Mean	8.525		7.838	
Variance	11.551		7.816	
Std Dev	3.399		2.796	
D.F.	3806		3652	

Table 4.3 – DF analysis time-domain results, experiments 1-10.

The comparison of the pooled data provided an F-stat value of 1.478, and a p-value less than 0.0001. It must be noted that for the time-domain analysis, the large number of samples (N>3500 for both sets) can allow slight mean difference to be significant, and hence the minute p-value.

The time-domain data for the additional five rabbits of 2007 is summarized as follows.

	Pre		Gap		Complete	
	Left	**Right**	**Left**	**Right**	**Left**	**Right**
N	1152	1106	1285	1122	1200	1177
Mean	8.310	8.308	9.156	8.507	8.438	8.884
Std Dev	2.983	3.292	3.248	3.301	2.962	3.233
Var	8.900	10.837	10.550	10.897	8.773	10.455
	Pooled		**Pooled**		**Pooled**	
N	2258		2407		2377	
Mean	8.309		8.853		8.659	
Variance	9.844		10.707		9.602	
Std Dev	3.138		3.272		3.099	

Table 4.4 – DF analysis time-domain results, gap-lesion experiments 11-15.

The F-stat for comparison of variances of Pre vs. Gap was found to be 0.919, with a p-value of 0.0214. For Pre vs. Complete, the F-stat was 1.025, with a p-value of 0.274. For Gap vs. Complete, the F-stat was calculated at 1.115, with a p-value of 0.00388.

Signal Morphology Analysis

Morphology analysis was performed on the 2008 set of rabbit heart experiments (N=9). In each experiment, non-paced electrocardiograms in both sinus rhythm and VF were recorded before and after ablation. The total recording time for a specific situation (pre-ablation, sinus rhythm, etc.) ranged from 10 seconds to 270 seconds. This range came from a lack of specified experimental recording duration. In three hearts, visual inspection of the TTC-stained heart, post-experiment, showed an incomplete (non-transmural) lesion. The data for this analysis was separated into two categories, Incomplete and Complete Lesion.

Sinus Rhythm

Correlation Values

Sinus rhythm recordings were taken for every heart, and every one-second segment was analyzed using the morphology comparison algorithm.

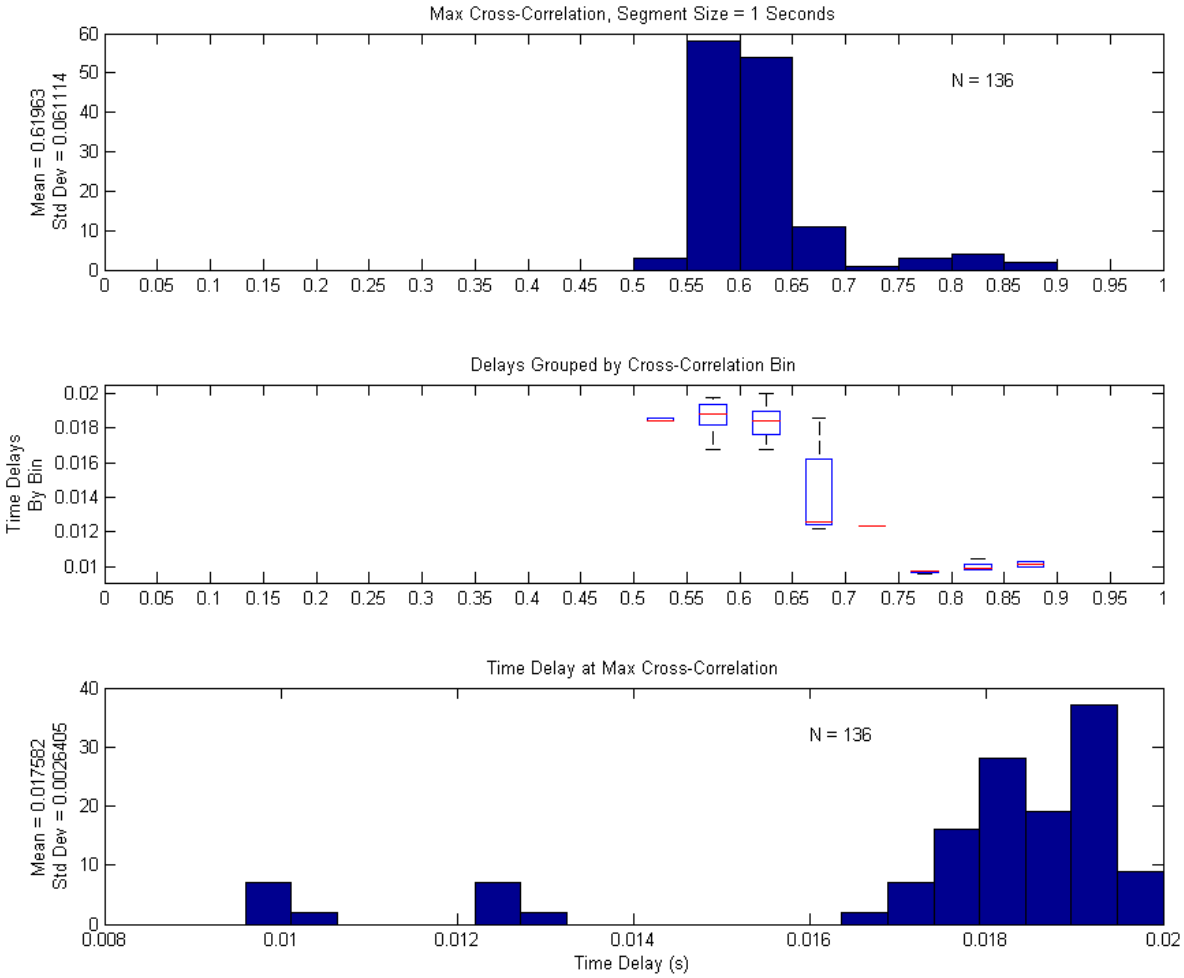


Figure 4.3 – Morphology comparison example for sinus rhythm recording. Upper Plot: Histogram of magnitudes of ***r****. Middle plot: Box plots of time delay grouped by correlation value. Lower Plot: Histogram of time delays.*

The above example charts are from the May 28, 2008 heart. The upper chart shows the histogram of groups of correlation values. Below that, box plots for each group show the time delays that occurred for each group of correlations. For sinus rhythm recordings, the means and standard deviations of correlation values are shown below.

	Pre, Sinus			Post, Sinus		
Date	**N**	**Mean**	**Dev**	**N**	**Mean**	**Dev**
Incomplete:						
1/25/2008	9	0.594	0.101	9	0.486	0.055
1/30/2008	118	0.774	0.045	118	0.568	0.054
2/26/2008	118	0.787	0.031	118	0.787	0.029
Complete:						
2/11/2008	118	0.632	0.053	118	0.500	0.086
5/7/2008	195	0.803	0.055	195	0.558	0.074
5/8/2008	136	0.368	0.079	136	0.792	0.037
5/12/2008	136	0.536	0.044	136	0.489	0.057
5/21/2008	136	0.648	0.083	136	0.526	0.040
5/28/2008	136	0.620	0.061	136	0.647	0.038

Table 4.5 – Sinus rhythm mean correlation values.

In the above table, the correlation coefficients from N segments analyzed in each heart were combined to find a mean and standard deviation. N shows the number of one-second segments analyzed separately. A paired t-test for the grouped incomplete lesion hearts between pre- and post-ablation showed a p-value of 0.220, showing the mean correlation coefficients are not significantly different. For the grouped complete lesion cases, a paired t-test showed a value of 0.876. Neither of these p-values shows a significant non-zero change in mean correlation coefficient after ablation. We are not aware of a straightforward method to statistically test changes in variance of grouped data.

To examine the single-heart changes from pre- to post-ablation during sinus rhythm, two statistical tests were performed to examine changes in mean correlation coefficient and variance.

Date	Mean Diff	Dev Diff	Variance	Mean
Incomplete:			**F-Test**	**T-Test**
1/25/2008	-0.107	-0.0462	0.051	**0.012**
1/30/2008	-0.206	0.0088	0.**027**	**4.2E-87**
2/26/2008	-0.00041	-0.0020	0.236	0.917
Complete:				
2/11/2008	-0.132	0.0321	**2.99E-07**	**1.39E-33**
5/7/2008	-0.244	0.0194	**1.59E-05**	**1E-128**
5/8/2008	0.424	-0.0424	**1.11E-17**	**1.6E-151**
5/12/2008	-0.046	0.0129	**0.0015**	**8.93E-13**
5/21/2008	-0.122	-0.0431	**2.64E-16**	**6.73E-39**
5/28/2008	0.028	-0.0233	**2.2E-08**	**1.11E-05**

Table 4.6 – Sinus rhythm mean correlation comparisons. F- and T-test results below the p=0.05 value are highlighted in bold.

Delays

From every cross-correlation calculation, a delay was found that best aligned the two signals. This delay was taken from the maximum correlation value of the cross-correlation, and by our tests correctly aligns two signals. For sinus rhythm recordings, the following delays were calculated.

		Pre, Sinus			Post, Sinus	
Date	**N**	**Mean**	**Dev**	**N**	**Mean**	**Dev**
Incomplete:						
1/25/2008	9	0.00530	0.00113	9	0.02793	0.02368
1/30/2008	118	0.00659	0.00058	118	0.02724	0.00595
2/26/2008	118	0.00827	0.00045	118	0.01226	0.00035
Complete:						
2/11/2008	118	0.01177	0.00815	118	0.00888	0.02052
5/7/2008	195	0.00235	0.00123	195	0.00860	0.02303
5/8/2008	136	0.05132	0.05737	136	0.00178	0.00083
5/12/2008	136	0.02331	0.01478	136	0.00434	0.01236
5/21/2008	136	0.02331	0.01115	136	0.01609	0.00381
5/28/2008	136	0.01758	0.00264	136	0.03532	0.01481

Table 4.7 – Sinus rhythm mean delay values.

For this set of data, paired t-tests of the mean delays between pre- and post-ablation showed p-values for incomplete and complete lesions of 0.117 and 0.384, respectively, indicating that the change in delay as a set could not be shown to be different from zero for either case (incomplete or complete). Variances were not compared because no statistical test for grouped variances could be identified.

The individual hearts were also analyzed for specific changes in mean delay and variance of those delays. This summary table shows the results of F-tests and T-tests for the sinus rhythm data.

Date	Mean Diff	Dev Diff	Variance	Mean
Incomplete:			**F-Test**	**T-Test**
1/25/2008	0.02263	0.02255	**9.37E-10**	**0.011**
1/30/2008	0.02065	0.00537	**0**	**5.1E-101**
2/26/2008	0.00399	-0.00011	**0.0021**	**3E-167**
Complete:				
2/11/2008	-0.00290	0.01237	**0**	0.155
5/7/2008	0.00625	0.02180	**0**	**0.000178**
5/8/2008	-0.04954	-0.05654	**6.20E-210**	**1.85E-20**
5/12/2008	-0.01897	-0.00242	**0.0192**	**3.98E-25**
5/21/2008	-0.00722	-0.00733	**8.04E-31**	**8.17E-12**
5/28/2008	0.01774	0.01217	**0**	**5.94E-33**

Table 4.8 – Sinus rhythm mean delay comparisons. F- and T-test results below the p=0.05 threshold are highlighted in bold.

The zero values shown in the results of the F-Test are Excel approximations of the p-value. While all cases showed a difference in the variances between pre- and post-ablation, there is no consistent increase or decrease in delay variance. In addition, every heart in both groups showed a change in variance between pre- and post-ablation, as showed by F-tests on single-heart variances. For the mean delays, all cases except for the Feb. 11 heart showed a significant change in mean, but as in the variance, there is no consistent positive or negative

change in delay after ablation. Because of this, we cannot determine a difference in the change in mean delay after ablation between incomplete and complete lesions.

Ventricular Fibrillation

Correlation Values

Recordings during ventricular fibrillation were taken for N=8 hearts. Fibrillation could not be induced in the May 21, 2008 rabbit heart post-ablation, so morphology analysis was not performed on the VF case for that heart.

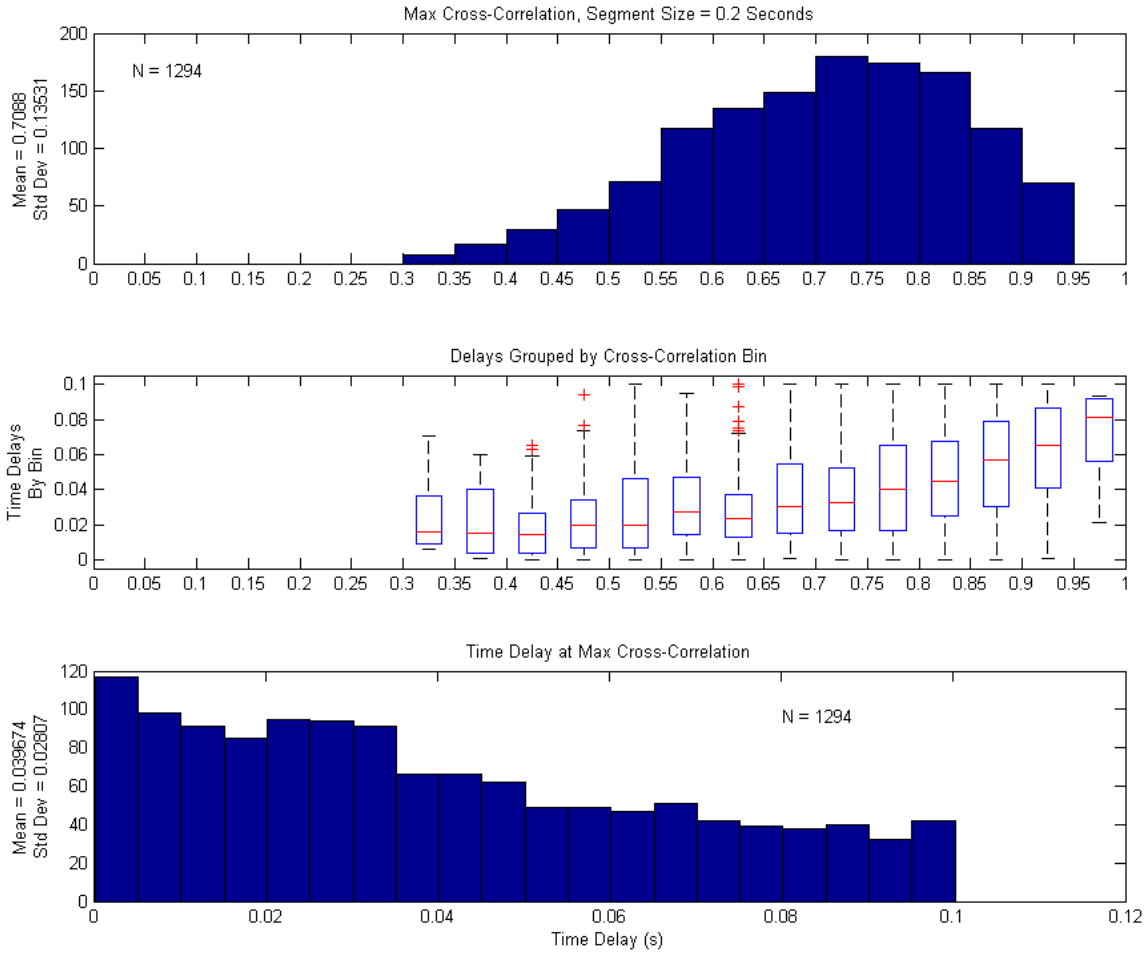


Figure 4.4 – Morphology comparison example for VF recording.

These charts are an example from the May 28, 2008 heart. As the segments are less uniform, there is generally a larger variance in correlation coefficients. The VF correlation data taken from each heart is summarized below.

		Pre, VF			Post, VF	
Date	**N**	**Mean**	**Dev**	**N**	**Mean**	**Dev**
Incomplete:						
1/25/2008	98	0.622	0.144	196	0.641	0.146
1/30/2008	299	0.670	0.154	598	0.672	0.156
2/26/2008	598	0.643	0.138	598	0.644	0.136
Complete:						
2/11/2008	598	0.650	0.137	598	0.608	0.132
5/7/2008	1343	0.618	0.135	1343	0.629	0.144
5/8/2008	1294	0.694	0.140	1294	0.650	0.143
5/12/2008	1294	0.723	0.163	147	0.731	0.151
5/28/2008	995	0.639	0.140	1294	0.709	0.135

Table 4.9 – VF mean correlation values.

For the incomplete lesion cases, a paired t-test of the mean correlation values returned a p-value of 0.353, above the 0.05 threshold. A paired t-test on the complete lesion set of mean correlation values returned a p-value of 0.984. This suggests there is no significant change in mean correlation value after ablation, incomplete or complete. Analysis of individual hearts yielded the following results.

Date	Mean Diff	Dev Diff	Variance	Mean
Incomplete:			**F-Test**	**T-Test**
1/25/2008	0.0189	0.0022	0.440	0.297
1/30/2008	0.0016	0.0014	0.436	0.887
2/26/2008	0.0008	-0.0027	0.314	0.921
Complete:				
2/11/2008	-0.0422	-0.0046	0.202	**6.95E-08**
5/7/2008	0.0112	0.0087	**0.012**	**0.039**
5/8/2008	-0.0444	0.0026	0.252	**2.11E-15**
5/12/2008	0.0078	-0.0118	0.123	0.582
5/28/2008	0.0698	-0.0048	0.122	**1.75E-32**

Table 4.2 – VF mean correlation comparisons. F- and T-test results below the p=0.05 threshold are highlighted in bold.

For incomplete lesions, there were no cases with any significant ($p<0.05$) differences in variance or mean correlation after ablation. For complete lesions, one heart (May 7 experiment) showed a significant rise in variance post-ablation. This could be due to the large number of samples for that experiment (N=1343 for pre- and post-ablation). Four of the five analyses showed a significant change in mean correlation values after ablation.

Delays

Although the signals were not as consistent as in sinus rhythm, delays were found in VF segments for the best alignment of morphologies. The following table shows the collected delay data.

		Pre, VF			Post, VF	
Date	**N**	**Mean**	**Dev**	**N**	**Mean**	**Dev**
Incomplete:						
1/25/2008	98	0.03995	0.02890	196	0.04419	0.03076
1/30/2008	299	0.04113	0.02780	598	0.04344	0.02975
2/26/2008	598	0.04146	0.02851	598	0.04163	0.02946
Complete:						
2/11/2008	598	0.04413	0.03005	598	0.04025	0.02860
5/7/2008	1343	0.04157	0.02874	1343	0.04084	0.02906
5/8/2008	1294	0.04024	0.02846	1294	0.04153	0.02904
5/12/2008	1294	0.04467	0.03140	147	0.03772	0.03115
5/28/2008	995	0.04199	0.02938	1294	0.03967	0.02807

Table 4.3 – VF mean delay values.

From this data, paired t-tests were performed for both incomplete and complete lesions to test for a consistent change in mean delay. For the incomplete lesion cases, a p-value of 0.197 was found; for complete lesions, a p-value of 0.146 was returned. These values suggest there is not a consistent positive or negative change in mean delay after ablation. Analysis of individual hearts was also performed, and summarized in the following table.

Date	**Mean Diff**	**Dev Diff**	**Variance**	**Mean**
Incomplete:			**F-Test**	**T-Test**
1/25/2008	0.0042	0.0019	0.247	0.257
1/30/2008	0.0023	0.0019	0.092	0.262
2/26/2008	0.0002	0.0010	0.211	0.922
Complete:				
2/11/2008	-0.0039	-0.0015	0.113	**0.022**
5/7/2008	-0.0007	0.0003	0.341	0.517
5/8/2008	0.0013	0.0006	0.235	0.255
5/12/2008	-0.0069	-0.0003	0.461	**0.011**
5/28/2008	-0.0023	-0.0013	0.062	0.055

Table 4.4 – VF mean delay comparisons. F- and T-test results below the p=0.05 threshold are highlighted in bold.

In the hearts with incomplete lesions, there was no cases with a significant ($p<0.05$) change in the mean delay or variance of delays. In hearts with complete lesions, two of the five hearts showed significant decreases in the mean delay; however, there were no hearts that had significant changes in deviation of the delay.

Translesion Stimulus Excitation Delay Pacing Analysis

Slow Pacing in Sinus Rhythm

The pacing regimen was established for the 2008 set of rabbits. In sinus rhythm, slow pacing proved successful, with highly regular stimulus responses. The stimulus response values that were calculated using the analysis algorithm were plotted on a histogram, as well as the peak stimulus excitation delay over time.

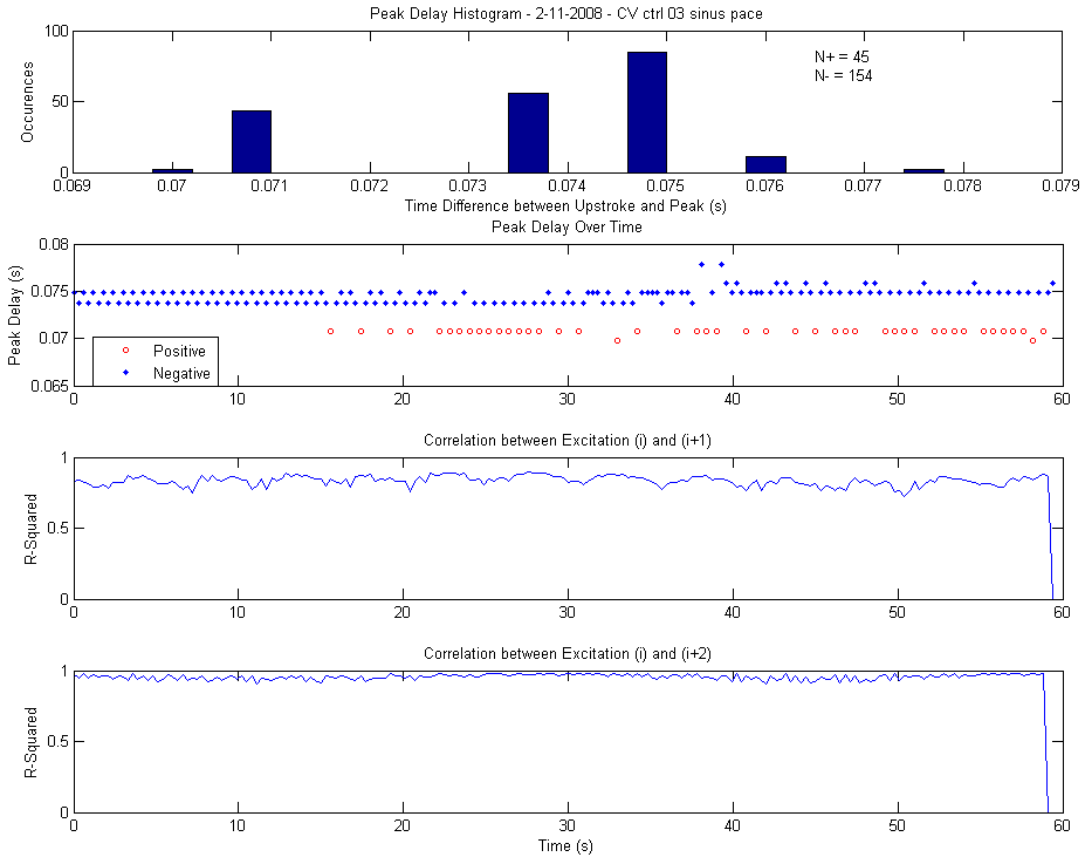


Figure 4.5 – TED in slow pacing example. Upper Plot: Histogram of TED. Upper Middle Plot: TED vs. Stimulation Time. Lower Plots: Correlation between beats shows variability throughout recording.

The final two plots in Figure 4.5 show the morphology correlation between consecutive stimulus responses, and between a beat (*i*) and beat (*i+2*). The beats are highly grouped in the above histogram. The resolution of the delays is limited by the sampling rate (5-10 kHz). The peak delay sign (positive or negative) was also calculated, and for sinus rhythm pacing, it was very common to see all peak delays have one sign, as is the case in the example plot above. The analysis is summarized in the following table showing mean delays and standard deviations.

File			Delay	
Pre-Ablation	**Length (s)**	**N**	**Mean**	**Std Dev**
1-25-2008 - CV ctrl pacing	10	31	0.0867	0.0346
1-25-2008 - CV ctrl pacing 2	10	32	0.0659	0.0005
1-30-2008 - CV ctrl pace01	60	109	0.0801	0.0007
1-30-2008 - CV ctrl pace02	60	199	0.0798	0.0001
2-11-2008 - CV ctrl 03 sinus pace	60	199	0.0737	0.0017
2-11-2008 - CV ctrl 04 sinus pace	60	199	0.0725	0.0025
2-26-2008 - CV Ctrl 03 Sinus Pace	60	199	0.0435	0.0005
2-26-2008 - CV Ctrl 04 Sinus Pace	60	199	0.0435	0.0005
Post Ablation				
1-25-2008 - CV post pacing	10	33	0.0850	0.0034
1-25-2008 - CV post pacing 2	10	32	0.0868	0.0027
1-30-2008 - CV post pace01	60	199	0.0965	0.0008
1-30-2008 - CV post pace02	60	199	0.0980	0.0006
2-11-2008 - CV post 16 sinus pace	60	199	0.0682	0.0017
2-11-2008 - CV post 17 sinus pace	60	199	0.0656	0.0004
2-26-2008 - CV Post 10 Sinus Pace	60	199	0.0721	0.0086
2-26-2008 - CV Post 11 Sinus Pace	60	199	0.0720	0.0087

Table 4.5 – Slow pacing TED results.

In order to compare each heart pre- vs. post-ablation, the means and standard deviations of each run for a specific heart were pooled, or combined together using the formulae listed in the Dominant Frequency results section. In 3 of the 4 rabbit hearts (one with 20 s of data pre-ablation and 20 s post-ablation, and 2 with 120 s pre and post), the mean delay increased after ablation. In one rabbit heart (with 120 s pre and post) the mean

delay decreased. The sign test applied to mean delays pre- and post-ablation in these 4 rabbits did not show that the probability of finding a greater or smaller delay after ablation was different from 0.5. To confirm this finding, a paired T-test was performed on the pooled means. A p-value of 0.188 was found for all hearts (N=4 pairs). Because the 1-25-2008 heart data had noticeably less samples, it was suggested to exclude its data from a paired T-test as well. Excluding this heart, a p-value of 0.325 was found. Both these results suggest that there is no consistent statistical change in mean peak delay between pre- and post-ablation slow-pacing recordings.

Date	N	Mean	Variance	Std. Dev.
Pre				
1/25/2008	63	0.0761	5.79E-04	0.0241
1/30/2008	308	0.0799	1.69E-07	0.0004
2/11/2008	398	0.0731	4.68E-06	0.0022
2/26/2008	398	0.0435	2.07E-07	0.0005
Post				
1/25/2008	65	0.0859	9.52E-06	0.0031
1/30/2008	398	0.0973	4.84E-07	0.0007
2/11/2008	398	0.0669	1.52E-06	0.0012
2/26/2008	398	0.0720	7.45E-05	0.0086

Table 4.6 – Slow pacing pooled results.

An F-test was performed on the standard deviations to examine the effect of ablation. Because of the large number of samples and very small variances, the p-values were minute. For the 1-30-2008 rabbit heart (120 s pre-ablation, 120s post-ablation), a p-value of 2.82E-21 was found. For the other three cases, the p-value was so small that Microsoft Excel calculated the value to be 0. However, a sign test applied to these deviations shows that probability of finding a greater or smaller standard deviation after ablation is not different from 0.5.

Ventricular Fibrillation Pacing

Pacing was also attempted during ventricular fibrillation, with much less success. Because of the lack of an established pacing regimen, the data collected varied in pacing frequencies between hearts.

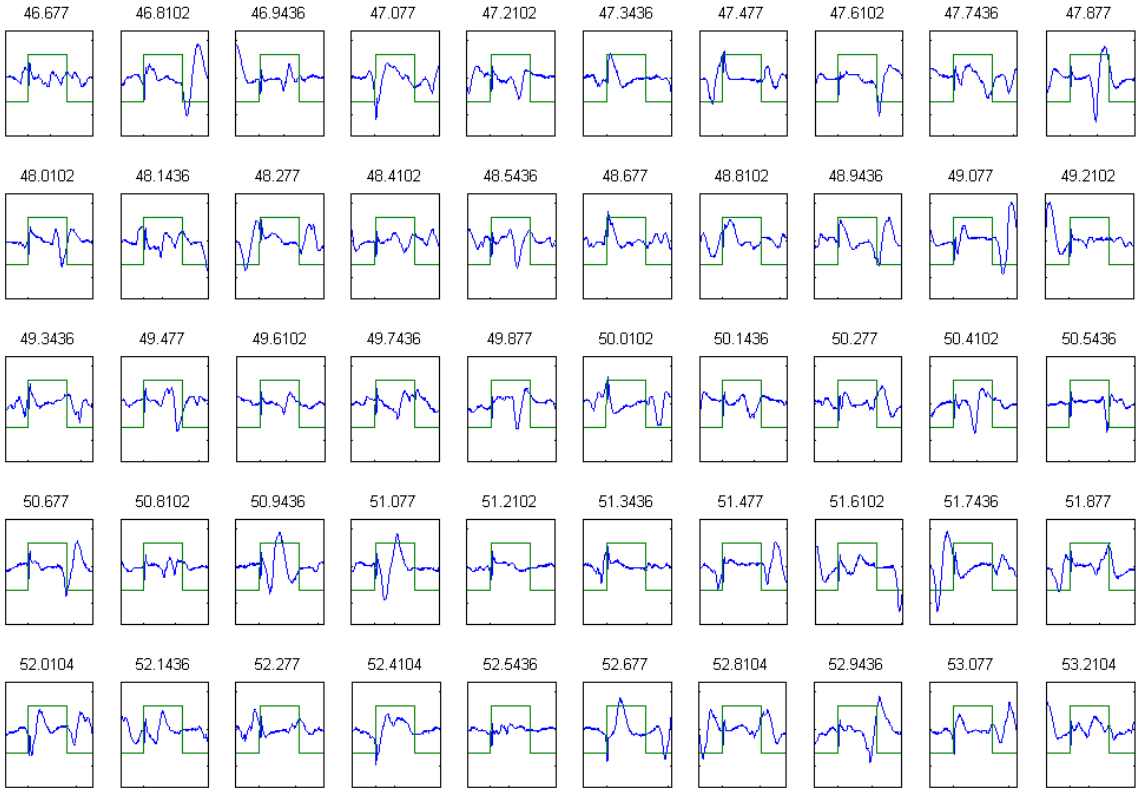


Figure 4.6 – Stimulus excitation response in VF.

Figure 4.6 above shows an example of the varied response to stimuli during VF. The green square wave shows the stimulation events (the upstroke of the wave). Small tics on the border of each graph shows the delay measured for that segment. For the analysis, pre-ablation files were compared with post-ablation files with the same pacing frequency. Although the pacing regimen was not consistent between experiments, within an experiment the same pacing frequencies were tested both pre- and post-ablation. For a statistical analysis

of the dependence of stimulus excitation delay on pacing frequency, rabbit, and ablation state (pre-ablation vs. post-ablation), an ANOVA analysis was performed using the SAS statistical analysis software package. Each stimulation segment was used as a separate observation, totaling 15,384 observations over 3 rabbit hearts. ANOVA analysis shows a $p<0.001$ indicating that stimulus response delay is dependent on both frequency and ablation state. The analysis also shows that the delay is not statistically dependent on the heart from which the observation originated. See Appendix C for the results page of the SAS ANOVA Analysis.

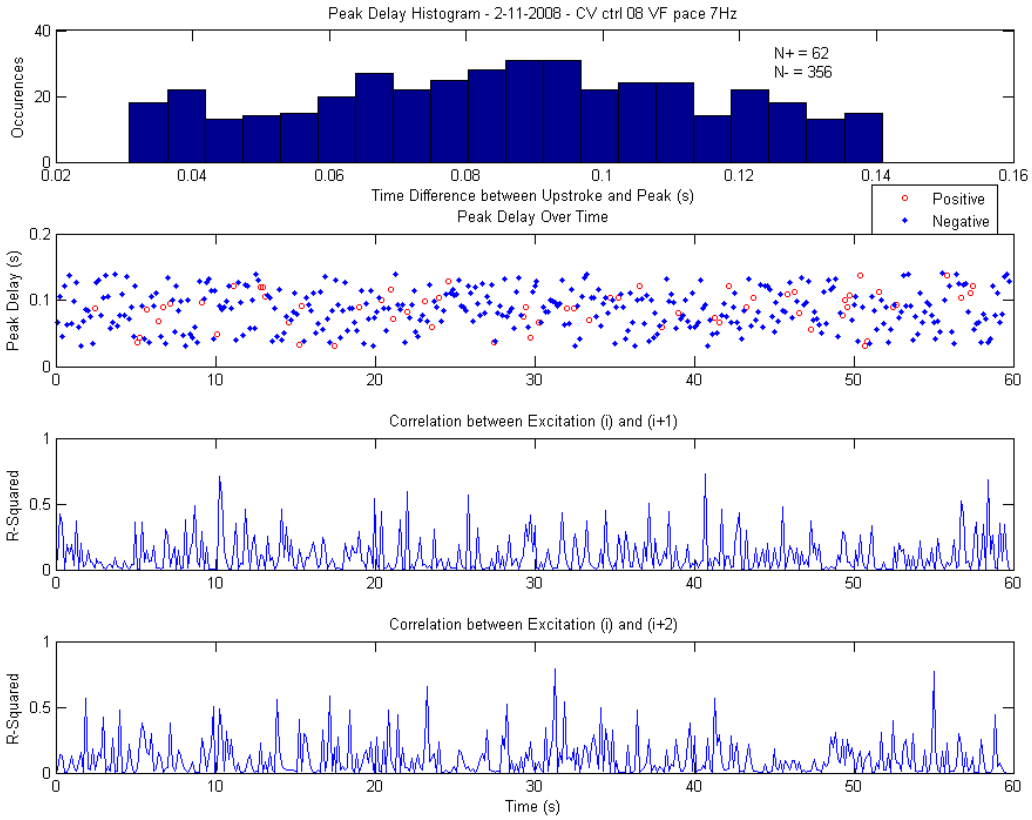


Figure 4.7 – TED in VF pacing example. High variability of response suggests lack of capture in most stimuli. Positive and negative values in the second plot indicate the sign of the maximum slope found in each segment.

Figure 4.7 above shows an example of the dispersion of peak delays over the entire search window. From each recording, a mean and standard deviation of the stimulus

response delays was calculated, with the stimulus frequency and number of pacing segments recorded as well.

Pre-Ablation Files	N	Hz	Mean Delay	Std Dev
1-25-2008 - c VF pace 100	49	5	0.1121	0.0516
1-25-2008 - c VF pace 105	49	5	0.1111	0.0557
1-25-2008 - c VF pace 95	49	5	0.1060	0.0546
2-11-2008 - c VF pace 6Hz	359	6	0.1000	0.0370
2-11-2008 - c VF pace 7Hz	418	7	0.0858	0.0290
2-11-2008 - c VF pace 8Hz	479	8	0.0759	0.0256
2-11-2008 - c VF pace 9Hz	538	9	0.0695	0.0236
2-11-2008 - c VF pace 10Hz	599	10	0.0624	0.0208
2-11-2008 - c VF pace 11Hz	659	11	0.0587	0.0177
2-11-2008 - c VF pace 12Hz	719	12	0.0557	0.0159
2-26-2008 - c VF pace 750	449	7.5	0.0765	0.0286
2-26-2008 - c VF pace 800	479	8	0.0731	0.0285
2-26-2008 - c VF pace 850	509	8.5	0.0725	0.0251
2-26-2008 - c VF pace 900	539	9	0.0692	0.0240
2-26-2008 - c VF pace 950	569	9.5	0.0667	0.0227
2-26-2008 - c VF pace 1000	599	10	0.0647	0.0210
2-26-2008 - c VF pace 1050	629	11	0.0600	0.0196

Table 4.7 – VF TED results, pre-ablation.

For each recording pre-ablation, there is a corresponding recording with the same frequency post-ablation.

Post-Ablation Files	N	Hz	Mean Delay	Std Dev
1-25-2008 - p VF pace 100	49	5	0.1023	0.0516
1-25-2008 - p VF pace 105	49	5	0.0940	0.0233
1-25-2008 - p VF pace 95	49	5	0.1073	0.0560
2-11-2008 - p VF pace 6Hz	359	6	0.0970	0.0360
2-11-2008 - p VF pace 7Hz	419	7	0.0862	0.0320
2-11-2008 - p VF pace 8Hz	479	8	0.0749	0.0281
2-11-2008 - p VF pace 9Hz	539	9	0.0673	0.0251
2-11-2008 - p VF pace 10Hz	599	10	0.0641	0.0219
2-11-2008 - p VF pace 11Hz	659	11	0.0595	0.0188
2-11-2008 - p VF pace 12Hz	719	12	0.0549	0.0166
2-26-2008 - p VF pace 750	449	7.5	0.0813	0.0314
2-26-2008 - p VF pace 800	479	8	0.0752	0.0294
2-26-2008 - p VF pace 850	509	8.5	0.0701	0.0262
2-26-2008 - p VF pace 900	539	9	0.0699	0.0245
2-26-2008 - p VF pace 950	569	9.5	0.0623	0.0212
2-26-2008 - p VF pace 1000	599	10	0.0607	0.0209
2-26-2008 - p VF pace 1050	629	11	0.0666	0.0234

Table 4.8 – VF TED results, post-ablation.

To compare these values, a paired *t*-test was performed to test for a change in mean delays from pre- to post-ablation. This resulted in a p-value of 0.264 (N=17 pairs), suggesting that there was no consistent non-zero change in mean stimulus response delay after ablation. An F-test was performed first to compare the standard deviations in single heart and frequency between pre- and post-ablation. In addition, a two-sample *t*-test was performed on each set of recordings (pre- and post-ablation for the same heart and frequency). The results are summarized below.

Pre-Ablation File Name	F-Test	T-Test
1-25-2008 - c VF pace 5 Hz	0.500	0.352
1-25-2008 - c VF pace 5 Hz	1.000	**0.050**
1-25-2008 - c VF pace 5 Hz	0.435	0.904
2-11-2008 - c VF pace 6Hz	0.704	0.274
2-11-2008 - c VF pace 7Hz	**0.023**	0.844
2-11-2008 - c VF pace 8Hz	**0.023**	0.556
2-11-2008 - c VF pace 9Hz	0.081	0.137
2-11-2008 - c VF pace 10Hz	0.096	0.160
2-11-2008 - c VF pace 11Hz	0.052	0.447
2-11-2008 - c VF pace 12Hz	0.103	0.309
2-26-2008 - c VF pace 7.50	**0.023**	**0.015**
2-26-2008 - c VF pace 8.00	0.264	0.247
2-26-2008 - c VF pace 8.50	0.176	0.132
2-26-2008 - c VF pace 9.00	0.317	0.623
2-26-2008 - c VF pace 9.50	0.948	**0.00081**
2-26-2008 - c VF pace 10.00	0.549	**0.00102**
2-26-2008 - c VF pace 10.50	**4.96E-06**	**7.86E-08**

Table 4.9 – VF mean TED comparison. F- and T-test results below the p=0.05 threshold are highlighted in bold.

In bold are the F-test and T-test p-values that are $p<0.05$, to show significance. Using a path length of 1 cm pre-ablation, and 3.16 cm post-ablation, the mean conduction velocity of each recording was calculated. Of the 17 sets of recordings, only four showed a significant ($p<0.05$) difference in standard deviation by way of the F-test. Five sets showed a significant ($p<0.05$) difference in mean stimulus excitation delay.

CHAPTER 5

DISCUSSION

The main findings of this study are the following:

1. DF analysis variance decrease in both time and frequency domains.

Our analysis of the variance in mean dominant frequency between pre- and post-ablation recordings has shown that there is a statistically significant difference observable in both frequency and time-domain. In comparisons between variance pre-and post-ablation (N=10 hearts) there was a significant decrease in variance after ablation. Using the FFT to determine dominant frequency, there was a decrease in variance by a factor of 1.51 ($p<0.001$). In our time-domain analysis, we found a similar decrease in variance by a factor of 1.48($p<0.001$). In the experiments with a gapped lesion (N=5), there was also a significant drop in dominant frequency variance using the FFT analysis after ablation, between both control (pre-ablation) and gap lesion ($p=0.015$), and control and complete lesion ($p=0.008$). Between control and complete lesion, variance decreased by a factor of 1.45. There was not a significant difference between gap and complete lesion dominant frequency variance. With the time-domain analysis, there was an increase in variance seen between control and gap lesion ($p=0.021$), but a decrease in variance between gap and complete lesions by a factor of 1.12 ($p=0.004$). There was no significant difference in variance between control and complete lesions ($p=0.27$, N=5 hearts with control, gap, and complete lesions).

As summarized in Abashian et al., the general trend of decrease in variance of dominant frequency may result due to "an increase in organization of the arrhythmia, producing frequencies that are more similar."[7]

Time-domain analysis has been shown to be comparable to FFT analysis for finding the dominant frequency in VF signals. By using the filter as per Ng et al. we have examined peaks in the signal and found similar frequencies that are found in FFT analysis. Frequency-domain analysis was able to distinguish between gap vs. complete lesions, while the time-domain analysis failed to do so. However, this may be due to the larger dominant frequency variance found in gap lesions, an increase from the control recordings. Further studies related to dominant frequency may examine the Power Spectral Density, which would allow for a more continuous analysis rather than the segmenting as we performed.

2. TED during sinus rhythm shows a significant change in mean and standard deviation after ablation.

However, there is neither a consistent increase nor a consistent decrease in either the mean or the standard deviation of TED when examining individual hearts. In combined data of all hearts, when all TED measurements from all hearts were combined together for pre- and post-ablation, TED increased from 68.0 ± 25.5 ms to 79.1 ± 14.0 ms. Both the increase in mean delay and decrease in standard deviation were statistically significant ($p<0.0001$). However, this required 380 seconds of pacing data before and after ablation, which may not be realistic for a clinical setting. Additionally, the change in TED did not quantitatively

agree with the factors of increase of 2.6-3.1 seen by Himel et al. in their study of TED as an indicator for quality of lesion[3].

3. Pacing during VF did not show a consistent statistical change in TED mean or standard deviation.

While ANOVA analysis of variables frequency, ablation state, or rabbit heart showed that there was a statistical relationship between TED and pacing frequency and whether or not ablation has been performed (See Appendix C), no consistent positive or negative change in the TED was measured for fibrillatory episodes. With only three of 17 pacing cases showing a significant change in mean TED after ablation, we can only conclude that either the stimulation current was not powerful enough to cause capture, or our algorithm could not accurately detect the stimulation response. Additionally, because of the complex nature of VF, it may not be possible to consistently induce capture between the electrodes, especially after the disruption of pathways by ablation. As well, this study has a possible limitation in that there is an unknown pathway for conduction of excitations that may change beat-to-beat.

The histogram in figure 4.7 showing stimulus delays highlights an inherent problem in the peak search algorithm. Because we limited the search window from 30 ms past the stimulus pulse until 2 ms before the following stimulus pulse, the window is inherently limited by the pacing frequency. The distribution of delays is limited by the window, and thus any comparison of TED between different pacing frequencies cannot be accurate. The upper limit of the window was included because of the subsequent stimulus pulse, and the large stimulus artifact that follows. The large deviation in delays suggests that the algorithm

needs to filter out segments that did not show capture. By visual inspection of pacing segments, some samples show upstrokes that suggest capture, while many lack a definite stimulus response, leading the current algorithm to choose a possibly incorrect value for the stimulus response delay. Future studies in this area should determine a technique to identify which TED segments were produced by the stimulus excitation and not by VF.

In 13 of 17 cases, standard deviation of stimulation delays increased post-ablation (three with $p<0.05$). In pre-ablation recordings, any captured beats had good chance to propagate directly between the electrodes since distance was small (recording and stimulation electrodes located 1 cm apart, perpendicular to axis of ablation, which would suggest a response time of 64.4±32.7 ms for transverse propagation, or 25.8±8.4 ms for longitudinal propagation)[10]. Post-ablation, the propagation distance is increased around the end of the lesion (smallest path 3.2 cm) producing greater time that would have to be available for the stimulation-induced excitation to arrive at recording electrode. But since VF is occurring, there is a greater chance one of the VF excitations would excite the recording area first. That could happen at any time relative to the stimulation, increasing the standard deviation.

Using the mean stimulus excitation delays, conduction velocities were calculated for each recording. Conduction velocities were estimated by dividing the minimum path length by the mean TED for each recording. Mean conduction velocities for all experiments of 13.5 ± 2.7 cm s^{-1} pre-ablation, and 42.4 ± 8.0 cm s^{-1} post-ablation are not consistent with those found by Knisley and Hill[10] for non-VF paced excitations. However, in our experiments the rapid pacing and VF may have led to the Na^{+} not being as fully recovered as in Knisley

and Hill; additionally, the conduction pathways would not be the same. Thus, the different situations (VF vs. slow pacing) may make comparisons between the two experiments invalid. Using a two-sample *t* test we determined that these values are significantly different ($p=2.6x10^{-15}$), but the extreme change in conduction velocity suggested by this data does not agree with Himel et al.[3] and suggests that pacing during VF does not detect TEDs with any consistency. However, Himel only examined conduction velocities in sinus rhythm, and additionally used optical mapping to estimate the propagation of stimulus across the surface of the heart. Additionally, in Himel's protocol, a higher current was used in stimulation, which may have led to higher rates of capture of stimulation beats.

4. Morphological comparison of translesion signals did not show a change after ablation.

There was not a significant change in correlation of signals from pre- to post-ablation in either sinus rhythm or VF recordings. Our hypothesis was that the lesion would disrupt potential re-entrant circuits and cause a decrease in morphology correlation values, and additionally increase the standard deviation of correlation measurements. Neither of these hypotheses was conclusively shown to occur in our analysis. While some individual hearts showed a statistical change in correlation, the sign test did not determine with probability different from 0.50 that the change in correlation would be positive or negative when examining the data set as a whole. We also hypothesized that delays associated with the correlation would increase as there was a greater path for the beats that did capture to travel from one electrode to the other. Many delays did show a significant change, but there was no consistent increase or decrease in delay after ablation.

In sinus rhythm, while eight of nine hearts showed a difference in mean correlation values (N=2 of 3 for incomplete lesions, N=6 of 6 for complete lesions), there was no consistent increase or decrease (See Table 4.6). Only one of three hearts with incomplete lesions showed a significant difference in variance of correlation values, while all hearts with complete lesions showed a significant change in variance. However, only half (3) showed an increase in variation, suggesting that there is not a probability different from 0.5 that variation would increase or decrease after ablation. The delays associated with these correlation coefficients did generally show a change after ablation. The variance of delays changed significantly after every complete and incomplete lesion, but did not show a consistent increase or decrease. The mean delays changed with statistical significance ($p<0.05$) in all incomplete lesion hearts, and in five of the six complete lesion hearts, but there was no consistent positive or negative change in delay associated with the creation of a lesion.

In our examination of the results of the VF morphology comparisons, we observed a general trend for the mean correlation to have a significant change post-ablation for complete lesions. Table 4.10 shows the summary of this analysis. For incomplete lesions (N=3), there were no hearts with significant change in mean correlation after ablation (paired t-test p-value = 0.35). For complete lesions (N=5), however, four of the five hearts showed a significant change in correlation; yet, there was no consistent increase or decrease in the correlation after complete lesions were administered (paired t-test p-value = 0.98). Only one heart (5/7/2008, complete lesion) showed a statistical change (p=0.012) in variance after ablation. All other hearts, with complete and incomplete lesions, had no significant change in variance of correlation after ablation.

Correlation delays found during VF did not show a significant change after ablation (Table 4.12). In incomplete lesions, there was an average of 2.2 ms rise in delay after ablation (N=3). However, these differences were not statistically significant (paired t-test p-value = 0.20). Individually, none of the three cases showed a significant change in mean delay (t-test p-values of 0.26, 0.26, 0.92). The standard deviation in these cases also increased in all cases an average of 1.5 ms, although these rises were not of statistical significance (f-test p-values of 0.24, 0.09, 0.21). In hearts with complete lesions, there was average drop in the mean delay of 2.5 ms after ablation (N=5). Four of five hearts showed a decrease in delay after ablation, yet these decreases were not statistically significant (paired t-test p-value = 0.15). Further experiments would increase N and potentially cause the p-value to approach 0.05. When analyzing individual heart data, only two of five hearts showed a significant change in mean delay (p-values of 0.022 and 0.011, both decreases in mean delay). There was no statistical change in the variance of delay in this group of hearts after ablation. In three hearts with complete lesions, there was a non-significant decrease in variance after ablation. Probability of having a deviation increase or decrease after ablation is not shown to be different from 0.5.

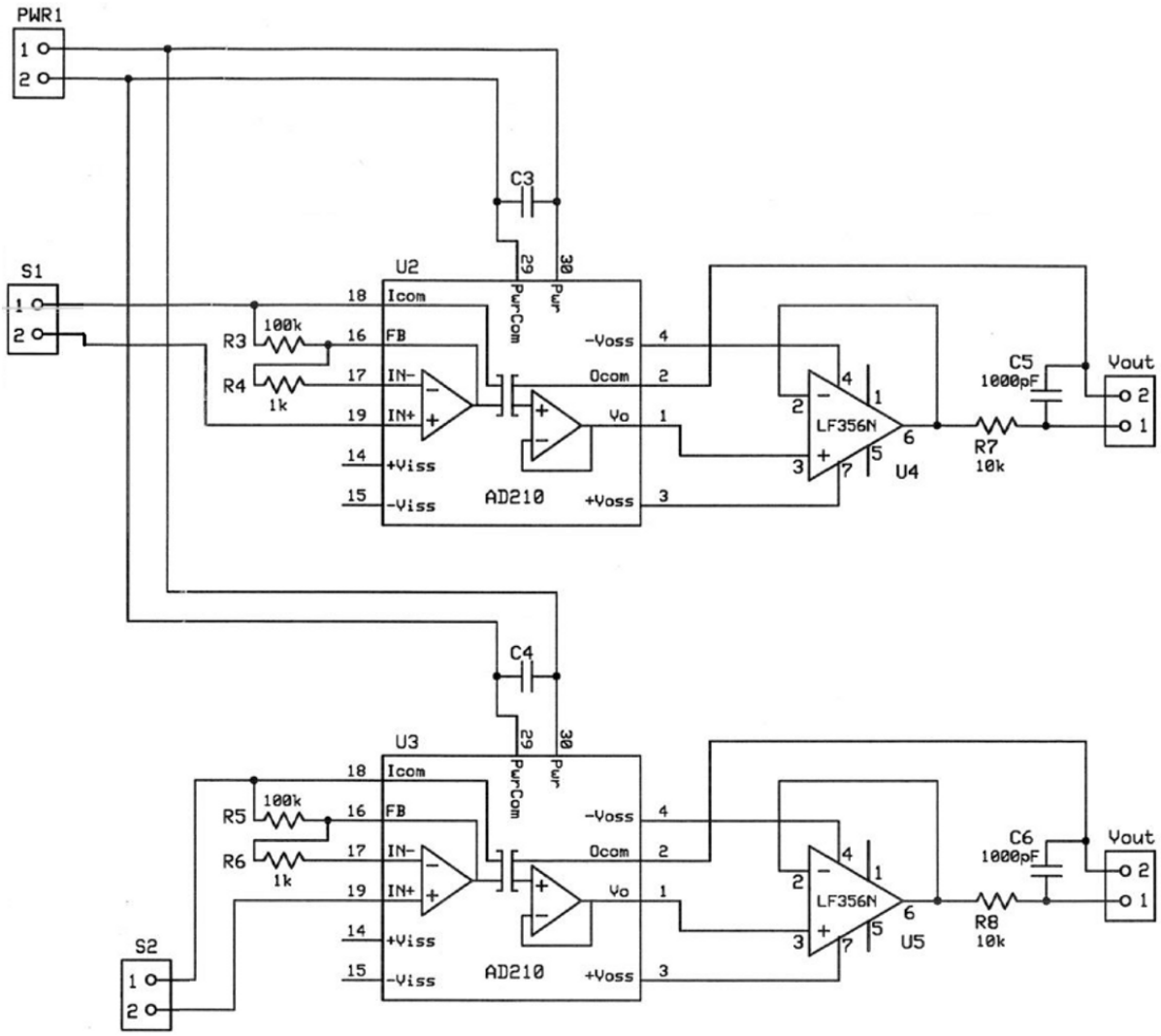
We observed a tendency for higher delays to result in higher correlation values. This can be seen in figure 4.4 in that the median delay in each box plot tended to increase at the higher correlation bins. We hypothesize that this is due to the fact that the more truncated two signals are; the remaining segments will be highly correlated. This agrees with the comparison of those two truncated segments that returned the highest cross-correlation.

Comparing the morphology of signals measured 1 cm apart on a heart in fibrillation presents several problems. Because of the constant creation and alteration of re-entrant

circuits on the surface of the cardiac tissue, the morphology of the two signals may be vastly different. While the FFT analysis was able to compare specific components of the fibrillatory signal, the morphology algorithm compares simple waveform segments to return a value of similarity. The addition of filtering improved our ability to isolate the desired components of the signal; however, we are unable to isolate only the segments that showed apparent capture from one electrode to the other. However, the addition of filtering and other processing may detract from patterns in the original data.

In summary, certain algorithms for the analysis of VF signals show promise as a metric for lesion completeness. Because the variance in dominant frequency decreased significantly after ablation in both time and frequency-domains, the dominant frequency algorithm is most applicable in predicting the desired outcome of ablation. TED pacing during VF did not reveal a significant decrease in excitation delay after ablation, as predicted in our hypotheses. Similarly, comparing signal morphology before vs. after ablation during VF only showed non-significant trends. There is however an overall limitation to the application of these findings to ablation in human atria because of anatomical differences. While the thickness of the human atria is similar to the ventricular thickness of rabbit hearts, the fiber orientation and anatomy is not similar, so results of clinical studies based on these experiments may have differing findings.

APPENDIX A: SIGNAL CONDITIONING CIRCUIT DIAGRAM



APPENDIX B: MATLAB ANALYSIS CODE

I. Dominant Frequency Analysis Code:

```
%************************************************************************
% zerophase.m           Sevan Abashian 2007-2009
%------------------------------------------------------------------------
%
%                   analyses and compares in order to
%                   find a correlation between the two analyses.
%
%                   This latest version, zerophase.m, will show the
%                   filtering we are applying to each data set and also
%                   apply Hann Windowing (aka Hanning) per Ng et al,
%                   "Understanding and Interpreting DF..." and use
%                   the zero-phase filter suggested in that paper.
%
%************************************************************************
%This version used Ng filter in FFT part and in time domain part.

% Need to select directory called First 10 exp using the command window's
% current directory.

clear all;
close all;
format compact;

%% Choose File
filelist='10Exp FileList.csv';
st=strcat(cd,'\',filelist);
fid=fopen(st,'r');

i=0;
while feof(fid)~=1
    i=i+1;
    filename{i}=fgetl(fid); %#ok<AGROW>
end
fclose(fid);
filename' %#ok<NOPTS>

%Choose files to analyze
startpt=input('Input start file number:  ');
endpt = input('Input end file number:   ');

% Initialize empty arrays for values to be added in during analysis
freqs=[];       %all frequencies of ch 1 in   frequency domain
freqs2=[];      %all frequencies of ch 2  in  frequency domain
freqdiffs=[];   %all frequency differences in frequency domain 1-2
pht11 = [];     %Ch1 Magnitude at DF1
pht22 = [];    %Ch2 Magnitude at DF2
pht12 = [];    %Ch1 Magnitude at DF2
pht21 = [];     %Ch2 Magnitude at DF1
phasediff1 = [];    %  phase 1 - phase 2 at DF1 in frequency domain
phasediff2 = [];    %  phase 1 - phase 2 at DF2 in frequency domain

timefreqs=[];   % all frequencies of ch 1 from time domain
timefreqs2=[];  % all frequencies of ch 2 from time domain
totaltf=[];     %all frequency differences in  time domain
totaltp=[];     %all     phase differences in  time domain
```

```
count=1;
%z = input('Starting Point: ');
for z = startpt:endpt
    tp1=[];
    tf1=[];

    %%  Read Data from File
    comma = findstr(filename{z},',');
    filepath = filename{z}(1:comma-1) %#ok<NOPTS>
    [data,time,numchans,scanrate] = readdata(filepath);

    %% FREQUENCY ANALYSIS Section

    Gain=[100 100];
    UF1=data(1,:)/Gain(1);
    UF2=data(2,:)/Gain(2);
    UF1=UF1-mean(UF1);
    UF2=UF2-mean(UF2);

    % Add pre-processing of Ng et al. Heart Rhythm 2006;3:1295-1305.
    % Bandpass 40-250 Hz
    % Sample rate is 10,000. Half of that is 5,000.
    % For low cutoff of 40 Hz, want 40/5000.
    % For high cutoff of 250 Hz, want 250/5000.

    order = 3; %Order of filter
    Wn = [40 250]/3000; %The bandpass from 40-250 Hz
    [b,a] = butter(order,Wn);

    offset=0;       %To test whether the filter removes DC
    X2=UF2+offset;     %To test whether the filter removes DC
    y = filtfilt(b,a,X2) ;   %The bandpass from 40-250 Hz
    rect=abs(y); %  To rectify
    [b,a] = butter(order,20/5000,'low');  %The lowpass from DC-20 Hz
    F2 = filtfilt(b,a,rect) ;   %The lowpass from DC-20 Hz

    order = 3; %Order of filter
    Wn = [40 250]/3000; %The bandpass from 40-250 Hz
    [b,a] = butter(order,Wn);

    offset=0;       %To test whether the filter removes DC
    X1=UF1+offset;     %To test whether the filter removes DC
    y = filtfilt(b,a,X1) ;   %The bandpass from 40-250 Hz
    rect=abs(y); %  To rectify
    [b,a] = butter(order,20/5000,'low');  %The lowpass from DC-20 Hz
    F1 = filtfilt(b,a,rect) ;   %The lowpass from DC-20 Hz

    %Now, both V1 and V2 have been filtered into F1 and F2 as in Ng et. al
    %%%%%%%%%% End of Ng Filtering %%%%%%%%%%%%%%%

    %Split data into subsections based on user preference
    s=5;    % Set to 5 for 2-second segments

    sublength = floor(length(data(1,:))/s);
    clear dataarray1 dataarray2
    for i = 1:s
        lowindex = (i-1)*(sublength)+1;
        highindex = sublength*i;
        if highindex > length(data)
            highindex = length(data);
        end
        dataarray1(i,:)= F1(lowindex:highindex);
        dataarray2(i,:)= F2(lowindex:highindex);
```

```
    timeindex(i,:) = [lowindex highindex];
end

%Perform Analysis on subsections (now in matrix [length/s s] dimens.)
for i = 1:s
    subtime = time(timeindex(i,1):timeindex(i,2));
    Vh1=dataarray1(i,:)/Gain(1);
    Vh2=dataarray2(i,:)/Gain(2);

    % Windowing Here on Vh1 and Vh2
    Vh1 = Vh1.*hann(length(Vh1))';
    Vh2 = Vh2.*hann(length(Vh2))';

    % Calculate FFT
    p=19;
    Y1=fft(Vh1,2^p);
    Y2=fft(Vh2,2^p);

    %Power Spectrum
    Pyy1 = Y1.* conj(Y1) / 2^p;
    Pyy1=Pyy1(1:(1+2^(p-1)));
    Pyy2 = Y2.* conj(Y2) / 2^p;
    Pyy2=Pyy2(1:(1+2^(p-1)));
    Freq = scanrate*(0:(2^(p-1)))/(2^p);    %frequency axis

    %Phase Spectrum
    Ph1 = unwrap(angle(Y1)); %Angle is in radians. % Unwrap gets rid
    % of jumps in consecutive angles in radians. It does not force
    % angles to be in -2pi to 2pi!
    Ph1 = Ph1(1:length(Freq));
    Ph2 = unwrap(angle(Y2));   % Phase angles, can still exceed 2pi.
    Ph2 = Ph2(1:length(Freq));
    PhDiff = Ph2-Ph1;

    %Get the desired values from the data
    pointsper =length(Freq)/max(Freq);
    Freq1=Freq(ceil(pointsper)+1:length(Freq));     % Scaled freq axis
    Pyy11=Pyy1(ceil(pointsper)+1:length(Pyy1));     % Power Spectrum
    Pyy21=Pyy2(ceil(pointsper)+1:length(Pyy2));
    Phase1=Ph1(ceil(pointsper)+1:length(Ph1));      % Phase angles
    Phase2=Ph2(ceil(pointsper)+1:length(Ph2));
    peakindex1=find(Pyy11==max(Pyy11));             % MAX peak
    peakindex2=find(Pyy21==max(Pyy21));
    peakfreq1 = Freq1(peakindex1);                  % DF in Channel 1
    peakfreq2 = Freq1(peakindex2);                  % DF in Channel 2

    peakdiff = peakfreq1-peakfreq2;                 % DF differences
    freqdiffs = [freqdiffs peakdiff];
    freqs = [freqs peakfreq1];
    freqs2 = [freqs2 peakfreq2];

    peakheight11 = Pyy11(peakindex1);            % Ch1 Magnitude at DF1
    peakheight22 = Pyy21(peakindex2);            % Ch2 Magnitude at DF2
    peakheight12 = Pyy11(peakindex2);            % Ch1 Magnitude at DF2
    peakheight21 = Pyy21(peakindex1);            % Ch2 Magnitude at DF1
    p11 = cuttopi(rem(Phase1(peakindex1),2*pi)); % Ch1 Phase at DF1.
    % In -pi to +pi because the rem brings it into -2pi to 2pi,
    % and the cuttopi brings it into  -pi to pi.
    p22 = cuttopi(rem(Phase2(peakindex2),2*pi));    % Ch2 Phase at DF2
    p12 = cuttopi(rem(Phase1(peakindex2),2*pi));    % Ch1 Phase at DF2
    p21 = cuttopi(rem(Phase2(peakindex1),2*pi));    % Ch2 Phase at DF1

    pht11 = [pht11 peakheight11];                   % Ch1 Mag at DF1
```

```
    pht22 = [pht22 peakheight22];                   % Ch2 Mag at DF2
    pht12 = [pht12 peakheight12] ;                  % Ch1 Mag at DF2
    pht21 = [pht21 peakheight21] ;                  % Ch2 Mag at DF1

    phd1 = p11-p21;                                 % Ph1 - Ph2 at DF1.
    phd2 = p12-p22;                                 % Ph1 - Ph2 at DF2.
    %But this difference can exceed -pi to pi.
    %So do cuttopi again to bring it into -pi to pi.
    phd1 = cuttopi(rem(phd1,2*pi));                 % Phase diff at DF1
    phd2 = cuttopi(rem(phd2,2*pi));                 % Phase diff at DF2
    phasediff1 = [phasediff1 phd1];
    phasediff2 = [phasediff2 phd2];

    clear Pyy11 Pyy21 Pyy1 Pyy2 Freq Y1 Y2
end

%%  TIME DOMAIN ANALYSIS
Gain=[100 100];
Vh1=data(1,:)/Gain(1);     %Has length 100,000, i.e., all 10 seconds.
Vh2=data(2,:)/Gain(2);     %

for j=1:5                   %Getting Rid of Drift in Channel 1
    sectionstart = length(Vh1)/20+(j-1)*length(Vh1)/5;
    sectionend   = length(Vh1)/20+(j-1)*length(Vh1)/5+length(Vh1)/10;
    Y(j)=mean(Vh1(sectionstart:sectionend));
    X(j)=time(length(Vh1)/10+(j-1)*length(Vh1)/5);
end
splinedbase = pchip(X,Y,time);
Vh1=Vh1-splinedbase;
Vh1=Vh1-mean(Vh1);

for j=1:5                   %Getting Rid of Drift in Channel 2
    sectionstart = length(Vh2)/20+(j-1)*length(Vh2)/5;
    sectionend   = length(Vh2)/20+(j-1)*length(Vh2)/5+length(Vh2)/10;
    Y(j)=mean(Vh2(sectionstart:sectionend));
    X(j)=time(length(Vh2)/10+(j-1)*length(Vh2)/5);
end
splinedbase = pchip(X,Y,time);
Vh2=Vh2-splinedbase;
Vh2=Vh2-mean(Vh2);


%Now filter per Ng.
% Channel 2
order = 3; %Order of filter
Wn = [40 250]/3000; %The bandpass from 40-250 Hz
[b,a] = butter(order,Wn);
offset=0;       %To test whether the filter removes DC

X2=Vh2+offset;     %To test whether the filter removes DC
y = filtfilt(b,a,X2) ;   %The bandpass from 40-250 Hz
rect=abs(y); %  To rectify
[b,a] = butter(order,20/5000,'low');  %The lowpass from DC-20 Hz
Vh2= filtfilt(b,a,rect) ;   %The lowpass from DC-20 Hz

% Channel 1
order = 3; %Order of filter
Wn = [40 250]/3000; %The bandpass from 40-250 Hz
[b,a] = butter(order,Wn);

offset=0;       %To test whether the filter removes DC
X2=Vh1+offset;     %To test whether the filter removes DC
y = filtfilt(b,a,X2) ;   %The bandpass from 40-250 Hz
```

```
rect=abs(y); %  To rectify
[b,a] = butter(order,20/5000,'low');  %The lowpass from DC-20 Hz
Vh1= filtfilt(b,a,rect) ;   %The lowpass from DC-20 Hz

% NOW, BOTH V1 AND V2 HAVE BEEN FILTERED AS DONE BY Ng ET AL.
% Here it was done to each subsegment separately.

Vh1=Vh1-min(Vh1(1000:(length(Vh1)))); %Make the minimum past 1000
Vh2=Vh2-min(Vh2(1000:(length(Vh2)))); %samples be zero for each Chan.

%Calculate time between indices:
dt = (time(length(time))-time(1))/length(time); %dt=seconds/point.

% Find Peaks
threshold1 = max(Vh1(1000:(length(Vh1))))/4; %%  Thresholds
threshold2 = max(Vh2(1000:(length(Vh2))))/4;
peakarray1 = find(Vh1>threshold1);  %VH1 INDICES THAT EXCEED THRESHOLD
peakarray2 = find(Vh2>threshold2);

%Filter electrodes to only take peaks that have width >= 1ms
%Channel 1
i=1;
keeparray1=[];
while i<length(peakarray1)
    j=0;
    while peakarray1(i)==peakarray1(i+j+1)-(j+1)
        j=j+1;
        if (i+j+1)>length(peakarray1)
            break
        end
    end
    if j>=10 && (i+j+1)<length(peakarray1) % Peak Width 1 (10 points)
        maxpeak = i-1+find(Vh1(peakarray1(i):peakarray1(i+j))==max(...
            Vh1(peakarray1(i):peakarray1(i+j))));
        %INDEX OF peakarray1 WHERE VH1 IS MAXIMUM W/I FILTERING WIDTH
        keeparray1(length(keeparray1)+1)=peakarray1(maxpeak);
        %INDEX OF Vh1 WHERE JUST MAXIMA OF Vh1 W/I FILTER WIDTH LOCATED
    end
    i=i+j+1;
end

%Channel 2
i=1;
keeparray2=[];
while i<length(peakarray2)
    j=0;
    while peakarray2(i)==peakarray2(i+j+1)-(j+1)
        j=j+1;
        if (i+j+1)>length(peakarray2)
            break
        end
    end
    if j>=10 && (i+j+1)<length(peakarray2) % Peak Width 2 (10 points)
        maxpeak = i-1+find(Vh2(peakarray2(i):peakarray2(i+j))==max(...
            Vh2(peakarray2(i):peakarray2(i+j))));
        keeparray2(length(keeparray2)+1)=peakarray2(maxpeak);
    end
    i=i+j+1;
end

% Figure to show peaks found on top of data plot
peakspike1 = zeros(1,length(Vh1));
for i=1:length(keeparray1)
```

```
    peakspike1(keeparray1(i))=1;
end
peakspike1=peakspike1*max(Vh1);

figure;
subplot(2,1,1);
plot(time,Vh1,time,peakspike1);
title('Time Data')
ylabel('Channel 1 - mV')

peakspike2 = zeros(1,length(Vh2));
for i=1:length(keeparray2)
    peakspike2(keeparray2(i))=1;
end
peakspike2=peakspike2*max(Vh2);

subplot(2,1,2);
plot(time,Vh2,time,peakspike2);
ylabel('Channel 2 - mV')
xlabel('Time (s)')
%End of figure

peakarray1 = keeparray1;        %ARRAY OF INDICES of Vh1
peakarray2 = keeparray2;        %ditto for Vh2
peakdiffs1=diff(peakarray1*dt);     %  PEAK DIFFS IN SECONDS
%peakdiffs1 SHOULD BE THE TIMES BETWEEN PEAKS, IN SECONDS
%THEIR LENGTH IS ONE LESS THAN LENGTH OF peakarray1
peakdiffs2=diff(peakarray2*dt);

timefreqs = [timefreqs 1./peakdiffs1];      % SHOULD BE IN HZ
timefreqs2 = [timefreqs2 1./peakdiffs2];

% Finding centers of intervales
for i=1:length(peakarray1)-1
    center1(i) = (peakarray1(i)+peakarray1(i+1))/2;
end
for i=1:length(peakarray2)-1
    center2(i) = (peakarray2(i)+peakarray2(i+1))/2;
end

% Finding closest center for channel 2 to channel 1.
for i=1:length(center1) % DO THIS FOR EACH INDEX of a center IN center1
    a = center1(i); % a IS AN INDEX OF Vh1.
    b = abs(center2-a);

    matchto1(i) = find(b==min(b));    % closest center 2 index to a
    c = center2(matchto1(i));    % c is an index of Vh2

    centerdiff=(a-c)*dt;    % Time difference of centers
    aveint = (peakdiffs1(i) + peakdiffs2(matchto1(i))) / 2;

    tp1(i) = (centerdiff / aveint) * 2 * pi; %NOW PHASE DIFF IN RADIANS

    %the rem would bring it into -2pi to 2pi, and the cuttopi brings it
    %into -pi to pi.
    tp1(i) = cuttopi(tp1(i));

    %% Difference in frequency (1/width1-1/width2)
    tf1(i)=1./peakdiffs1(i)-1./peakdiffs2(matchto1(i));
end

totaltf = [totaltf tf1];    %all frequency diffs in time domain 1-2
totaltp = [totaltp tp1];    %all phase diffs in time domain 1-2
```

```
    clear center1 center2 matchto1 peakdiffs1 peakdiffs2
end

%% Plotting Histograms to Compare Time and Frequency Domains Analyses %%

figure('Position',[100 100 800 650]); %I think this defaults as figure 1
barwidth = 0.7;
edges= 0:1:20;

subplot(6,6,1:2);
bar(edges,histc(timefreqs,edges),barwidth,'k');
% all frequencies of ch 1 from time domain
title(strcat('Time Domain Analysis, Runs ',num2str(startpt),'-',...
    num2str(endpt),', ',filelist,', 0<f<20Hz'));
ylabel(strcat('N=',num2str(length(timefreqs)),',Kept=',num2str(length(...
    timefreqs(find(timefreqs>0 & timefreqs<=20))),3)));
xlabel('Channel 1, Frequency (Hz)');
xlim([0 20]);
a=ylim;
text(21,a(2)*0.8,strcat('Mean=',num2str(mean(timefreqs(find(timefreqs>0 ...
    & timefreqs<=20))),3)));
text(21,a(2)*0.6,strcat('Median=',num2str(median(timefreqs(find(...
    timefreqs>0 & timefreqs<=20))),3)));
text(21,a(2)*0.4,strcat('StDev=',num2str(std(timefreqs(find(timefreqs>0 ...
    & timefreqs<=20))),3)));

subplot(6,6,4:5);
bar(edges,histc(freqs,edges),barwidth,'k');
%all frequencies of ch 1 in   frequency domain
title(strcat('Frequency Domain Analysis, Runs ',num2str(startpt),'-',...
    num2str(endpt)));
ylabel(strcat('N=',num2str(length(freqs)),',Kept=',num2str(length(freqs(...
    find(freqs>0 & freqs<=20))),3)));
xlabel('Channel 1, Frequency (Hz)');
xlim([0 20]);
a=ylim;
text(21,a(2)*0.8,strcat('Mean=',num2str(mean(freqs(find(freqs>0 & ...
    freqs<=20))),3)));
text(21,a(2)*0.6,strcat('Median=',num2str(median(freqs(find(freqs>0 & ...
    freqs<=20))),3)));
text(21,a(2)*0.4,strcat('StDev=',num2str(std(freqs(find(freqs>0 & ...
    freqs<=20))),3)));

subplot(6,6,7:8);
bar(edges,histc(timefreqs2,edges),barwidth,'k');
% all frequencies of ch 2 from time domain
ylabel(strcat('N=',num2str(length(timefreqs2)),',Kept=',num2str(length(...
    timefreqs2(find(timefreqs2>0 & timefreqs2<=20))),3)));
xlabel('Channel 2, Frequency (Hz)');
xlim([0 20]);
a=ylim;
text(21,a(2)*0.8,strcat('Mean=',num2str(mean(timefreqs2(find(...
    timefreqs2>0 & timefreqs2<=20))),3)));
text(21,a(2)*0.6,strcat('Median=',num2str(median(timefreqs2(find(...
    timefreqs2>0 & timefreqs2<=20))),3)));
text(21,a(2)*0.4,strcat('StDev=',num2str(std(timefreqs2(find(...
    timefreqs2>0 & timefreqs2<=20))),3)));

subplot(6,6,10:11);
bar(edges,histc(freqs2,edges),barwidth,'k');
%all frequencies of ch 2  in  frequency domain
ylabel(strcat('N=',num2str(length(freqs2)),',Kept=',num2str(length(...
```

```
    freqs2(find(freqs2>0 & freqs2<=20))),3)));
xlabel('Channel 2, Frequency (Hz)');
xlim([0 20]);
a=ylim;
text(21,a(2)*0.8,strcat('Mean=',num2str(mean(freqs2(find(freqs2>0 & ...
    freqs2<=20))),3)));
text(21,a(2)*0.6,strcat('Median=',num2str(median(freqs2(find(freqs2>0 & ...
    freqs2<=20))),3)));
text(21,a(2)*0.4,strcat('StDev=',num2str(std(freqs2(find(freqs2>0 & ...
    freqs2<=20))),3)));

diffedges = -20:2:20;
subplot(6,6,13:14);
%totaltf = abs(totaltf); % TO GET MAGNITUDES OF THE DIFFERENCES
bar(diffedges,histc(totaltf,diffedges),barwidth,'k');
% All freq diffs in time domain 1-2 for paired nearest
% centers closest to each center1
ylabel(strcat('N=',num2str(length(totaltf)),',Kept=',num2str(length(...
    totaltf(find(totaltf>=-20 & totaltf<=20))),3)));
xlabel('Peak Difference 1-2, Frequency (Hz)');
xlim([-20 20]);
a=ylim;
text(22,a(2)*0.8,strcat('Mean=',num2str(mean(totaltf(find(totaltf>=-20 ...
    & totaltf<=20))),3)));
text(22,a(2)*0.6,strcat('Median=',num2str(median(totaltf(find(totaltf>=...
    -20 & totaltf<=20))),3)));
text(22,a(2)*0.4,strcat('StDev=',num2str(std(totaltf(find(totaltf>=-20 ...
    & totaltf<=20))),3)));

subplot(6,6,16:17);
%freqdiffs = abs(freqdiffs); % TO GET MAGNITUDES OF THE DIFFERENCES
bar(diffedges,histc(freqdiffs,diffedges),barwidth,'k');
%all frequency differences in frequency domain 1-2
ylabel(strcat('N=',num2str(length(freqdiffs)),',Kept=',num2str(length(...
    freqdiffs(find(freqdiffs>=(-20) & freqdiffs<=20))),3)));
xlabel('Peak Difference 1-2, Frequency (Hz)');
xlim([-20 20])
a=ylim;
text(22,a(2)*0.8,strcat('Mean=',num2str(mean(freqdiffs(find(freqdiffs>=...
    (-20) & freqdiffs<=20))),3)));
text(22,a(2)*0.6,strcat('Median=',num2str(median(freqdiffs(find(...
    freqdiffs>=(-20) & freqdiffs<=20))),3)));
text(22,a(2)*0.4,strcat('StDev=',num2str(std(freqdiffs(find(freqdiffs>=...
    (-20) & freqdiffs<=20))),3)));



%%%%%%%%%%
%Phase difference plots
factor = 180/pi;%TO CONVERT FROM RADIANS TO DEGREES
barwidth = 0.7;
phedges = -180:18:180;
xcor = ((21/20)*180); %puts text just right of the phase graph


phased1 = phasediff1 * factor; %NOW PHASE IS IN DEGREES.
%  phase 1 - phase 2 at DF1 in frequency domain
subplot(6,6,22:23); %
bar(phedges,histc(phased1,phedges),barwidth,'k');
ylabel(strcat('N=',num2str(length(phased1)),',Kept=',num2str(length(...
    phased1(find(phased1>=(-180) & phased1<=180))),3)));
xlabel('Phase Diff 1-2 in FD at DF1, (degrees)');
xlim([-180 180])
```

```
a=ylim;
text(xcor,a(2)*0.8,strcat('Mean=',num2str(mean(phased1(find(phased1>=...
    (-180) & phased1<=180))),3)));
text(xcor,a(2)*0.6,strcat('Median=',num2str(median(phased1(find(phased1...
    >=(-180) & phased1<=180))),3)));
text(xcor,a(2)*0.4,strcat('StDev=',num2str(std(phased1(find(phased1>=...
    (-180) & phased1<=180))),3)));

phased2= phasediff2 * factor; %NOW PHASE IS IN DEGREES.
%  phase 1 - phase 2 at DF2 in frequency domain
subplot(6,6,28:29); %
bar(phedges,histc(phased2,phedges),barwidth,'k');
ylabel(strcat('N=',num2str(length(phased2)),',Kept=',num2str(length(...
    phased2(find(phased2>=(-180) & phased2<=180))),3)));
xlabel('Phase Diff 1-2 in FD at DF2, (degrees)');
xlim([-180 180])
a=ylim;
text(xcor,a(2)*0.8,strcat('Mean=',num2str(mean(phased2(find(phased2>=(-...
    180) & phased2<=180))),3)));
text(xcor,a(2)*0.6,strcat('Median=',num2str(median(phased2(find(phased2...
    >=(-180) & phased2<=180))),3)));
text(xcor,a(2)*0.4,strcat('StDev=',num2str(std(phased2(find(phased2>=...
    (-180) & phased2<=180))),3)));

tottp=totaltp * factor; %NOW PHASE IS IN DEGREES AND IS CALLED tottp.
% all phase differences in  time domain  1-2 for paired nearest
% centers closest to each center1
subplot(6,6,19:20);  %
bar(phedges,histc(tottp,phedges),barwidth,'k');
ylabel(strcat('N=',num2str(length(tottp)),',Kept=',num2str(length(tottp...
    (find(tottp>=(-180) & tottp<=180))),3)));
xlabel('Phase Diff 1-2 in TD at center 1, (degrees)');
xlim([-180 180])
a=ylim;
text(xcor,a(2)*0.8,strcat('Mean=',num2str(mean(tottp(find(tottp>=(-180)...
    & tottp<=180))),3)));
text(xcor,a(2)*0.6,strcat('Median=',num2str(median(tottp(find(tottp>=...
    (-180) & tottp<=180))),3)));
text(xcor,a(2)*0.4,strcat('StDev=',num2str(std(tottp(find(tottp>=(-180)...
    & tottp<=180))),3)));

%RELATIVE MAG OF POWER SPECTRUM AT ONE SIDE OF THE LESION MEASURED AT THE
%DOMINANT FREQUENCY OF THE OTHER SIDE OF LESION
relmag = pht21./pht11;
other = pht12./pht22;
relmag = [relmag other];  %both combined for single plot


magedges = 0:0.05:1;
xmax = 2; %probably most are under 1
xcor = (21/20)*xmax;

subplot(6,6,25:26);  %
bar(magedges,histc(relmag,magedges),barwidth,'k');
ylabel(strcat('N=',num2str(length(relmag)),',Kept=',num2str(length(...
    relmag(find(relmag>=(0) & relmag<=xmax))),3)));
xlabel('Relative magnitude of spectrum at DF of other side');
xlim([0 xmax])
a=ylim;
text(xcor,a(2)*0.8,strcat('Mean=',num2str(mean(relmag(find(relmag>=(0)...
    & relmag<=xmax))),3)));
text(xcor,a(2)*0.6,strcat('Median=',num2str(median(relmag(find(relmag>=...
    (0) & relmag<=xmax))),3)));
```

```
text(xcor,a(2)*0.4,strcat('StDev=',num2str(std(relmag(find(relmag>=...
    (0) & relmag<=xmax))),3)));

disp('Analysis Completed:')
disp(['Channel 1 Mean = ' ...
    num2str(mean(freqs(find(freqs>0 & freqs<=20))))]);
disp(['Channel 1 StDv = ' ...
    num2str(std(freqs(find(freqs>0 & freqs<=20))))]);
disp(['Channel 2 Mean = ' ...
    num2str(mean(freqs2(find(freqs2>0 & freqs2<=20))))]);
disp(['Channel 2 StDv = ' ...
    num2str(std(freqs2(find(freqs2>0 & freqs2<=20))))]);
disp(['Peak Diff Mean = ' ...
    num2str(mean(freqdiffs(find(freqdiffs>=(-20) & freqdiffs<=20))))]);
disp(['Peak Diff StDv = ' ...
    num2str(std(freqdiffs(find(freqdiffs>=(-20) & freqdiffs<=20))))]);
disp('Time Domain:');
disp(['Channel 1 Mean = ' ...
    num2str(mean(timefreqs(find(timefreqs>0 & timefreqs<=20))))]);
disp(['Channel 1 StDv = ' ...
    num2str(std(timefreqs(find(timefreqs>0 & timefreqs<=20))))]);
disp(['Channel 2 Mean = ' num2str(mean(timefreqs2(find(timefreqs2>0 & ...
    timefreqs2<=20))))]);
disp(['Channel 2 StDv = ' num2str(std(timefreqs2(find(timefreqs2>0 & ...
    timefreqs2<=20))))]);
```

II. Signal Morphology Analysis

```
%*************************************************************************
% vfcorrtrun.m           Sevan Abashian 2008-2009
%-------------------------------------------------------------------------
% VERSION HISTORY:  vfcorrtrun.m is the analysis program for the 2008
% morphology experiments data.  It uses xcorr to find the delay and
% truncates the shifted signals before finding a correlation value.
%*************************************************************************
% Need to select directory using the command window's current directory.

clear all;
close all;
format compact;

figs = 0;

%% Read File
csvs = dir(fullfile(cd,'*.csv'));
filelist=csvs(1).name;
st=strcat(cd,'\',filelist);
fid=fopen(st,'r');

i=0;
while feof(fid)~=1
    i=i+1;
    filename{i}=fgetl(fid);
end
fclose(fid);
filename' %#ok<NOPTS>

%Choose Files for Analysis
startpt=input('Input file numbers and/or range:  ');

% Length of Time for Segment
ts = input('Input length of time segment: ');
if isempty(ts)
    ts = 0.2; % Default for VF
end

allscorr = [];
alltcmax = [];
for z = 1:length(startpt)

    clear ch1 ch2 data time
    comma = findstr(filename{startpt(z)},',');
    filepath = filename{startpt(z)}(1:comma-1);
    disp(filepath)
    % Get Data from filepath
    [data,time,numchans,scanrate] = readdata(filepath);

    %% ANALYZE FILE DATA
    Gain=[100 100];
    ch1=data(1,:)/Gain(1);
    ch2=data(2,:)/Gain(2);

    [ch1,ch2]=vffilter(ch1,ch2,time,scanrate);

    % Begin sectioning data by "ts"
    tstart = 1;
    tend = find(time>=ts);
```

```
    tend = tend(1);
    winstart=0.25; % Window for searching for xcorr max mag
    i=1;
    while tend < length(time)
        % Take xcorr of segments and find maximum magnitude within winstart
        c=xcorr(ch1(tstart:tend),ch2(tstart:tend),'coeff');
        csub=c(floor(winstart*length(c)):floor((1-winstart)*length(c)));
        cmax=find(abs(csub)==max(abs(csub)));
        cmax=cmax(1);
        % Delay in index points
        delay=cmax+floor(winstart*length(c))-length(ch1(tstart:tend));
        % Calculate correlation value by aligning and truncating signals
        if delay>=0
            scorr(i)=abs(corr2(ch1(tstart+delay:tend),...
                ch2(tstart:tend-delay)));
        else
            scorr(i)=abs(corr2(ch1(tstart:tend-abs(delay)),...
                ch2(tstart+abs(delay):tend)));
        end
        tcmax(i)=abs(delay/scanrate); % Delay, in seconds
        tbegin(i) = time(tstart); %#ok<*AGROW>

        if (i+1)*ts <= max(time)
            tstart = tend;
            tend = find(time>=(i+1)*ts);  % Next segment
            tend = tend(1);
        else
            tend=length(time)+1;
        end
        i=i+1;
    end
    allscorr=[allscorr scorr];
    alltcmax=[alltcmax tcmax];
end

%% Plot Results
numbins = input('Number of bins:  ');
figure('Position',[100,100,1000,800]);
while numbins > 0
    subplot(3,1,1)
    edges=(1/numbins)*(0:numbins-1);
    bincenters = edges + 0.5/numbins;
    bar(bincenters,histc(allscorr,edges),1)
    set(gca,'XTick',[edges 1])

    title(['Max Cross-Correlation, Segment Size = ' num2str(ts) ...
        ' Seconds'])
    ylabel({['Mean = ' num2str(mean(allscorr))];['Std Dev = ' ...
        num2str(std(allscorr))]})
    xlim([0 1])
    y=ylim;
    text(0.8,y(2)*0.8,['N = ' num2str(length(allscorr))])

    subplot(3,1,2);
    for i=1:length(allscorr)
        bin(i)=ceil(allscorr(i)*numbins)/numbins;
        if bin(i)<=0
            bin(i)=1/numbins;
        end
    end
    boxplot(alltcmax,bin)
    xlim([0.5 numbins+0.5])
    xlabel('Maximum Limit of Bin')
```

```
    ylabel({'Time Delays';'By Bin'})

    subplot(3,1,3);
    hist(alltcmax,numbins);
    title('Time Delay at Max Cross-Correlation')
    ylabel({['Mean = ' num2str(mean(alltcmax))];['Std Dev = ' ...
        num2str(std(alltcmax))]})
    y=ylim;
    text(max(alltcmax)*0.8,y(2)*0.8,['N = ' num2str(length(alltcmax))])
    numbins = input('Number of bins:  ');
end

disp(['N = ' num2str(length(allscorr))])
disp(['Mean Xcorr = ' num2str(mean(allscorr))])
disp(['StndardDev = ' num2str(std(allscorr))])
disp(['Mean Delay = ' num2str(mean(alltcmax))])
disp(['StndardDev = ' num2str(std(alltcmax))])
```

III. TED Pacing Analysis

```
%*************************************************************************
% pacecorr.m           Sevan Abashian 2008-2009
%-------------------------------------------------------------------------
% VERSION HISTORY:  pacecorr.m is the analysis program for the 2008 pacing
% experiments data.  It finds the peak TED after a stimulus.
%*************************************************************************
%Need to select directory called First 10 exp using the
%command window's current directory.

clear all;
close all;
format compact;

%% Choose & Read File

group = input('Which group of data? (Pre=1, Post=2) ');
if group == 1
    csvs = dir(fullfile(cd,'*Pre*.csv'));
else
    csvs = dir(fullfile(cd,'*Post*.csv'));
end

filelist=csvs(1).name;
st=strcat(cd,'\',filelist);
fid=fopen(st,'r');

i=0;
while feof(fid)~=1
    i=i+1;
    filename{i}=fgetl(fid);
end
fclose(fid);
filename' %#ok<NOPTS>

%Choose Files to Analyze
startpt=input('Input start file number:  ');
endpt = input('Input end file number:   ');
if endpt == []; %#ok<BDSCA>
    endpt = startpt;
end

allpeakdiff = [];
% For every file chosen run the following
for z = startpt:endpt
    tp1=[];
    tf1=[];

    comma = findstr(filename{z},',');
    filepath = filename{z}(1:comma-1);
    if group == 1
        filepath2 = ['\PREAblation\' filepath];
    else
        filepath2 = ['\POSTAblation\' filepath];
    end
    % Get Data from filepath
    [data,time,numchans,scanrate] = readdata(filepath2);

    %% ANALYZE FILE DATA
    Gain=[100 100 100 100];
```

```
ch1=data(1,:)/Gain(1);
ch2=data(2,:)/Gain(2);
ch3=data(3,:)/Gain(3);
ch1=ch1-mean(ch1);
ch2=ch2-mean(ch2);
ch3=ch3-mean(ch3);

% Find Trigger Upstrokes
d3dt = diff(ch3);
spikes=find(d3dt>max(d3dt)/2);
trigger=oneshot(spikes);

for i=1:length(trigger)-1
    s=ch2(trigger(i)+150:trigger(i+1)-10);
    avgs(i)=mean(s);
end
baseline = mean(avgs);

posneg=[];                          % Positive or Negative Peak
peakdiff=[];                        % Time Difference Upstroke to Peak
p1corr=[];
p2corr=[];
nrows = ceil(length(trigger)/10);
for i=1:length(trigger)-1
    %New Figure every 50 plots
    if rem(i,50)==1
        figure;
    end

    if trigger(i) > 200
        t1=trigger(i)-200;
    else
        t1=trigger(i);
    end

    t2=t1+scanrate*0.150;    % 250 ms window
    if t2>length(time)
        t2=length(time);
    end

    %Individual Plot
    subplot(5,10,rem(i-1,50)+1)
    plot(time(t1:t2),ch2(t1:t2),time(t1:t2),ch3(t1:t2)/...
        max(ch3(t1:t2))*max(ch2)/2)
    xlim([time(t1) time(t2)])
    ylim([min(ch2) max(ch2)])
    set(gca,'YTickLabel',[]);
    set(gca,'XTickLabel',[]);
    title(num2str(time(t1)))

    samp=ch2(trigger(i)+(0.03*scanrate):trigger(i+1)-(0.002*...
        scanrate))-baseline;
    sampdec = decimate(samp,5);
    sampdiff = diff(sampdec);
    sampmax=find(sampdiff==max(sampdiff));   % Find max value of sample
    sampmin=find(sampdiff==min(sampdiff));   % Find min value of sample
    % To find samppeak, the highest mag of [sampmax sampmin]
    if sampdec(sampmax(1))>abs(sampdec(sampmin(1)))
        samppeak = sampmax(1);
        posneg(i)=1; %#ok<*AGROW>
    else
        samppeak = sampmin(1);
        posneg(i)=0;
```

```
    end

    peakdiff(i)=(samppeak*5+(0.03*scanrate)-1)/scanrate; %Delay from trigger

    if i<length(trigger)-1
        s1=samp;
        s2=ch2(trigger(i+1)+150:trigger(i+2)-10)-baseline;
        [s1,s2]=zeropad(s1,s2);
        p1corr(i)=corr2(s1,s2)^2;  %Corr the sample with next sample
    end
    if i<length(trigger)-2
        s3=ch2(trigger(i+2)+150:trigger(i+3)-10)-baseline;
        [s1,s3]=zeropad(s1,s3);
        p2corr(i)=corr2(s1,s3)^2;  %Corr the sample with the i+2 sample
    end
    % Sets tick mark locations to trigger and response times.
    set(gca,'XTick',[time(trigger(i)) time(trigger(i)+99+samppeak)]);
end

allpeakdiff=[allpeakdiff peakdiff];
disp([filepath '  Peak Delay Mean = ' num2str(mean(peakdiff)) ...
    '  StdDev = ' num2str(std(peakdiff))])
p1corr = [p1corr 0];
p2corr = [p2corr 0 0];

% Histogram of Peak Delays
figure;
subplot(4,1,1)
hist(peakdiff,20)
x=xlim;
y=ylim;
text(0.75*(x(2)-x(1))+x(1),0.75*(y(2)-y(1))+y(1),strvcat(['N+ = ' ...
    num2str(length(find(posneg==1)))],['N- = ' ...
    num2str(length(find(posneg==0)))])); %#ok<VCAT>
title(['Peak Delay Histogram - ' filepath])
xlabel('Time Difference between Upstroke and Peak')
ylabel('Occurences')

% Scatter Plot of peak delays
subplot(4,1,2)
C = [];
S = [];
peaktime=time(trigger(1:end-1));
pospeak=[];
postime=[];
poscount=1;
negpeak=[];
negtime=[];
negcount=1;
for i=1:length(posneg)   % Sorting Positive & Negative Peaks
    if posneg(i)==1
        pospeak(poscount)=peakdiff(i);
        postime(poscount)=peaktime(i);
        poscount=poscount+1;
    else
        negpeak(negcount)=peakdiff(i);
        negtime(negcount)=peaktime(i);
        negcount=negcount+1;
    end
end
plot(postime,pospeak,'or',negtime,negpeak,'*b','MarkerSize',4)
%     scatter(time(trigger(1:end-1)),peakdiff,4,C,'*')
title('Peak Delay Over Time')
```

```
    ylabel('Peak Delay (s)')
    legend('Positive','Negative')
    orient landscape;

    subplot(4,1,3)
    plot(time(trigger(1:end-1)),p1corr);
    ylabel('R-Squared')
    title('Correlation between Excitation (i) and (i+1)')

    subplot(4,1,4)
    plot(time(trigger(1:end-1)),p2corr);
    ylabel('R-Squared')
    title('Correlation between Excitation (i) and (i+2)')
    xlabel('Time (s)')
end
disp(['Overall Peak Delay Mean = ' num2str(mean(allpeakdiff)) ...
    '  StdDev = ' num2str(std(allpeakdiff))])
```

APPENDIX C: ANOVA STATISTICAL ANALYSIS

ANOVA results for TED pacing in VF. Independent variables listed in Class. For FREQ, pacing frequencies of 5-12 Hz listed. OUTCOME describes the ablation state of the heart (Pre- or Post-Ablation). HEART represents which from which heart the recording originated. DELAY is the dependent variable.

The GLM Procedure

Class Level Information

Class	Levels	Values
FREQ	12	5 6 7 7.5 8 8.5 9 9.5 10 10.5 11 12
OUTCOME	2	1 2
HEART	3	1 2 3

Number of Observations Read 15384
Number of Observations Used 15384

19:30 Wednesday, July 1, 2009 2

The GLM Procedure

Dependent Variable: DELAY DELAY

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	13	3.03901227	0.23377017	315.36	<.0001
Error	15370	11.39357863	0.00074129		
Corrected Total	15383	14.43259090			

R-Square	Coeff Var	Root MSE	DELAY Mean
0.210566	40.09153	0.027227	0.067911

Source	DF	Type III SS	Mean Square	F Value	Pr > F
FREQ	10	1.48773028	0.14877303	200.70	<.0001
OUTCOME	1	0.02171450	0.02171450	29.29	<.0001
HEART	1	0.00160113	0.00160113	2.16	0.1417

The final table details the results of the ANOVA analysis showing the dependency of the DELAY variable on each of the independent variables. Both FREQ and OUTCOME are shown to have highly probable dependency ($p < 0.0001$). From which heart the recording came is not as statistically significant ($p = 0.1417$).

REFERENCES

1. *Atrial Fibrillation*. 2009, American Heart Association.

2. *Ventricular Fibrillation*. 2009, American Heart Association.

3. Himel, H.D.t., et al., *Translesion stimulus-excitation delay indicates quality of linear lesions produced by radiofrequency ablation in rabbit hearts.* Physiol Meas, 2007. **28**(6): p. 611-23.

4. Dumas, J.H., 3rd, *Myocardial electrical impedance as a metric of completeness for radiofrequency ablation lesions,* in *Biomedical Engineering*. 2007, University of North Carolina at Chapel Hill: Chapel Hill.

5. Faes, L., et al., *A method for quantifying atrial fibrillation organization based on wave-morphology similarity.* IEEE Trans Biomed Eng, 2002. **49**(12 Pt 2): p. 1504-13.

6. Ng, J., A.H. Kadish, and J.J. Goldberger, *Effect of electrogram characteristics on the relationship of dominant frequency to atrial activation rate in atrial fibrillation.* Heart Rhythm, 2006. **3**(11): p. 1295-305.

7. Abashian, S.M., et al. *Effect of ablation on local activation intervals and dominant frequencies of fibrillation.* in *Computers in Cardiology, 2007*. 2007.

8. Ng, J. and J.J. Goldberger, *Understanding and interpreting dominant frequency analysis of AF electrograms.* J Cardiovasc Electrophysiol, 2007. **18**(6): p. 680-5.

9. Cao, J.M., et al., *Spatiotemporal heterogeneity in the induction of ventricular fibrillation by rapid pacing: importance of cardiac restitution properties.* Circ Res, 1999. **84**(11): p. 1318-31.

10. Knisley, S.B. and B.C. Hill, *Effects of bipolar point and line stimulation in anisotropic rabbit epicardium: assessment of the critical radius of curvature for longitudinal block.* IEEE Trans Biomed Eng, 1995. **42**(10): p. 957-66.