DYNAMIC MECHANICAL RESPONSE OF AIRWAY MUCOSAL MEMBRANE

by

LU WANG

B. Medicine, The Capital Institute of Medicine, Beijing, China 1991

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF

DOCTOR OF PHILOSOPHY

in

THE FACULTY OF GRADUATE STUDIES

THE FACULTY OF APPLIED SCIENCE

(Department of Chemical and Bio-Resource Engineering)

We accept this thesis as conforming
to the required standard

THE UNIVERSITY OF BRITISH COLUMBIA

October, 1997

© Lu Wang, 1997
In presenting this thesis in partial fulfilment of the requirements for an advanced degree at the University of British Columbia, I agree that the Library shall make it freely available for reference and study. I further agree that permission for extensive copying of this thesis for scholarly purposes may be granted by the head of my department or by his or her representatives. It is understood that copying or publication of this thesis for financial gain shall not be allowed without my written permission.

Department of Chemical and Bio-Resource Engineering

The University of British Columbia
Vancouver, Canada

Date October 6, 1997
Abstract

Folding of the mucosal membrane of the airways during bronchoconstriction can provide an elastic load [Lambert 1991, 1994] which impedes the airway smooth muscle shortening. The mechanical properties of the membrane, both in tension and in compression, are needed to quantify this load. To measure the mechanical properties of the airway mucosal membrane in tension, a high accuracy stress-strain-time apparatus was designed and constructed. 40 New Zealand White female rabbits of ages between 2 and 35 months were studied for the mechanical response of the airway mucosal membrane. A pair of membrane strips (one in the circumferential, the other in the longitudinal direction of the airway) were dissected from each trachea and mounted in the apparatus. Static uniaxial tensile tests, sinusoidal oscillations and pulse tests were used to measure the steady-state stiffness as well as the frequency response of the membrane in tension. The results of the pulse tests were fitted to a Kelvin model, from which the elastic component, viscous component and the time constants were calculated. The tested strips were histologically processed after the mechanical experiments. The elastic fiber orientation in the airways was measured using a new geometrical approach. The volume fraction of the elastic fibers in the mucosal membrane was measured using the classical point counting technique. Statistically significant linear relationships were found between the Kelvin model parameters and the morphometrical measurements. In summary, the rabbit airway mucosal membrane is viscoelastic. Age (2-35 months) has no statistically significant effect on either the mechanical properties of the membrane or the morphometrically measured parameters of the elastic fibers. The membrane is stiffer in the longitudinal than in the circumferential
direction of the airway. The volume fraction of the elastic fibers is linearly related to the steady-state stiffness of the membrane. The viscous component in the dynamic response is inversely related to the percentage of the fibers that have components in both circumferential and longitudinal directions (wavy fibers). The time constants are proportional to the percentage of the wavy fibers.
# TABLE OF CONTENTS

Abstract

List of Tables

List of Figures

Acknowledgments xvii

Chapter One: Introduction 1

1.1. Mechanics of Airway Narrowing 1

1.1.1. Elastic Loads on ASM during Shortening in Vivo 2

1.1.2. Airway Mucosal Membrane as an Additional Load on ASM Shortening 4

1.2. Testing Tissue Mechanical Properties 6

1.2.1. The Mechanical Properties 6

1.2.2. Experimental Techniques 7

1.2.3. The Range of Elongation 9

1.2.4. Data Analysis and Modeling 9

1.3. Tissue Structure: Collagen and Elastic Fibers 12

1.4. How Are the Structure and the Mechanical Behavior Linked? 15

1.5. Scope of the Study 17

Chapter Two: Equipment Design 19
2.1. The Necessity of Designing and Building

Equipment for Dynamic Testing of Biological Tissues

2.1.1. Experimental Layout: the Basis of the Equipment Design
2.1.2. Physiological Requirements
2.1.3. Mechanical and Electronic Requirements

2.2. Mechanical Support

2.3. Hardware Design

2.4. Equipment Control Design

2.4.1. Control of the Stepper Motor and Linear Table
2.4.2. Reading the Position of the Stepper Motor
2.4.3. Reading the Tension From the Strain Gage
2.4.4. Computer Interface Software Design

2.5. Equipment Validation

2.5.1. Basic Validation
2.5.2. Performance Validation
2.5.3. Grounding of the Equipment
2.5.4. Testing of Standard Spring

2.6. Equipment Limitations

2.6.1. Sensitivity at Very Low Loads
2.6.2. Choice of Motor Step Size
2.6.3. Noise Generated by the Movement of the Stepper Motor

Chapter Three: Physiological Experiments
3.1. Introduction

3.2. Experimental Procedure

3.2.1. Equipment Preparation 49
3.2.2. Tissue Sample Preparation 50
3.2.3. Sample Mounting 51
3.2.4. Experimental Protocol

3.2.4.1. Preliminary Tests 53
3.2.4.2. Experiments 56
3.2.4.3. Measuring the Cross Sectional Area of the Tissue Samples 63

3.3. Data analysis method and modeling

3.3.1. Data 65
3.3.2. Data Conversion 71
3.3.3. Modeling of the Data

3.3.3.1. In time domain 73
3.3.3.2. In frequency domain 78
3.3.3.3. Determination of the Parameters of the Kelvin Model 93
3.3.3.4. Sine Wave Dynamic Analysis 102
3.3.3.5. Step Function Analysis 102

3.4. Results

3.4.1. Steady-State Stiffness of the Airway Mucosal Membrane

3.4.1.1. Results of Static Experiments 103
3.4.1.2. Results of Dynamic Experiments (Pulse Test) 106
3.4.1.3. Comparison of the Results of the Static and Dynamic Experiments 109
3.4.1.4. The Elastic Constants Found in the Literature 110
3.4.2. Time Constants of the Airway Mucosal Membrane

3.4.2.1. $\tau_e$ and $\tau_\alpha$ of the Circumferential Samples

3.4.2.2. $\tau_e$ and $\tau_\alpha$ of the Longitudinal Samples

3.4.2.3. $\tau_e$ and $\tau_\alpha$ for Different Strain Values

3.4.2.4. Kelvin Model Parameters ($\mu_1$, $\mu_2$, and $\eta$)

3.4.3. Storage and Loss Moduli

Chapter Four: Morphometrical Measurements

4.1. Introduction

4.2. Histological Processing for Morphometrical Measurement

4.2.1. Section Preparations

4.2.2. Staining

4.3. Morphometrical Equipment Setup

4.4. Measuring the Elastic Fiber Orientation

4.5. Measuring the Relative Volume of the Elastic Fibers

4.6. Analysis

4.6.1. Elastic Fiber Orientation

4.6.1.1. Classification of the Elastic Fiber Bundles of Each Section

4.6.1.2. Re-Classification of the Type D Fiber Bundles

4.6.1.3. The Orientation of the Elastic Fibers in the Airway

4.6.2. The Relative Volume of the Elastic Fibers

4.7. Results

4.7.1. The Elastic Fiber Orientation

4.7.1.1. Measurements of Single Fibers
Chapter Five: Correlations of Experimental and Morphometrical Results

5.1. Introduction

5.2. General Considerations of Tests of Correlation

5.2.1. Linear Correlation and the Goodness of Fit

5.2.2. Selection of the Morphometrical Parameters

5.3. Tests of Correlations of the Experimental Data at 30% Strain

5.3.1. Data from Longitudinal Samples

5.3.2. Data from Circumferential Samples

5.4. Tests of Correlation of the Experimental Data at 20% Strain

5.4.1. Data from Longitudinal Samples

5.4.2. Data from Circumferential Samples

5.5. Tests of Correlation of the Experimental Data at 10% Strain

5.6. Summary of Correlations

Chapter Six: Conclusions
List of Tables

Table 2.1 Comparison of the set and measured oscillatory frequency 41
Table 2.2 Comparison of the set and measured sampling frequency 42
Table 3.1 Ingredients of the altered Kreb's solution (g/l): pH=7.4-7.5 49
Table 3.2 Comparison between sine wave technique and pulse testing 79
Table 3.3 Comparison of the known and the estimated parameters of the Kelvin model 97
Table 3.4 Comparison of the actual data/Kelvin and the simulated data/Kelvin model 100
Table 3.5 Comparison of known (in simulated data) and the estimated parameters (Kelvin) 101
Table 3.6 Steady-state stiffness of airway mucosal membrane 110
Table 3.7 The Young's moduli of several biological materials 111
Table 5.1 Correlations between the mechanical properties and morphometrical observations (longitudinal samples at 30% strain) 175
Table 5.2 Correlations between the mechanical properties and morphometrical observations (circumferential samples at 30% strain) 182
Table 5.3 Correlations between the mechanical properties and morphometrical observations (longitudinal samples at 20% strain) 184
Table 5.4 Correlations between the mechanical properties and morphometrical observations (circumferential samples at 20% strain) 186
Table 5.5 Correlations between the mechanical properties and morphometrical observations (longitudinal samples at 10% strain) 188
Table 5.6 Correlations between the mechanical properties and morphometrical observations (circumferential samples at 10% strain) 188
Table 5.7 Summary of correlations between mechanical properties and morphometrical characteristics 191
List of Figures

Figure 2.1 The arrangement of tissue bath and sample 21
Figure 2.2 Sketch of the hardware design 24
Figure 2.3 Schematic diagram of the equipment control design 27
Figure 2.4 The table position controlled by the index pulse and direction 28
Figure 2.5a Time interval at the peak (or bottom) of a sine wave (oscillatory frequency = 60 Hz) 32
Figure 2.5b Time interval at the peak (or bottom) of a sine wave (motor step size = 0.5 μm) 32
Figure 2.6 Reading the position signal 34
Figure 2.7 Reading the tension signal 35
Figure 2.8 Bode diagram of the standard spring 44
Figure 2.9 Tension response of the tissue sample to stepwise length change 47
Figure 3.1 Experimental layout 52
Figure 3.2 Uniformity of the sample length change 55
Figure 3.2a Measured strain between markers on the tissue sample 55
Figure 3.3a Preconditioning of tissue samples 58
Figure 3.3b Preconditioning of tissue samples 58
Figure 3.4 Tracing of the static preloading (10% strain) 66
Figure 3.5 Tracing of the single pulse test (10% strain, height = 15% of the present length) 67
Figure 3.6 Tracing of the sinusoidal oscillation (10% strain, amplitude = 3% of the present length, frequency = 1 Hz) 68
Figure 3.7 Tracing of the static preloading (10% and 30% strain) 70
Figure 3.8 Voigt model 74
Figure 3.9 Tracing of the static preloading (10% strain) with Voigt model 75
Figure 3.10 Kelvin model
Figure 3.11 Tracing of the static preloading (10% strain) with Kelvin model
Figure 3.12a Position signal in frequency domain
  (pulse data at 10% strain, cp4)
Figure 3.12b Tension signal in frequency domain
  (pulse data at 10% strain, cp4)
Figure 3.13 Bode diagram of experimental data
Figure 3.14a Fitting data (absolute gain) with Voigt model
Figure 3.14b Fitting data (phase angle) with Voigt model
Figure 3.15a Fitting data (absolute gain) with Kelvin model
Figure 3.15b Fitting data (phase angle) with Kelvin model
Figure 3.16 Smoothing the pulse data with digital filters
Figure 3.17 The algorithm of the fitting of the Kelvin model
Figure 3.18 Bode diagram of actual and simulated data
Figure 3.19a Autocorrelation of experimental data, bandwidth of noise shown
Figure 3.19b Autocorrelation of simulated data, bandwidth of noise shown
Figure 3.20a Steady-state stress and strain (static experiment), circumferential
Figure 3.20b Steady-state stress and strain (static experiment), longitudinal sample
Figure 3.21a Steady-state stiffness (static experiments)
Figure 3.21b Steady-state stiffness (static experiments) Mean and SD
Figure 3.22 Steady-state stiffness at different strain values (dynamic experiments)
Figure 3.22a Steady-state stiffness at 10% strain (dynamic experiments)
Figure 3.22b Steady-state stiffness at 20% strain (dynamic experiments)
Figure 3.22c Steady-state stiffness at 30% strain (dynamic experiments)
Figure 3.23a Estimated time constants (Kelvin model),
circumferential samples, 10% strain
Figure 3.23b Estimated time constants (Kelvin model),
circumferential samples, 20% strain
Figure 3.23c Estimated time constants (Kelvin model),
circumferential samples, 30% strain

Figure 3.24a Estimated time constants (Kelvin model),
longitudinal samples, 10% strain

Figure 3.24b Estimated time constants (Kelvin model),
longitudinal samples, 20% strain

Figure 3.24c Estimated time constants (Kelvin model),
longitudinal samples, 30% strain

Figure 3.25 Mean time constants (Kelvin model)

Figure 3.26a The stiffness of the linear spring 1 (Kelvin model)
circumferential samples, 10% strain

Figure 3.26b The stiffness of the linear spring 1 (Kelvin model)
circumferential samples, 20% strain

Figure 3.26c The stiffness of the linear spring 1 (Kelvin model)
circumferential samples, 30% strain

Figure 3.27 Mean spring constant (Kelvin model)
measured from circumferential samples

Figure 3.28a The stiffness of the linear spring 1 (Kelvin model)
longitudinal samples, 10% strain

Figure 3.28b The stiffness of the linear spring 1 (Kelvin model)
longitudinal samples, 20% strain

Figure 3.28c The stiffness of the linear spring 1 (Kelvin model)
longitudinal samples, 30% strain

Figure 3.29 Mean spring constant (Kelvin model)
measured from longitudinal samples

Figure 3.30a The viscosity of the dashpot (Kelvin model)
Circumferential samples, 10% strain

Figure 3.30b The viscosity of the dashpot (Kelvin model)
Circumferential samples, 20% strain

Figure 3.30c The viscosity of the dashpot (Kelvin model)
Circumferential samples, 30% strain

Figure 3.31 Mean viscosity of the dashpot (Kelvin model) circumferential samples

Figure 3.32a The viscosity of the dashpot (Kelvin model) longitudinal samples, 10% strain

Figure 3.32b The viscosity of the dashpot (Kelvin model) longitudinal samples, 20% strain

Figure 3.32c The viscosity of the dashpot (Kelvin model) longitudinal samples, 30% strain

Figure 3.33 Mean viscosity of the dashpot (Kelvin model) longitudinal samples

Figure 4.1 Sections for morphometrical measurements

Figure 4.2 Measurements of the elastic fiber orientation (single fiber bundle)

Figure 4.3 Classification of the elastic fiber bundles

Figure 4.4 Estimation of the elastic fiber orientation

Figure 4.5a 2-D images of the collagen fibers, under a light microscope, oil lens × 2000

Figure 4.5b 2-D images of the elastic fibers with the vertical axis in the longitudinal direction of an airway, under a light microscope, oil lens × 2000

Figure 4.5c 2-D images of the elastic fibers (view of the cross section of an airway) under a light microscope, oil lens × 2000

Figure 4.6 3-D images of the collagen fibers

Figure 4.7 The average fiber bundle thickness

Figure 4.8a Elastic fiber orientation in the airway

Figure 4.8b Elastic fiber orientation in the airway (Mean and SD)

Figure 4.9a Area fraction of the elastic fiber bundles

Figure 4.9b Area fraction of the elastic fiber bundles (Mean and SD)

Figure 4.10a Volume fraction of the elastic fiber bundles
Figure 4.10b Volume fraction of the elastic fiber bundles (Mean and SD) 157

Figure 5.1 Correlation between stiffness $\mu_2$ and volume fraction (longitudinal samples, at 30% strain) 165

Figure 5.2 Correlation between stiffness $\mu_2$ and $(V_{\text{long}} \times L_{\text{percent}})$ (longitudinal samples, at 30% strain) 165

Figure 5.3 Correlation between viscosity $\eta$ and volume fraction (longitudinal samples, at 30% strain) 166

Figure 5.4 Correlation between viscosity $\eta$ and $L_{\text{percent}}$ (longitudinal samples, at 30% strain) 167

Figure 5.5 Correlation between viscosity $\eta$ and $(V_{\text{long}} \times L_{\text{percent}})$ (longitudinal samples, at 30% strain) 168

Figure 5.6 Correlation between viscosity $\eta$ and $T_{\text{percent}}$ (longitudinal samples, at 30% strain) 169

Figure 5.7 Correlation between viscosity $\eta$ and $T_{\text{percent}} / L_{\text{percent}}$ (longitudinal samples, at 30% strain) 170

Figure 5.8 Correlation between $\tau_c$ and $L_{\text{percent}}$ (longitudinal samples, at 30% strain) 171

Figure 5.9 Correlation between $\tau_c$ and $L_{\text{percent}}$ (longitudinal samples, at 30% strain) 171

Figure 5.10 Correlation between $\tau_c$ and $T_{\text{percent}}$ (longitudinal samples, at 30% strain) 172

Figure 5.11 Correlation between $\tau_c$ and $T_{\text{percent}}$ (longitudinal samples, at 30% strain) 172

Figure 5.12 Correlation between $\tau_c$ and $T_{\text{percent}} / L_{\text{percent}}$ (longitudinal samples, at 30% strain) 173

Figure 5.13 Correlation between $\tau_c$ and $T_{\text{percent}} / L_{\text{percent}}$ (longitudinal samples, at 30% strain) 174

Figure 5.14 Correlation between stiffness $\mu_1$ and volume fraction
Figure 5.15 Correlation between viscosity $\eta$ and volume fraction (circumferential samples, at 30% strain)

Figure 5.16 Correlation between $\tau_e$ and $L_{\text{percent}}$ (circumferential samples, at 30% strain)

Figure 5.17 Correlation between $\tau_e$ and $T_{\text{percent}}$ (circumferential samples, at 30% strain)

Figure 5.18 Correlation between $\tau_e$ and $T_{\text{percent}} / L_{\text{percent}}$ (circumferential samples, at 30% strain)
Acknowledgments

I wish to express deep gratitude to my thesis supervisors Dr. Kenneth L. Pinder, Dr. Joel L. Bert, Dr. Mitsushi Okazawa, and Dr. Peter D. Paré for their invaluable guidance, continuous support, and encouragement throughout my study.

I thank Dr. Kenneth L. Pinder for the numerous discussions that so much benefited my research, for spending enormous amount of time and effort to help with the thesis organization and corrections. I thank him for his professional discipline and his compassion towards his students that have helped many to improve.

I thank Dr. Joel L. Bert, for providing me with his expertise in Biomedical Engineering research, for the challenging questions that he asks himself before asking his students, for his honesty.

I thank Dr. Mitsushi Okazawa, for his passion towards scientific research, for his support and guidance especially at the beginning of the project.

My very special thanks to Dr. Peter D. Paré, for his patience, his intelligence, and above all, for his faith in me that has made all the hardship worthwhile.

The construction of the experimental apparatus would have been impossible without the technical support of Mr. Alex Pepper, Mr. Chris Castles and Mr. Peter Roberts. Also I greatly appreciate the assistance of Ms. Lynne Carter and Mr. Dean English during the physiological experiments.
Many thanks to Ms. Julie Chow, who spent hours and hours preparing the microscopic sections that are crucial to obtaining the morphometrical measurements. I would also like to thank Mr. Randy Thompson for his valuable advice on the application of the Point Counting technique.

It's a pleasure to acknowledge Mr. Stuart Greene and Mr. Micheal Weis, who have provided professional photographs to illustrate the findings of this work.

Finally, I am thankful for Marek, my bridegroom after the completion of the thesis, my life companion. I thank you for your contribution to this project, for your love and selfless devotion, for your integrity and your constant belief in me. I am grateful to have found you and fallen in love with you.
Chapter One

Introduction

From the trachea down to thousands of peripheral branches embedded in the lung parenchyma, "airway" is a general term for a gas transporting passage in the respiratory system. It is essential for the airways to maintain their normal structure and dimensions in order to perform their physiological functions. Decrease in airway lumen area, known as airway narrowing, occurs in several pulmonary diseases, especially in asthma. Although swelling of the mucosal membrane has been observed in diseased tissues, there is no clear agreement on the role of the membrane in the airway wall and its interrelationship with smooth muscle with respect to their mechanical properties. The purpose of this study is to understand the mechanical behavior of the mucosal membrane, explore the relationship between the structural characteristics of the elastic components and the mechanical properties and, hence, provide information about the role the mucosal membrane plays in airway lumen narrowing.

1.1. Mechanics of Airway Narrowing

One characteristic feature of asthma is excessive airway narrowing caused by the airway smooth muscle (ASM) contraction and the inflammatory changes in the airways [James et al. 1988 and 1989]. Airway narrowing in excised canine lungs can be severe, as
was measured by high resolution computed tomography [McNamara et al. 1992], the lumen cross sectional area of airways with 2-4 mm diameters could decrease 56±4% after stimulation and airways with 4-6 mm diameters could narrow 59±3%.

Exaggerated airway narrowing in response to pharmacological agonist is a characteristic feature of patients who have asthma. Since the narrowing of the airway in response to pharmacological stimuli is predominantly related to ASM contraction [Moreno et al. 1986b], the factors which relate ASM stimulation and airway narrowing, such as the analysis of the elastic loads that ASM has to overcome during contraction, are of considerable interest.

1.1.1. Elastic Loads on ASM during Shortening in Vivo

It has been speculated [Moreno et al. 1986b] that the loads on ASM must limit ASM shortening because in vitro the muscle could shorten more than 70% [Stephens et al. 1977], which could completely occlude all airways if this level of shortening occurred in vivo.

According to Okazawa et al. [1993] the shortening of the ASM in vivo is neither isometric nor isotonic. Rather, it acts against elastic loads contributed to by structural elements in the airway wall and the surrounding tissues, namely the cartilage expansion in large airways and the lung elastic recoil in small airways [Moreno et al. 1986b]. It is possible that the increased responsiveness of the airway could be altered by the changing loads placed upon the muscle without necessarily changing the intrinsic properties of the muscle itself [Moreno et al. 1985].
Robinson et al. [1992] analyzed the tension-length curve of a cartilage-muscle unit in vitro and calculated an average elastic load of 1.7 g per each percent change in the length of ASM. The tracheal muscle in rabbits and dogs [Moreno et al. 1987, Okazawa et al. 1992] was found to shorten up to 70-80% of its starting length from the optimal length in vitro (i.e. without cartilage), although it could only shorten by around 20-30% in vivo. Meanwhile, Moreno et al. [1985, 1986a] showed that intravenous administration of papain in rabbits, which causes cartilage softening, resulted in an increased maximal airway response to intravenously administered acethocholine. Therefore, the geometrical arrangement and the stiffness of the cartilage are likely factors which can alter the ASM shortening in cartilaginous airways.

In the membranous bronchioles, which have no cartilage in their wall, the airways are surrounded by the lung parenchyma and supported by the alveolar attachments. The elastic recoil of the lung parenchyma [Evans et al. 1981] provides a load which restricts ASM shortening and limits airway narrowing [Butler et al. 1986, James et al. 1989]. The load applied to ASM during contraction was calculated [Okazawa et al. 1993] from carbachol induced airway narrowing in excised canine lung lobes. The pressure applied to the ASM by lung elastic recoil was $P_L$ when muscle was at resting position. As the ASM contracts and narrows the airway, the alveolar walls must be stretched and the alveoli immediately surrounding the airway must be distorted. This deformation results in the development of an increased transmural pressure ($\Delta P_X$) across the airway. Both $P_L$ and $\Delta P_X$ were calculated using equations developed by Lai-Fook et al. [1978]. The contraction of the ASM has to generate a force that has the magnitude to overcome $P_L + \Delta P_X$. Since
the internal perimeter or the luminal border of the epithelial surface, $P_i$, remains constant despite the ASM contraction and narrowing [James et al. 1988 a and b], the degree of ASM shortening that occurred in individual airways was calculated by comparing muscle perimeters of relaxed and contracted airways with the same $P_i$. It was found that the calculated stress of the muscle is less than the maximal stress that the muscle should theoretically be able to generate at that degree of contraction. It was therefore postulated that the actual stress generated by the muscle did not only overcome $P_i + \Delta P_X$ but also counteracted some additional, unmeasured, elastic loads. Otherwise, if these unmeasured loads had not existed, the muscle would have contracted more than observed.

1.1.2. Airway Mucosal Membrane as an Additional Load on ASM Shortening

Although not much work has been focused on airway mucosal membrane, its effect on ASM contraction in peripheral airways was suggested to be similar to that of the cartilage in large airways [James et al. 1988, Yager et al. 1989]. When the bronchial smooth muscle contracts and the diameter of the bronchus is reduced, the membrane develops folds (Figure 1a.1, Appendix 1A) to accommodate the reduction in diameter [Kresch et al. 1972, Yager et al. 1989]. In a recent morphometrical study of airway narrowing in canine lungs [Okazawa et al. 1993], it was found that the degree of ASM shortening was inversely related to relative mucosal area in the cross-section of the airway. This result suggests that the thicker the membrane, the less ASM shortening
occurs. Could the deformation of this connective tissue impart an additional load to the smooth muscle?

The airway mucosal membrane can be easily recognized in the trachea and the bronchial tree [Hayek 1960, Nagaishi 1972]. It contains 3 layers: the epithelium, the basal lamina, and the lamina propria. The epithelium covers the lumen surface and consists of epithelial cells. Underlying the epithelial cells is the connective tissue called lamina propria which is bound to the epithelium by the basal lamina. The basal lamina is a sheetlike extracellular structure which appears to be a dense layer since it is composed mainly of type IV collagen. The lamina propria contains a superficial dense matrix, underneath which is a loose connective tissue layer called sub-mucosa where the blood vessels and glands distribute. The superficial dense matrix is richly endowed with elastic and collagen fibers [Sobin et al. 1988].

Mechanically, the mucosal membrane can be viewed as a thin-walled elastic tube collapsible under a pressure difference across the wall. Lambert [1991] suggested a model to describe the behavior of the bronchial basement membrane during airway collapse. The hypothesis, based on mathematical and physical derivations, was that this thin-walled elastic tube is capable of supporting some of the pressure imposed by the contracting ASM. This ability increases with the number and depth of folds into which the tube collapses. Lambert et al. [1994] further investigated the folding of the mucosal membrane using data from 17 sheep. Based on the fact that the mucosal membrane must remain inside the boundary of the smooth muscle wall, that the mucosal mass is constant, and that the strain energy of the folding membrane is minimal, their model prediction agreed with
the observations that in cases of asthma, the thicker membranes develop fewer folds, but
have a similar increase in stiffness as the thin membranes. It is possible that the folding of
the mucosal membrane accounts for the discrepancy between the observed and the
predicted ASM shortening. The mechanical properties of the mucosal membrane are
important in airway mechanics.

The uniaxial tensile stiffness of ovine tracheal wall was measured [Codd et al.
1994] using the conventional static tensile test. Wang et al. [1997] reported the stiffness of
rabbit tracheal mucosal membrane not only using the static stress-relaxation technique,
which agreed with the measurements of Codd et al., but also revealed the viscoelastic
properties of the mucosal membrane by using sinusoidal oscillations.

1.2. Testing Tissue Mechanical Properties

1.2.1. The Mechanical Properties

Roughly speaking, elasticity, plasticity and viscoelasticity are referred to as the
mechanical properties of deformable solid materials.

An elastic body is defined as one in which all deformations are recoverable upon
removal of external forces, such as a spring that has not been overly stretched. The study
of a pure elastic material does not consider time as a parameter.
A plastic body, on the other hand, undergoes permanent (unrecoverable) deformations, that is, the deformation caused by the applied force will remain even if the force is removed. An example of a plastic body is an overly stretched spring.

As Fung [1994] pointed out, biological tissues are not purely elastic: the history of strain affects the stress. Soft tissues are regarded as viscoelastic bodies [Hildebrandt 1970, Mijailovich et al. 1993, Peslin et al. 1991, Robatto et al. 1991, Stamenovic et al. 1990, Woo et al. 1980]. Viscoelastic materials combine the viscosity of a fluid and the elasticity of a solid. A viscoelastic material will return to its original shape after any deforming force has been removed (i.e. show an elastic response) even though it will take time to do so (i.e. have a viscous component to the response). This does not rule out some studies on the viscoplasticity of connective tissue [Stamenovic et al. 1990].


1.2.2. Experimental Techniques

According to Vincent [1982], two major types of experiments have been performed on biological tissues, the transient and dynamic experiments. Transient
experiments involve deforming the material (by simple elongation or in shear) and following the response of the material with time. There are two types of transient experiments. One is the creep experiment in which the material is loaded and the change of deformation with time is noted [Haut et al. 1972, Woo et al. 1980]. The other transient experiment is the stress-relaxation experiment in which the material is deformed and the force required to maintain the deformation at a constant value is measured with time [Frisen 1969 a and b, Woo et al. 1980]. In the dynamic experiments, on the other hand, both stress and strain vary simultaneously within a fixed time period. Either stress or strain (usually strain) is varied cyclically (usually sinusoidally at different frequencies and amplitudes) or in the form of wave propagation and the response, either strain or stress, is measured [Hildebrandt 1969, Mridha et al. 1985 and 1992, Pereira et al. 1991, Peslin et al. 1991, Stamenovic et al. 1990, Torzilli 1984, Wang et al. 1997].

The transient method yields little information about the time dependent properties of the material, and the dynamic tests, although highly accurate and replete with as much frequency information as allowed by the testing system, are time consuming and expensive. The so-called “pulse test” applied in the process control industry since the 1960s [Hougen 1964, Banham 1965, Ogunnaike and Ray 1994], which can give the same dynamic information much more quickly, has not been applied to the testing of biological tissues. The principle of the pulse test is that the system transients can be completely represented by components in the complex frequency domain, and that these components bear corresponding relationships to those of periodic disturbances at corresponding
frequencies. One of the contributions of this research is the application of this technique to the mechanical testing of biological tissues.

1.2.3. The Range of Elongation

The strength of many biological materials is well documented [Yamada 1970]. These data came from experiments in which the stress-strain behavior of specimens was studied up to the point of rupture. Many specimens were reportedly stretched by more than 60% of their initial length before rupture. Although this behavior is important in terms of material properties, and possibly for the reduction of noise in the tension signals, it is of less interest from a functional point of view, as most tissues in vivo are subjected to small changes in length equivalent to the change of length at about 1/3 of the ultimate strength that can be measured in vitro. Since the stress-strain curve for most biological tissues are not linear beyond the elastic limit, which is usually much less than the ultimate strength of the tissue [Yamada 1970], the data on ultimate strength are not useful in calculating the elastic parameters, i.e. the tissue mechanical properties under physiological conditions.

1.2.4. Data Analysis and Modeling

There are three major theories for describing viscoelastic behavior [Silver 1987, Vincent 1982]. The first is the Boltzmann superposition principle which is sometimes called the integral representation of linear viscoelasticity because it is defined by an integral equation. The Boltzmann superposition theory may be stated as: (a) the creep in a specimen is a function of the entire loading history, and (b) each increment of load makes
an independent and additive contribution to the total deformation. The importance of Boltzmann's principle to the study of viscoelasticity is not so much that it provides any explanations but that it provides a starting point for mathematical models which can be tested against reality and refined to give a better fit [Vincent 1982]. The second theory leads to a linear differential equation [Nigul 1987, Fung 1990, Hildebrandt 1969] and is therefore called the differential representation. Most researchers use assemblages of Hookean springs and Newtonian viscous elements (dashpots) as models. An elastic material deforms, stores potential energy, and recovers deformations in a manner similar to that of a linear spring. Fluid behavior can be simulated by another basic mechanical element called a dashpot, which is a simple piston-cylinder or a syringe type of arrangement. A force applied in the piston will advance the piston in the direction of the applied force. The speed of the piston is dependent upon the magnitude of the applied force and the friction occurring between the contact surfaces of the piston and the fluid in the cylinder. Springs and dashpots connected to one another in various forms are used to construct empirical viscoelastic models. The most profitable approach with spring-and-dashpot models [Wilkinson 1960, Fung 1994] has been found to be that of combining a number of Maxwell, Voigt, or Kelvin elements to obtain a spectrum of time characteristics.

The third method is based on the limitation that in order to apply theories of linear viscoelasticity to potentially non-linear solids (as are most biological materials), the deformation applied has to be sufficiently small not to cause non-linear responses [Fredberg et al. 1991]. Therefore dynamic testing rather than stress-relaxation or creep
experiments is particularly suitable for tests under such limitations. If the material being tested is Hookean, then the stress will be proportional to the strain and the resulting stress-strain plot will be a straight line. But if the material is viscous and has no elastic component, the stress in the material will be highest at the highest strain rate. A viscoelastic material has a response which is partly viscous and partly elastic, a combination of the above two extremes. To extract the information from the stress-strain plot, a modulus $G$ can be calculated as the ratio of maximum stress to maximum strain [Vincent 1982, Fung 1994]. This modulus is a vector that can be viewed as the sum of loss modulus and storage modulus, indicating energy loss from a viscous component and energy stored by an elastic component in the material [Pereira 1991, Naimark 1992]. Dynamic testing provides a means for calculating the amount of energy storage and dissipation during deformation.

It is important to note that both the integral and differential models are only models and are not explanations of the biological tissue behavior. With the use of mathematical expressions derived from the models it is possible to determine constants which can be used as a basis for comparison or prediction, but it is not very likely that a biological material can be described in terms of a single spring and dashpot unit [Fung 1990].
1.3. Tissue Structure: Collagen and Elastic Fibers

Mechanical properties are dependent on the structural configurations of the elastic components [Viidik 1980]. The connective tissue matrices contain collagen, elastic fibers, and ground substances [Hukins 1984]. Methods of histochemical determination of the amount of collagen and elastin in biological tissues have been developed [Cardoso et al. 1993, Rosenbloom et al. 1993], but in this study a morphological approach was used.

Interactions between molecules are responsible for the tissue structure. For example, collagen molecules interact with each other to form fibrils; these fibrils interact with glycosaminoglycans which are themselves covalently linked to the protein core of a proteoglycan, and so on. The main concern of connective tissue research is to understand a complicated system of interacting macromolecules. Using well-defined antibodies to a number of macromolecules, studies have demonstrated that the basement membranes contain at least one collagen type (IV) and probably others including types V and VI [Kleinman 1993]. Elastic fibers are also found in the basement membranes [Pasquali-Ronchetti 1984]. In fact, controversial observations of the changes of the airway elastic fiber in asthma were reported: both fragmentation of the fibers combined with loss of elastin [Bousquet et al. 1996] and hyperplasia of elastic and collagen fibers [Gabbrielli et al. 1994] were found in the submucosa of asthmatic patients. For this project, the orientation of the elastic components, namely the collagen and elastic fibers, as well as their volume fractions in the airway mucosal membrane are of particular interest in terms of determining the mechanical properties in tension.
Collagen fiber is composed of bundles of fibrils formed by the protein collagen. The fibers are visible under the light microscope. They have diameters between 1 and 12 μm [Viidik 1980]. Isolated and straightened collagen fibers allow only minimum stretching [Morgan 1960, Stromberg et al. 1969]. They impart a unique combination of flexibility (when coiled) and strength (when straightened) to the tissue.

Elastic fibers are made of an amorphous core (elastin) surrounded by microfibrils that are partially embedded in thicker core fibers [Mijailovich et al. 1993]. The microfibrils have a diameter of 10-12 nm. The proportion of elastin and microfibrils may change with age [Hukins 1984] or diseases [Bousquet et al. 1996]. The extracted elastin is rubbery in a wet environment although it becomes brittle on drying. Tissues containing a high proportion of elastic fibers are able to attain high strains, that is, to be stretched by a factor of 2-2.3 with respect to its unloaded length without rupture, and return to their original shape when the forces are relaxed [Carton et al. 1962]. It was found [Hukins 1984] in tissues rich in elastic fibers, that the collagen fibers appear not to be highly oriented and presumably do not become strained and do not stiffen the tissue until the applied stress is sufficient to orient them.

The fiber orientation in the tissue can be determined by methods such as polarized light microscopy, transmission electron microscopy, scanning electron microscopy, and x-ray diffraction [Hukins 1984]. The orientation is different in different organs and locations in the body, depending on the biological function of the tissue. For example, the collagen fibers in a tendon are parallel so that they can withstand uniaxial tension; the collagen fiber in an artery reorient circumferentially when the internal pressure rises so that they can
reinforce the artery wall. On the other hand, the elastic fibers in bovine ligamentum nuchae are found to be oriented roughly parallel to the axis of the ligament and are connected by rare branching; in the lung a completely disordered interbranched meshwork of elastic fibers has been described [Pasquali-Ronchetti 1984].

According to the literature, age plays a role in the orientation and the mechanical properties of collagen and elastic fibers. Reorientation of the collagen fibers occurs during aging and appears to influence mechanical properties, for example, the age related increase in stiffness of the rat-tail tendon [Hukins 1984]. It was found that an increase of the tension of skin and of the wall of the uterus, similar to what occurs during aging, stimulates the production of new collagen [Takacs et al. 1968]. The volume fractions of both the collagen and elastic fibers in normal human dermis [Vitellaro-Zuccarello et al. 1994], calculated by a point counting method, were found to increase until 30-40 years of age then start to decrease. With respect to the changes in the elastic fibers during aging, as summarized by Kádár [1984], there is most likely no change in the amino acids composition of the elastin, while there is an increase in the number of cross-links [Rosenbloom 1993], leading to an increase in the rigidity of elastin. Calcium bound to aged elastin can also result in an increased rigidity of elastic fibers [Kádár 1984]. In a recent study, De Carvalho et al. [1996] found that the length of the elastic fibers of human heart increased significantly beyond the third decade of life which could be interpreted as a continuous formation of new fibers. Furthermore, they found that the amount of elastin increased and the number of microfibrils decreased with age, similar to that found in the age-related changes of elastic fibers in the superficial layer of the lamina propria of human
vocal cords [Sako et al. 1997]. A different distribution of the elastic fibers in the body during aging was also reported [Gigante et al. 1994]. It was found in New Zealand White rabbits that the elastic fibers are richly and homogeneously distributed in tendons and ligaments of younger animals, whereas they are rarely found in tendons of mature rabbits. In older animals, an abundance of elastic fibers is found in other connective tissues such as the articular capsule. A gradual process of maturation and specialization of the network of elastic fibers takes place with age.

Progressive losses in compliance or increasing stiffness with age have been observed throughout the body [Kohn et al. 1989], e.g., the heart, arteries, lung, and skin. This widespread increasing rigidity of tissues very likely plays an important role in the generalized physiological decline that characterizes the aging syndrome. The state of the fibrous proteins of connective tissue, i.e., collagen and elastin would be expected to alter the age-related mechanical properties of organs.

1.4. How Are the Structure and the Mechanical Behavior Linked?

The need for a combined structure/function approach to the study of the mechanical properties of connective tissue is increasingly recognized within the literature [Naimark et al. 1992, Trowbridge et al. 1989]. In studies of the pericardium, it was suggested that the number of layers of collagen and elastic fibers, the elastin content, and the orientation of the fibers in the tissue may be responsible for the differences in the mechanical properties among animal species. Some studies have suggested possible links
between the fiber orientation and the strength of tissue samples since for a two-or-three-dimensional meshwork, which does not have a pronounced "main fiber direction", the meshwork can be deformed before the fibers themselves start to deform [Hukins 1984, Viidik 1980]. There was also an attempt to develop a quasi-non-linear model that takes into account the fiber orientation and fiber length in biological tissues [Flaud et al. 1986]. A study on maturing rat skin [Belkoff et al. 1991] suggested that the observed collagen fibers being less wavy and more straightened with age, along with the increased diameter and fiber packing may account for the increase in the skin stiffness during aging. A study in a completely different field, the pulp and paper industry [De Grâce et al. 1976], investigated the relationship between the morphometry of the pulp fiber and the elasticity of paper. They also found that the higher the degree of curl and compression in the fibers, the more extensible is the paper, both in the elastic region and at rupture. No references were found where the measured morphometrical parameters of the elastic or collagen fibers were correlated with the mechanical behavior of the tissues in the biological fields.

A recent study [Mijailovich et al. 1993] suggested a mechanism for the energy distribution between the storage and loss modulus calculated from dynamic experiments. They set up an elastic fiber sliding model to test their hypothesis that the energy dissipation in the connective tissue matrix upon deformation occurs at the level of the frictional interaction. This leads to a new approach to the mechanisms that underlie connective tissue elasticity and energy dissipation during various types of loading.
1.5. Scope of the Study

Studies on the basement membrane of other organs showed "true elasticity" [Cook et al. 1975], but no stress-strain data within the elastic limit have been reported. The preliminary experiments in this work [Wang et al. 1997] showed the viscoelastic properties of rabbit's airway mucosal membrane. The focus of this thesis is to further test the viscoelastic properties of the airway mucosal membrane in tension and to correlate the mechanical properties with the fiber relative volume and orientation in order to reveal the link between the structure and function of the mucosal membrane in the airways.

We hypothesize that 1) the airway mucosal membrane displays viscoelastic properties and provides a significant elastic load which restricts ASM shortening to maintain airway caliber; 2) the mechanical properties of the mucosal membrane in tension can be mathematically predicted and morphometrically explained by the volume fraction and the geometrical orientation of elastic fibers.

The specific aims are: (a) to design and construct a high accuracy stress-strain-time apparatus, (b) to investigate the dynamic response of the membrane in tension and to determine its viscoelastic properties (the absolute gain at steady-state and the phase angle), (c) to develop a dynamic lumped model which allows the determination of meaningful time constants from the results, (d) to measure the volume fraction and the orientation of the elastic components of the mucosal membrane, (e) to correlate statistically the morphometrical measurements with the dynamic frequency response of the
membrane, (f) to observe the age effect on both the mechanical and structural characteristics.

To facilitate the study, the following assumptions have been made: (a) following the application of a deforming force, the cross-sectional area of the tissue samples returns to the value before the deformation, provided that the applied force is within the elastic limit, and (b) the shrinkage factor of tissue is the same in all dimensions after dehydration during histological processing.
Chapter Two

Equipment Design

To test the hypotheses set out in the Introduction, an experimental program was carried out. This program includes, not only a series of physiological experiments which will be described in the next chapter, but also the development of a stress-strain-time apparatus. This apparatus can be used to measure force to 10 mg and distance to 0.25 µm; apply changes in sample length in a wide range of static and dynamic patterns; display its own dynamic response only at frequencies greater than 100 Hz; and sample all variables and record them at adjustable sampling frequencies (from 1 Hz to 600 Hz).

2.1. The Necessity of Designing and Building Equipment for Dynamic Testing of Biological Tissues

Since the design and commissioning of the dynamic testing equipment used in the experiments is one contribution of this thesis, it will be covered in considerable detail. Although it is generally accepted [Ogunnaike and Ray 1994] that dynamic tests have more advantages than do the conventional static tensile tests, the actual errors caused by existing dynamic testing equipment have not been dealt with systematically. We have taken an engineering approach to the study of these artifacts [Wang et al. 1997]. Problems with previous dynamic tests arose because of the frequency response of the testing
equipment itself. These equipment responses introduce artifacts in the experimental results which require long and tedious analysis to detect and separate. The equipment that we designed and built is a result of our attempt to avoid the artifacts and to obtain only the true mechanical behavior of the tissue being tested. The new design uses the latest electronic and mechanical equipment, which not only meet the needs of the specific dynamic tests in this research project, but also can be used in various research areas concerning the viscoelastic properties of soft tissues. For example, it can be used to study the physiochemical, transport-related mechanical properties of connective tissues (e.g. dermis, mesentery) as a function of the degree of cellular and interstitial interactions.

2.1.1. Experimental Layout: the Basis of the Equipment Design

The equipment is designed so that the tissue sample sits vertically in the tissue bath (Figure 2.1). The vertical orientation was chosen for ease of tissue mounting, zero stress determination, and reduced play in the gears of the linear table. The bottom of the sample is fixed on a rod, which moves with a linear table whose movement is controlled by a stepper motor [Vroon 1992]. The top of the sample is mounted on a strain gage force transducer whose position is fixed. The sample clips are made of stainless steel tubing whose natural curvature allows the firm gripping of the tissue sample. Using a small pin, these clips are easy to open for tissue mounting.
The length of the sample can be stretched using three different programs: static stepwise preloading, sinusoidal oscillation, and single pulse test at each preload. This is achieved by controlling the movement of the motor which is connected to the bottom end of the tissue sample.

2.1.2. Physiological Requirements

For physiological materials the equipment must be stable and noise-free over a range of low frequencies from 0 to about 100 cycles/min which includes the breathing frequency of rabbits. It must also be very sensitive to force (or tension) changes since soft tissues generate small forces when stretched and these small forces must be accurately measured.
2.1.3. Mechanical and Electronic Requirements

The goal in the design of a stress-strain-time testing machine for soft tissue is to produce a highly accurate and flexible testing device using off-the-shelf parts. The components used in this design were chosen to ensure that no dynamic mechanical response, which originated from the equipment itself, would be found within the frequency range of interest. To accurately measure and control the input signal (position), a stepper motor, which is operated by digital pulses, is used. As required by the experimental protocol, the motor must be programmed to move a linear table to specific positions at adjustable rates; to oscillate the linear table at each position according to sinusoidal functions with different frequencies and amplitudes; and to move the linear table to generate a single triangular pulse with given height and duration. All parameters and commands are sent from a PC to the motor. At the same time, the position of the motor and the tension of the tissue sample must be recorded through the data acquisition system and displayed / recorded by the PC. Mechanical support for the machine parts must be designed to hold them in place and to protect them from external vibrations. Computer programs must be developed to produce and coordinate the movement of the motor and to acquire data.

2.2. Mechanical Support (Design Drawings See Appendix 2A)

The support structure consists of:
1. Isolation table (Figure 2a. M-1). Made from steel tubes and plates, the table serves as a sturdy support for the testing apparatus. The design used tennis balls between top plates and base legs to reduce vibration.

2. Linear table support (Figure 2a. M-3 & Figure 2a. M-4). Required by the shape of the linear table (Figure 2a. M-2), the support included a motor clearance notch as well as table mounting and adjustment slots.

3. Linear table connector (Figure 2a. M-5). This is an attachment for a stainless steel rod connecting the linear table and the tissue sample. The rod moves the tissue sample together with the linear table.

4. Force transducer holder (Figure 2a. M-6) and locking device (Figure 2a. M-7). The force transducer must be held in place so that it lines up with the stainless steel rod on the linear table to prevent twisting of the tissue sample. Due to the fragile nature of the transducer, a locking device was designed. A stainless steel plate was secured on the bottom of the transducer to isolate the tip of the transducer during tissue mounting. This plate is then released, by adjusting the screws on the sides, to take readings from the transducer during experiments.

5. Tissue bath (Figure 2a. M-8 & Figure 2a. M-9). The glass windows allow monitoring the tissue sample during experiment. Slots in the wall are for the heater installation. The bath slides up and down, as needed in the experiments, along a rod from the base plate.
6. Two heavy steel base plates, are separated to prevent vibration transmission between the motor and the force transducer.

2.3. Hardware Design

Figure 2.2 Sketch of the hardware design

The sketch of the design (Figure 2.2) and Figure 2app.1 (Appendix 2A) contains the basic hardware components of the equipment. To provide mechanical isolation, the whole assembly sits on an isolation table which is not shown in the figure. The hardware is composed of a motorized linear position table (#1 in Figure 2.2) that is run open loop by a stepper motor (#2 in Figure 2.2). The stepper motor drives a head screw that moves the linear table. The force transducer (#3 in Figure 2.2) is a strain gage type (Kulite transducer, BG series (model BG-10), Leonia, New Jersey). It has a 10 gram full scale output (10 mV/V), nominal full scale deflection of 0.0007", and a natural frequency 0.6
kHz. Operational mode may be either tension or compression. Overload: 400% of full scale load with no calibration change, 800% of full scale load with no mechanical failure.

Sensing principle: 4 arm strain gage bridge. Since the tissue sample is directly mounted on to the force transducer, a protective locking device was designed (for mechanical drawings see Appendix 2A) and installed on the transducer support. The purpose of the locking device is to carry all the force imposed on the transducer during the mounting of the tissue sample, which may well exceed the transducer bearing limit. The tissue sample is held by small stainless clips (#5 in Figure 2.2) attached to the testing arms. They are corrosion resistant, grip the tissue sample firmly, and make tissue mounting easy. The tissue bath (#4 in Figure 2.2) slides up and down along a fixed stainless steel support rod to allow easy mounting of the test samples. Before testing, the bath is slid down to the bottom of the support rod leaving the clamps free in the air for tissue mounting. After the tissue sample is mounted in place, the tissue bath is moved back up along the support rod to totally immerse the tissue sample in the physiological solution, which is being continuously renewed in the bath. An o-ring at the bottom of the tissue bath prevents the solution from leaking. Since the tissue bath is small, in order to avoid introducing disturbance to the tissue sample, the needed gas (mixture of 95% O₂ and 5% CO₂) bubbles are not released directly into the tissue bath. Instead, the physiological solution was saturated by the gas mixture in the reservoir outside the bath. This saturation was reached by continuous supply at a flow rate of approximately 3 milliliters per minute. The chamber of the bath has as many rounded corners as possible to aid cleaning. The bath also allows observation of the tissue through clear glass windows, which enabled the measurement of
the tissue dimensions using an optical micrometer. The bath is made of stainless steel with a heating cartridge embedded in one of the walls, which is electrically controlled to maintain the solution in the bath at a physiologically desired temperature: $37 \pm 0.2 \degree C$.

A manual control panel can be used for emergency stops of the motor, or for manual control of the motor movement during the set-up period.

### 2.4. Equipment Control Design

The equipment control design involves four functional areas as indicated in the schematic diagram (Figure 2.3): 1. directing the movement of the motor; 2. capturing the motor position; 3. obtaining the tension signal of the tissue sample; and 4. coordinating the above three actions. The control of the motor includes converting distance scale to index pulse number and time interval, downloading the calculation through a General Purpose Instrument Bus (GPIB) to the position indexer, and programming the motor to move according to the index pulse calculation. The position of the motor is "read" by digital input from the up-down counter at set time intervals. The force (or tension) signal of the test sample is collected from the strain gage transducer at the same set intervals by using an A/D converter. The control of the motor movement and simultaneous data acquisition provided a challenge in this design. For example, during sinusoidal testing, a personal computer (PC) would drive the motor with a digitized sine wave. While this is occurring, the force transducer signal from the strain gage output is read into the PC. The current design (Figure 2.3) provides control of the motor and data acquisition from the same PC
because the GPIB interface is used between the position indexer and the PC. Finally, the
above three functions are incorporated together in an operating software to serve the
purpose of running and monitoring the entire physiological experiments through the PC.
The software is a user friendly program developed using Visual Basic (Microsoft)
Professional Version 3.0. It is designed to have advanced capabilities such as starting up
and zeroing equipment, initiating calibration routine and adjusting calibration factors,
down-loading wave forms to controller, on line graphing of the tension and position
signals, high frequency sampling and data recording.

![Figure 2.3 Schematic diagram of the equipment control design](image)

**2.4.1. Control of the Stepper Motor and Linear Table**

The linear-motion table lead screw and Parker stepper motor driver were
configured to provide 0.5 μm incremental positioning in our study. The stepper motor is
controlled by digital index pulses, and can move the linear table with a resolution as small as 0.25 μm. The linear table moves in the vertical direction, and its motion is transferred to the tissue sample via a stainless steel rod. The direction of table motion is controlled by a level sensitive direction digital input to the Parker motor driver. For each pulse sent to the motor driver, the table will move 0.5 μm in the direction indicated by the direction level input.

As an example, a stepwise saw-tooth motion could be created as in Figure 2.4.

![Figure 2.4 The table position controlled by the index pulse and direction](image)

The selection of the step size of the linear table was rather arbitrary during the design stage, however, during equipment validation, a step size of 0.5 μm was found to produce the smoothest sine waves of correct frequencies and amplitudes. Once the step size is determined, it is hard wired and remains a constant for all future tests. By programming the timing of the index pulses and the direction of linear table movement, various wave forms can be generated. The timing of the index pulses also determines the
speed of the motor movement. For example, if the motor was to move at a speed of \(a\) \(\mu\text{m/s}\), then the time counts per step is calculated by \((\text{clock speed, in time count/s}) \times (\text{step size, in \(\mu\text{m/step}\}) / (\text{'}a\text{', in \(\mu\text{m/s}\}).\) The software clock speed is \(2 \times 10^6\) time counts per second.

During static preloading, the motor is programmed to move from one position to another along a straight line. The program includes calculation of the index pulse interval according to the assigned speed (strain rate), number of pulses according to the assigned distance, and the direction in which the motor moves.

To generate one complete sine wave, the number of index pulses determines the amplitude, the time between index pulses determines the frequency. The frequency range we are interested in is below 60 Hz. The amplitude range depends on the length of the test specimen. The length of our specimen (between markers) is about 4 mm. During static pre-loading, the length reaches 4.4 to 5.2 mm. If the amplitude is set to be 3\% of the length before oscillation, then the range of the amplitude is 132 to 156 \(\mu\text{m}\). Let \(A\) be the amplitude and \(\omega\) be the frequency of the sine wave to be generated. The time \(t\) when the linear table will be at displacement \(x\) (where \(-A \leq x \leq A\)):

\[
x = A\sin(\omega t), \quad t = \frac{1}{\omega}\sin^{-1}\left(\frac{x}{A}\right).
\]

After the frequency and amplitude of the sine wave are specified, the time interval \(t_n\) of the index pulses would be calculated for a quarter of the sine wave:
where \( t_0 = 0, \ x_n = n \cdot (\text{stepsize}) \)

(see Appendix 2B for the corresponding computer program (Chap2a.bas)). The index pulses will be sent to the motor drive according to the calculated interval. By alternating the direction signal 4 times, a complete sine wave would be generated due to the symmetrical nature of a sine wave. However, a limitation of the combination of frequency and amplitude is imposed by the motor control.

When the motor changes its direction, it requires about 200 \( \mu \text{sec} \) to do so [Parker Hannifin Corporation 1993]. We would have to "borrow" some time from in between index pulses sent to the motor to allow for this required time if the frequency was set high. The pulse width is 200 nsec which is not sufficient to allow the motor to reverse direction. According to mathematics, the motor velocity at the peaks or bottoms of a sine wave should be zero, then it would be possible to add adjustment to the adjacent two index pulses apart at the peak or the bottom of the sine wave (for program (Chap2a.bas) see Appendix 2B) to allow for this time. Calculations showed that the fact that the motor requires time to change direction could limit the possible settings of the frequency and amplitude of the sine wave. With a fixed frequency, the higher the amplitude, the narrower
the time interval at the peak or the bottom of the sine wave where the motor has to change its direction.

The frequency and amplitude limitations were calculated for our system. The time available for the motor to switch direction is plotted against settings of the oscillatory amplitudes as shown in Figure 2.5a and b. As pointed out previously, the amplitude range of interest for the physiological experiments is between 100 and 200 µm. The time needed for the motor to switch direction (200 µsec) is indicated with a horizontal line and an arrow in each figure. All symbols that fall below the horizontal line indicate settings that cannot be reached due to the nature of the motor. Figure 2.5a shows the choice of motor step size. The frequency tested is chosen to be 60 Hz, the upper limit of interest. The step size of the motor must be above or equal to 0.5 µm to allow enough time for the motor to switch direction. Figure 2.5b shows that with given step size, the higher the frequency, the shorter the time available for the motor. The calculations showed that it is still possible to manipulate the sine wave generation in order to achieve the ranges of frequency and amplitude of interest.
Figure 2.5a. Time interval at the peak (or bottom) of a sine wave
Oscillatory frequency = 60 Hz

Figure 2.5b. Time interval at the peak (or bottom) of a sine wave
Motor step size = 0.5 μm
Another consideration related to sine wave generation is whether the calculated time interval between index pulses should be the time interval between the "edge points" or "mid points". The "edge points" are the points when the motor makes a step, the "mid points" are the points between the two steps. Calculations showed that the difference resulting from the two choices is negligible.

To generate a single triangular pulse, the motor movement is controlled in a similar fashion to that during static preloading. The linear table is driven by the motor to move in one direction until the desired position is reached (the pulse height), then in the opposite direction until the initial position is reached. The speed of the motor was kept equal in both directions. The time between moving from the steady-state position and returning to the same point is called the duration of a pulse. The shorter the duration, the higher the frequency of the tissue response can be recovered. However, our system is limited, for all amplitudes, to generating pulses no shorter than 1.7 seconds. Once the height and the duration is specified by the PC, the position indexer is programmed to calculate the timing of the index pulse and direction according to the fixed motor step size, thus to control the movement of the motor.

2.4.2. Reading the Position of the Stepper Motor

To record the position of the linear table, we must keep track of the number of index pulses and the direction signal sent from the position indexer to the stepper motor driver. These are only incremental position data. In order to obtain the absolute position, the starting position must be known. We used a 12-bit up-down counter to obtain digital
position signals (direction and the number of index pulses), which were then read by the data acquisition system at the desired sampling frequencies (Figure 2.6).

![Diagram of position signal reading process](image)

**Figure 2.6** Reading the position signal

### 2.4.3. Reading the Tension From the Strain Gage

The analog voltage acquired from the strain gage force transducer was digitized and recorded in the PC (Figure 2.7) with the same sampling frequency used for reading the position signal. The analog signals were first passed through a multiplexer, then an A / D converter.
2.4.4. **Computer Interface Software Design**

The computer interface software which controls the communication between the PC and the GPIB, also captures the converted analog signals from the strain gage. The two signals are displayed on the computer screen simultaneously (real time display) and can be recorded at any specified sampling frequency.

1. Configuration of the interface board in the PC (configuration file see Appendix 2B: (das1600.cfg)); parameter setting see Appendix 2B: (wdas.ini))

The interface used is a Keithley Metrabyte DAS 1201 board. It can capture the digital and analog input signals simultaneously using the DMA acquisition mode. In DMA mode, the board acquires a single sample or multiple samples from one or more input channels. A hardware clock initiates conversions. Once the input operation begins, control...
is returned to the application program. The DMA mode provides the fastest data transfer rates. The board must first be configured for each application. The configuration includes specifying the board address, operation mode, communication ports, etc. It was necessary to use the standard acquisition mode, which is limited by the accuracy of the computer system clock when using Microsoft Windows. The fastest sampling rate was once every millisecond. Since Visual Basic (our operating program) can not use call back functions or interrupt service routines, time has to be pooled which means that the possible error of these samples would be in the range of ± 1 millisecond. A higher accuracy requires a significant increase in complexity of the programming.

2. Visual Basic program development (for program and captured computer screen see Appendix 2A)

The operating program was designed using Visual Basic Professional version 3.0. The detailed design and coding includes the following: functional design, form design, calibration algorithms and logic, interface to position indexer, interface to DAS1201 board, data storage, and data display (test and graphics) for analog input and digital input calibration, static preloading, single pulse initiation, sine wave oscillation and data capture.

The application framework includes the main window and main menu design according to the experimental layout. The main window (Appendix 2B.6) continuously displays the two signals: tension (in yellow) and position (in blue). A red vertical bar sweeps across the displaying area at a speed of 4 seconds per screen to renew the display of data. The actual values of the two signals as well as the time at which the signals occur are shown on the left hand side (LHS) boxes. The program allows for a continuous change
in amplification of the signals up to 100 times by redefining the offset and scale for display. The buttons on the upper LHS are used to start or stop an oscillation or capture static and dynamic data after the parameters are defined through the top panel. The top panel consists of four function groups. "File" is used to save the captured data under a file name or to exit the program. "View" changes the graphing on the screen between two modes. One is the position and tension signals vs. time, the other is the so-called x-y graphics, that is, the two signals are plotted against each other. During oscillation, the moment when the system is considered to have reached a pseudo-steady-state is when the x-y graph becomes repeatable in time. Data are captured after this steady-state is reached.

"Configuration" facilitates the position and tension signals to be calibrated, and provides dialog boxes for users to assign sampling frequency and number of samples needed for data capture during continuous acquisition, static, and dynamic tests. Using "Configuration", frequency and amplitude of the desired sine waves are also specified before initiating each oscillation. "Utilities" is the function group through which the movement of the motor is controlled for static preloading and pulse tests. The command sent from "Utilities" initiates the deformation of the tissue sample.

There are three different ways of deforming the tissue sample, or three different tests in our experimental design. Each test is controlled by its own dialog box under "Utilities".

1. During static preloading, the dialog box called "Controller Manual Move" (Figure 2app.2) is opened. The speed of the motor (equivalent to the strain rate) and the distance the motor should travel (the amount of elongation of the tissue sample), as well
as the direction in which the motor moves are defined. When the motor moves in the
direction labeled "down", the sample is stretched. If the direction indicated "up", then the
sample is released. Upon clicking the "start" button, the motor moves according to the
above commands. The "stop" button is for emergency stopping of the motor after starting.

2. Sine wave generation is controlled from two different locations in the main
Window. The frequency and amplitude are specified under "Dynamic Configuration"
(Figure 2app.3) and the actual command to start / stop a sinusoidal oscillation is
controlled from the buttons on the LHS of the main window. When the "start" button is
clicked, the command (specified frequency and amplitude) is first downloaded to the
position indexer in the form of the index pulse interval and the number of index pulses.
The calculated wave form can be previewed from the "Utilities" panel before starting the
movement of the motor. Then the indexer controls the motor to move according to the
calculated wave form.

3. During the single pulse tests, the command is again sent from the "Utilities"
panel. Once the pulse height, pulse duration, and therefore the pulse speed is assigned,
clicking the "start" button initiates and directs the motor movement accordingly.

Data recording can begin at any time. When the data are recorded, an indicator
will appear on the screen indicating the percentage of the required number of samples that
has been recorded.
2.5. Equipment Validation

Without equipment validation, the experimental results can not be trusted. Tests were designed and carried out to validate the equipment both mechanically and electrically.

2.5.1. Basic Validation

1. Tissue bath leakage. If the tissue bath is not well sealed, especially if a poor contact between the o-ring arrangement at the bottom and the stainless steel support rod occurs, the tissue sample could be exposed to air during experiments. This would not only interrupt the tension reading but also damage the tissue. The bath was tested over the time period required for a complete experiment and no leakage was found. Cold water, which has a very small evaporation rate, was used to fill the tissue bath for the test. There were no droplets observed on the outside of the tissue bath. The water in the tissue bath was removed regularly and measured. The volume was found to be a constant.

2. The heater temperature control. The tissue bath was filled with cold water, the temperature of the water was monitored throughout the time period required by the experiment. It took about 10 minutes for the temperature of the water in the bath (17 ml) to increase from room temperature to $37.2 \pm 0.2 \, ^\circ\text{C}$. This temperature remained constant until the temperature controller was turned off.
2.5.2. Performance Validation

1. The force transducer was calibrated for linearity in air, deionized water and Krebs solution. The results of the tests were in agreement with the properties specified in the certificate supplied by the manufacture. For results see Appendix 2C.

2. Position calibration. This can be easily tested when the motor is moved in one direction only. The distance that the linear table travels is measured with an optical micrometer. The optical micrometer has a display of 0.001 cm, which means it can read accurately to 10 µm. This measurement was compared with the position signal displayed on the PC. We found very good agreement between the two measurements. For results see Appendix 2C.

3. Oscillatory frequency. The set oscillatory frequency is compared with that measured using a stopwatch, and with that calculated from the recorded data (Table 2.1). The reason for using a stop watch is that the accuracy of only using the recorded data is limited by the sampling frequency, which is another parameter to be validated. With respect to the calculations listed in the table below, the sampling frequency used to record data was set at 100 data points/cycle, or 100 times the oscillatory frequency.
### Table 2.1 Comparison of the set and measured oscillatory frequency

<table>
<thead>
<tr>
<th>Set oscillatory frequency (Hz)</th>
<th>Stopwatch measured oscillatory frequency (s/10 cycles)</th>
<th>Calculated oscillatory frequency from recorded data (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.01</td>
<td>257.60</td>
<td>0.039</td>
</tr>
<tr>
<td>0.04</td>
<td>198.81</td>
<td>0.050</td>
</tr>
<tr>
<td>0.1</td>
<td>91.30</td>
<td>0.110</td>
</tr>
<tr>
<td>0.2</td>
<td>47.37</td>
<td>0.211</td>
</tr>
<tr>
<td>0.5</td>
<td>18.90</td>
<td>0.529</td>
</tr>
<tr>
<td>1</td>
<td>8.72</td>
<td>1.147</td>
</tr>
<tr>
<td>2</td>
<td>4.53</td>
<td>2.208</td>
</tr>
</tbody>
</table>

A comparison of columns 1, 3, and 4 shows that there is reasonable agreement except at the two lowest frequencies. Even though the step size of the motor has been tested to minimize the discrepancy between the set and the actual oscillatory frequencies, the motor failed to follow the set frequency at these two lowest settings. This is a limitation of the equipment. The measurements at higher frequencies showed that the motor moves according to the set oscillatory frequency with an acceptable accuracy. The motion of the motor was difficult to monitor with a stop watch at frequencies above 2 Hz. Because of the unreliability of the set frequencies, the actual frequencies were calculated from the captured input data in the data analysis.

4. Sampling frequency. According to the Nyquist criteria [Eckman 1962] the sampling frequency must be at least 10 data points/cycle to depict the actual sinusoidal tracing with an acceptable accuracy. Our equipment can reach a 500-600 Hz sampling
frequency without graphic display, and about 110 Hz with real time graphic display. The
difference is due to the limitation of the interface clock and the fact that the command for
sampling the motor position was sent at the same time as the continuous data display
occurred. The set sampling frequency (without graphics) was also compared with the one
measured using a stop watch (time required for recording 100 data points) (Table 2.2).

Table 2.2 Comparison of the set and measured sampling frequency

<table>
<thead>
<tr>
<th>Set sampling frequency (Hz)</th>
<th>stop watch time (sec)</th>
<th>Calculated actual sampling frequency (1/100 sec)</th>
<th>Calculated actual sampling frequency (Hz)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10</td>
<td>63</td>
<td>9.41</td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>87</td>
<td>17.04</td>
</tr>
<tr>
<td>50</td>
<td>2</td>
<td>71</td>
<td>36.9</td>
</tr>
<tr>
<td>80</td>
<td>2</td>
<td>03</td>
<td>49.26</td>
</tr>
<tr>
<td>100</td>
<td>1</td>
<td>64</td>
<td>60.98</td>
</tr>
</tbody>
</table>

Comparing columns 1 and 4, larger and larger discrepancies at higher and higher
sampling frequencies. It is considered common sense by many that, the higher the
sampling frequency, the better the recorded data depicts the actual tracing. The actual
sampling frequency was calculated from the captured data, using the computer time, in the
analysis program.

2.5.3. Grounding of the Equipment

The equipment must be properly grounded to ensure a meaningful tension reading
and minimize the noise in the data. It is a strong indication of the equipment not being
properly grounded if the tension signal of the mounted tissue sample increases or decreases erratically. This can be further confirmed if any metal parts that are immersed in the ionized solution in the tissue bath, display a deposit of bubbles and salt. This is because, if there is an electric current across the system hardware, the ionized solution in the tissue bath will be electrolyzed to release bubbles and produce salt crystals.

All components of the equipment must be electrically grounded to prevent noisy signals or electrolytic reactions. Using an ohmmeter, the equipment was checked for a common ground. The motor and the stand were grounded with a copper wire. The connection between the force transducer and the interface board of the PC was also examined to ensure the correct electrical linkage. For example, the shielding should be connected to the real ground instead of the signal output ground; the tip of the force transducer must be connected to the real ground instead of the floating ground of the PC. The latter means that the force transducer must use a separate power supply instead of the power from the PC. A separate DC power supply was therefore specifically made for the transducer to provide the excitation voltage (5 volts). This power supply operates on a battery and the voltage is monitored to check that it remains at a level well above the required 5 volts.

2.5.4. Testing of Standard Spring

To test the operating program and the frequency response of the entire equipment, tests were performed on a standard spring (spring constant \( k = 1 \text{ g/cm} \)) following the same experimental protocol (Chapter Three) for testing biological tissues. The spring was
calibrated with a standard weight (Appendix 2C, Figure 2a2). Sinusoidal oscillations (Appendix 2C, Figure 2a3) and single pulse tests (Figure 2a4) were also performed.

From the analysis of the pulse test, using the same analysis method for the tissue samples, to be described in Chapter Three, a Bode diagram (Figure 2.8) was obtained.

As expected from a standard spring, a Hookean material, there was no dynamic response revealed from the Bode diagram. The results from testing a standard spring indicated that there was no presence of machine response within the frequency range of interest. Thus the experimental results obtained from this equipment will contain only the tissue sample responses. This is an important improvement over previously available equipment. It simplifies the data analysis procedure and makes the tissue response visible directly from the experiments.
The tests of the standard spring also confirmed that the safe operating ranges for amplitude and frequency are amplitude below 200 μm and frequency between 0.1 and 30 Hz. Any substantial alteration of the ranges would require major changes in the hardware.

2.6. Equipment Limitations

2.6.1. Sensitivity at Very Low Loads

Because of the delicate nature of the tissue sample, the force transducer must be able to sense very small readings. The chosen force transducer is very sensitive and both the physical and electrical noise in the surroundings will also be picked up. This results in noisy signals when measuring very small forces.

2.6.2. Choice of Motor Step Size

The motor step size was set to 0.5 μm. To produce linear signals the amplitude of a sine wave is usually fixed at 3% of the tissue sample length. The more steps the motor moves, or the smaller the steps are, the smoother the sine wave. But the steps can not be too small because the time interval between index pulses is limited by the GPIB, whose maximum count is 65,000 per second. If we chose the minimum step size of the motor, 0.25 μm, as seen in Figure 2.5a, the time interval at the peak of a sine wave would be too small, and the system produces irregularly shaped sine waves. If we chose the motor step
size of 1 µm, sine waves at low frequencies appeared to be saw toothed. Limited by the
system, the step size of the motor was chosen to be 0.5 µm.

Due to the step size, the time needed to reach the desired pulse height during
single pulse tests was predetermined. Imposing a 30% strain on a 4 mm sample, 4080
steps were needed for a pulse. For stable operation the minimum period attainable was 1.7
seconds. At the point when the motor is changing direction, added time was needed. This
also contributed to prolong the pulse duration.

2.6.3. Noise Generated by the Movement of the Stepper Motor

As will be discussed in the next chapter, the tension of the tissue samples responds
to each step of the length change in an exponential fashion as shown in Figure 2.9. During
sinusoidal oscillation or single pulse test, the motor moves in multiple steps to generate
the desired wave form. Since the motor moves in steps, the change in the length of the
tissue sample is also achieved in multiple steps. With a sensitive force transducer like the
one used in this system, the tension tracing could be made up of multiple exponential-like
curves. Therefore even though the length changes according to a sine wave, the tension
tracing may have a slight deviation from a perfect sine wave. This deviation appears
mostly at the peaks of every exponential-like curves, which could be considered non-
random noise added to the true signal due to the motion of the motor.
Length change

Tension

Time

Figure 2.9 Tension response of the tissue sample to stepwise length change
Chapter Three

Physiological Experiments

3.1. Introduction

Physiological experiments are used to determine the mechanical properties of the biological tissues by their stress-strain relations. A set of in vitro experiments was designed and carried out on tracheal mucosal membrane samples from 40 rabbits using the equipment described in Chapter Two. Since the tissue samples were obtained from living animals, the age and the overall health of the animals before they were sacrificed may cause a difference in the tissue response. The age effect on the mechanical response was studied by randomly selecting animals of different ages (from 6-8 weeks to 35 months) as sample donors. Only healthy female rabbits were selected to exclude the possible influence of diseases or the sex of the animals. The purpose of the experiments was to develop a consistent routine for sampling the mucosal membrane of rabbits of various ages and to develop a dynamic lumped model to allow the determination of steady-state stiffness and meaningful time constants from the results. Detailed descriptions of both the experimental procedure and the data analysis are presented. At the end of this chapter, findings of the mechanical properties of the mucosal membrane are reported. The significance of these
results as well as their correlation with stereological measurements of the tested samples will be discussed in the following chapters.

3.2. Experimental Procedure

3.2.1. Equipment Preparation

The operation of the equipment was checked by testing the response of a standard spring. Before each experiment, the electrical and mechanical components of the equipment were examined to ensure proper connection since the experiments should not be interrupted by equipment repair. The calibrations (see Appendix 2B) of both the tension reading of the force transducer and the position reading from the PC were confirmed before each tissue sample was mounted for testing. The physiological solution (Krebs) (Table 3.1) was made and purged through the tubing connecting the solution reservoir and the tissue bath. The classic Krebs solution was altered to eliminate the Ca \(^{++}\) ion which may facilitate the denaturation of collagen in the tissue [Miller 1984] and thus may alter the mechanical properties of the samples.

Table 3.1 Ingredients of the Altered Krebs Solution (g/l): pH=7.4-7.5

<table>
<thead>
<tr>
<th>NaCl</th>
<th>KCl</th>
<th>MgSO(_4) . 7H(_2)O</th>
<th>KH(_2)PO(_4)</th>
<th>NaHCO(_3)</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.9</td>
<td>0.35</td>
<td>0.29</td>
<td>0.16</td>
<td>2.1</td>
<td>2.0</td>
</tr>
</tbody>
</table>
The solution in the reservoir was bubbled with 95% oxygen - 5% carbon dioxide [West 1990]. The heater of the tissue bath was turned on to maintain the Krebs at 37°C in the bath. By continuously circulating the solution through the tissue bath, with an optimum flow rate (27 drops/minute), the pH was maintained at 7.4-7.5 (see Appendix 3D). If the flow rate is too low, the Krebs solution in the tissue bath is not renewed often enough to supply necessary nutrients or to remove the waste sufficiently to maintain tissue physiological functions. This can be observed by following changes in the pH. On the other hand, if the flow rate is too high, turbulence or vibrations of the fluid may occur, which impose additional unmeasurable deformation to the mounted sample and disturb the tension measurement. Additional Krebs solution was also set aside in which the tissue samples were kept as soon as they were obtained.

3.2.2. Tissue Sample Preparation

The material tested in this investigation was the tracheal mucosal membrane obtained from New Zealand female white rabbits (with animal care approval). The tracheal mucosal membrane was chosen because it is more easily removed, free of smooth muscle, than is the mucosal membrane from other airways. The animals were sacrificed with a Katamine hydrochloride injection (i.m.) at 150 mg/kg, or until pain response was no longer present. The area above the trachea was shaved. An incision from clavicle to larynx was cut. After the connective tissue under the skin was removed using blunt dissection, the chest was opened. The trachea was removed and kept in the prepared oxygenated Krebs solution. The trachea was cut open from the posterior membranous portion to
eliminate the possibility of including the airway smooth muscle in the tissue samples. The trachea was fixed on a dissection plate with its inner surface (the mucosal membrane) facing the magnifying glass. Mucosal membrane strips were dissected out using a razor blade, one from the longitudinal and the other from the circumferential direction of the trachea. Care was taken that the cartilage under the mucosal membrane was not included in the tissue samples. This was further confirmed under the microscope after the sample was histologically processed at the end of the experiments. Both strips of tissue sample were tested individually in the experiment but they were not dissected out at the same time. A particular strip was only dissected immediately before it was mounted in the equipment, to maintain the best possible physiological condition. The size of the dissected specimens was approximately 6 mm long, 3 mm wide and 1 mm thick. Both ends of the specimen were held by small aluminum clips. Cyanoacrylate glue was used to prevent slippage between tissue and clips. Tests had shown that using the glue did not affect the experimental results. Immediately following the sample preparation, the specimen was submerged in the tissue bath filled with oxygenated Krebs solution.

3.2.3. Sample Mounting

The specimen was mounted vertically between two clip holders. The upper and lower holders were set and fixed at the appropriate angles to avoid twisting of the sample during elongation. One of the holders was attached to the force transducer, which remained in a fixed position; the other holder was attached to the motor arm (the stainless steel rod attached onto the linear table) that is moved along the long axis of the specimen.
by the stepper motor (Figure 3.1). The motor arm was used to apply uniaxial deformations from one end of the tissue sample. The tension in the specimen was continuously monitored by the force transducer.

![Figure 3.1 Experimental layout](image)

**3.2.4. Experimental Protocol**

In order to capture the tissue behavior at conditions as close as possible to those under physiological conditions, all of the tests were performed and completed within 6-8 hours of the death of the animals [Hildebrandt 1969]. One of the two samples (circumferential or longitudinal) was tested first in each experiment, closer to the time when the animal was sacrificed. The choice of the “first sample” was alternated in the subsequent experiment to ensure comparable freshness among all samples. Preliminary tests were carried out to measure the elastic limit of the mucosal membrane and the
uniformity of the strain distribution across the sample. Duplicate tests were also made to
test reproducibility. The results were found to be within the experimental error.

The mucosal membrane in the trachea was most likely under some stress in situ
due to its attachment to the cartilage. Since the trachea was opened from the posterior
membranous portion before exposing the mucosal membrane, it was difficult to obtain an
accurate measurement of the length of the sample strips in situ.

3.2.4.1. Preliminary Tests

3.2.4.1.1. Measuring the Elastic Limit of the Rabbit Tracheal Mucosal Membrane

Almost all materials have their own elastic limit [Reiner et al. 1967]. We define the
elastic limit as the strain within which the length of the tissue sample will return to its
initial length upon removal of the external forces. When a tissue sample is elongated
beyond this strain value, the length does not return to the initial value even if the external
force is removed. In other words, the sample may be overly stretched and a nonlinear
stress-strain response may occur. This condition is not physiological and may be an
indication that the sample structure is altered [Fung 1990, 1994].

In the preliminary studies, the elastic limit of rabbit tracheal mucosal membrane
was first tested. For example, before any external force was imposed, the length of a tissue
sample was measured to be 2.48 mm. When the sample was stretched to 3.43 mm, a strain
of 38%, the force was removed and the sample length was measured to be 2.5 mm. This
indicates that the 38% strain is still within the elastic limit because the length of the tissue
sample was preserved within a reasonable error for biological tissues. The same test was
repeated for various values of strain and on both longitudinal and circumferential samples from 10 rabbits. The results showed that the elastic limit of the rabbit mucosal membrane is about 40±5%. The strain during the stress-strain experiments was not allowed to exceed 30%.

3.2.4.1.2. Uniformity

When one end of the tissue sample undergoes a stretching process, the elongation of the regions that are closer to the clips may be different from that of the region which is closer to the center of the sample. In order to detect whether the elongation was distributed evenly throughout the sample length, both longitudinal and circumferential samples were marked with four dots at equal distances along the long axis of the sample (Figure 3.2). The strain, or the percentage of length change, of each segment marked by the dots was measured when the tissue sample was under different magnitudes of tension. An example is shown in Figure 3.2a.
Figure 3.2 Uniformity of the sample length change

Figure 3.2a Measured strain between markers on the tissue sample
The length change of all segments followed a similar increasing trend with tension. The length changes of segments F and G were noisier than those in the middle segments, which can be seen in Figure 3.2a. Within the B segment, which is between marker 1 and 4, the elongation was rather uniform. Therefore the distance between these two markers should be measured and used to calculate the average strain of the tissue sample. However, the equipment was designed to record the position of the lower clip, that is, the distance between points a and b in Figure 3.2. The length change of any other segment was difficult to monitor especially during dynamic experiments unless a video camera was used. A closer look at the results suggests that the measurement variations in G and F may cancel out making the measurement of segment A close to that of the segment B or D. Macroscopically, the length change between the two clips, which is the value used in the data analysis, is still roughly equal to the relative length change of the center region of the sample. A duplicate test verified this result.

3.2.4.2. Experiments

3.2.4.2.1. Tissue Sample Preconditioning

The tissue sample was preconditioned [Fung 1994] when first mounted in the apparatus. Repeated loading and unloading of the same specimen to successively higher stress levels were performed until the length reached 130% of the initial length (Figure 3.3a &b). The curves would be expected [Fung 1994] to shift to the right during roughly the first three cycles and become repeatable, provided the specimen has been dissected out
and mounted onto the testing apparatus without being subjected to any tensile stresses [Fung 1994].

As shown in Figure 3.3 a and b, the force was less than 500 mg for longitudinal samples, and 200 mg for circumferential samples. The readings of the force transducer at this magnitude could be quite noisy, which makes it difficult to trace the exact loading and unloading part of the curve. Nevertheless, the trend of curves shifting to the right during the first few cycles was not observed. All seven cycles appeared to be reproducible within the error range of the force transducer.

The purpose of the preconditioning was to allow the tissue sample to reach a state at which the response stays the same even if the sample undergoes several more loading-unloading cycles. Preconditioning can be viewed as a preparation step to ensure reproducible experimental results. It is likely that the structure of the tissue sample alters with the deformation, and this reorganization of the tissue structure became less random and more predictable after the preconditioning.

At the end of this process, the specimen was allowed to equilibrate at zero tension for about 15 minutes. The distance between the two clips was measured with a traveling microscope and recorded as the initial length.
Figure 3.3a Preconditioning of tissue samples

Longitudinal sample, 02/16/96

Figure 3.3b Preconditioning of tissue samples

Circumferential sample, 02/16/96
3.2.4.2.2. Measurement Procedures: Capturing the Position and Tension Signals

The three measuring techniques used in these experiments are static preload, single pulse test, and sinusoidal oscillation. It was shown in the preliminary experiments that when at least 10-15 minutes waiting time was allowed for the tension of the tissue sample to stabilize in between different techniques, the sequence of using the techniques had no significant influence on the measurements. All experiments were started with the smallest extension, and/or the lowest frequency. These parameters were then gradually increased to the maximum settings. This was to minimize the time needed for the tension to reach an equilibrium. The single pulse test was performed before the sinusoidal oscillation at each preload even though there were larger extensions in the pulse test than in the sinusoidal oscillations. The pulse tests gave much more information about the tissue's dynamic behavior and used much less time than the sinusoidal tests did. It was therefore, preferred to obtain pulse test results when the tissue sample was as fresh as possible.

Three strain values were chosen as the static preloads: 10%, 20% and 30%. At each of the three levels of preload, the single pulse tests and sinusoidal oscillation were performed. Then the tissue sample was elongated to the next preload level. The position and the tension signals were monitored throughout the duration of all deformations, and recorded during selected time periods. The recorded position and tension signals were analyzed to obtain the mechanical properties of the tissue sample.
3.2.4.2.2.1. Static Preload

By changing the position of the motor arm with a speed of 200 \( \mu \text{m/sec} \), the specimen was stretched uniaxially by 10% of its initial length as the first preload. The tension was observed to first increase to a certain level and then gradually decrease in an exponential-like fashion. About 15-30 minutes after the extension, the tension approached a constant value (plateau). The tension vs. time tracing gives important evidence about the viscoelastic properties of a material (for an example, see Appendix 3E Figure 3a.1).

3.2.4.2.2.2. Single Pulse Test

Single triangle pulse tests were initiated after the plateau of tension was reached at the end of static preload. Pulse duration was set at 1.7 seconds. Two different pulse heights were used: 15% and 20% of the actual length of the specimen. At each pulse height setting, five consecutive tests were recorded at a 100 Hz sampling rate, with 800 data points for each test, and were used in the data analysis to obtain mean and standard deviation values of the fitting parameters (see Eqn. 3.3). After the pulse tests, the specimen was again, allowed to reach an equilibrium before the sinusoidal oscillations were initiated at the same level of preload.

The setting of the parameters for pulse tests was determined based on a series of preliminary evaluations. The pulse height was tested for three different settings, 9%, 15%, and 20% of the present length. The 9% setting could not drive the system hard enough to give a large enough signal-to-noise ratio for analysis. On the other hand, if the system was driven too hard, the tissue sample may be stretched over the elastic limit which was not desirable. Depending on the sample length after dissection, a 20% setting of the pulse
height could overly stretch the sample in some cases. A 15% setting was first used to collect data in case permanent damage was done when the 20% setting pulse test was used.

Different settings of pulse duration were also tested. According to the literature [Hougen 1964], the shorter the duration, the better the data can reveal the frequency characteristics of the material. The shortest possible duration was 0.1 second, but at this setting the position of the motor failed to return to the initial value, which disqualified the deformation as a pulse, by definition. This may complicates the data analysis. The pulse would not close (either tension or position signal does not return to the initial value) until a pulse duration of 1.7 seconds was used.

It was observed that the higher the sampling rate, the better the data depicted the actual tracing. However, there existed an upper limit of the sampling rate due to the nature of the operating system of the computer. The pulse was monitored on the computer screen at the same time the data were captured. This slowed down the maximum sampling rate. The actual time interval between data points were calculated in the analysis program.

3.2.4.2.2.3. Sinusoidal Oscillation

The amplitude of the oscillations was set at 3% of the actual length of the specimen. This amplitude was chosen from the literature [Fredberg et al. 1991], as well as from observations of actual tests. Apart from the limitations on the amplitude imposed by the equipment, in some cases, especially when the tissue sample was under a preload of 30%, a 5% amplitude caused the cross plot of the tension and position signals to turn into
"banana" shaped lines rather than a straight line. This non-linearity complicates the data analysis. It could be caused by over stretching of the sample for a long time. In order to be consistent throughout all preloads, the amplitude had to be below 5% of the present sample length. On the other hand, if the amplitude was as low as 1%, noise in the tension signal could be of a similar magnitude to that of the true signal, especially when the preload was about 10%. Taking these observations into account, the amplitude was chosen to be 3% for all preloads. The oscillatory frequencies used were between 0.04 and 30 Hz (0.25 and 188.4 rads/sec). The upper limit of the range was set by the equipment (Chapter Two). The actual oscillatory frequencies were selected so that they would be somewhat evenly distributed on a log scale for ease of frequency response analysis (described in the later sections). The selected frequencies were: 0.04, 0.1, 0.2, 0.5, 1, 2, 5, 10, 20, 30 Hz. It took, on average, 5-6 cycles for the specimen to reach a pseudo-steady-state after initiating the oscillation. After reaching this steady-state, 3 cycles of response at each oscillatory frequency were recorded at a sampling rate of 100 data points/cycle which is more than sufficient according to the Nyquist criteria [Ramirez 1985]. However, when the oscillatory frequency exceeded 10 Hz, due to the limitation of the computer as mentioned in the pulse testing, the sampling rate was reduced. In the program for analysis, the time interval between data points was calculated from the captured data. The tension of the specimen and the position of the motor arm, as well as a plot of one against the other, were monitored from the computer screen. An example of the former is shown in Appendix 3E Figure 3a. 2.
After the sinusoidal oscillation, the tissue sample was elongated to the next preload level. The procedure described above was repeated until the preload reached 30% of strain.

3.2.4.2.3. Recovery of the Original Length of Sample after Deformation

At the end of a complete set of experiments on a tissue sample, the external force was removed, with the sample still mounted in place, to allow the length of the sample to return from 30% strain to its initial value. The tension of the sample was monitored until it stabilized. On average, it took about 5-10 minutes for the tension reading to return to zero. The fact that the tension returned to zero was a proof that the sample had been exercised only within its elastic limit throughout the experiments. Otherwise, the recorded data could not be used for further analysis.

3.2.4.3. Measuring the Cross Sectional Area of the Tissue Samples

After the physiological experiments, the tissue samples were fixed in 10% formalin under no load. Following the conventional procedures, samples were embedded vertically in paraffin [Gordon et al. 1977] so that the cross-sectional area of the sample (the plane perpendicular to the direction of the applied force during experiments) can be measured. Sections of 3-5 μm thickness were cut and the areas of the cross-sections were measured. The cross-sectional area (CSA) was used in the analysis to normalize the tension readings, that is, to obtain stress.
3.2.4.3.1. Instrument Preparation for CSA Measurement

The morphometrical equipment used for measuring the CSA was a Bioquant System IV (copyright 1989 R&M Biometrics, Inc.). The system consists of a computer for image analysis, a light microscope with a mirror on the side eyepiece, and a digitizing tablet from which the image under the microscope can be sent to the computer. This system can determine length, area, perimeter, shape factor, and shortest or longest distance between two points. The smallest unit on the calibration ruler is 0.01 mm. The magnification factor was set by choosing the eyepiece of the microscope. The power of the microscope eyepiece was set at “x 4”. This setting was chosen to reveal the details of the image as much as possible, while including the complete image in the same field of view.

The system was calibrated to ensure no distortion of measurement horizontally, vertically, or diagonally. The measurements were stored as ASCII numbers for graphing or statistical analysis.

3.2.4.3.2. Measurements of CSA

Stained with Toluidene Blue O (TBO), the cross sections of the mucosal membrane displayed a purple tone with distinguishable epithelial cells, blood vessels, glands and loose submucosa. The total area of the image was measured by tracing the outermost contour. The same image was measured 5 times to obtain an average. A shrinkage factor (10%) [Weibel 1979] was taken into account when obtaining the actual cross sectional area of the tissue samples.
3.3. Data analysis method and modeling

The stress-strain relationships may be used to define the mechanical properties of solid materials, including biological tissues. The methods used in the analysis of the data from the three testing techniques will now be considered in detail. A dynamic model will be proposed which best fits these data and allows a better understanding of the macro-changes which are occurring.

3.3.1. Data

Representative tracings of position and tension signals are illustrated in Figures 3.4, 3.5, and 3.6. The dots represent the actual tension readings, the solid lines are tracings after smoothing.
Static preloading is a form of stress-relaxation experiment [Frisen et al. 1969a, b, Woo et al. 1980] in which the tissue sample is suddenly deformed (see position tracing) and the deformation is maintained constant. The corresponding tension induced in the sample was measured with time. The tracing indicated that the tension first increased to a certain level then decreased with time until it approached a plateau. This phenomena is called stress-relaxation [Fung, 1994] (as shown in Figure 3.4).
The single pulse test (as shown in Figure 3.5), which followed fifteen minutes after the preloading shown in Figure 3.4, is a type of dynamic experiment [Clement 1963, Hougen 1964] in which the position and the tension signal both start at steady-state. The position signal is then moved from steady-state through a certain pattern and returns after time $t$ to the initial value. The force signal changes in response to the strain and returns to its steady-state value. Notice that in the pulse test, the tension signal was less noisy than in the sinusoidal tracing (Figure 3.6), which followed the pulse test by about 10 - 15 minutes because the position change, thus the magnitude of tension change, was larger, and the relative noise smaller. In the pulse tests, it is important that the system be driven sufficiently hard so that the dynamics of the system be excited but not so hard that the capacity of the system to respond be exceeded [Hougen, 1964]. When there is relatively
less noise in the tension signal, more information on the tissue response can be extracted from the data.

The sinusoidal oscillation is the traditional dynamic stress-strain test [Mridha et al. 1985, 1992, Preslin 1991, Stamenovic et al. 1990, Torzilli 1984] in which the position signal is varied cyclically (sinusoidal for mathematical convenience) at different frequencies (tracing at 1 Hz is shown in Figure 3.6) and amplitudes. The corresponding tension was measured with time. The amplitudes of the position oscillation were very small (about 150 - 200 μm). It is suggested in the literature [Vincent 1982, Fung 1990] that small deformations are often used in sinusoidal oscillations for exploring viscoelastic properties which most biological tissues possess.
The data suggested a viscoelastic behavior of the mucosal membrane. A viscoelastic material will return to its original shape after any deforming force has been removed although it takes time to do so [Vincent 1982]. When the material is deformed, it responds to the deformation over a period of time [Jeronimidis et al. 1984]. This is seen in the results of the static preloading as the tension in the material relaxed to a final plateau value. If the material is deformed dynamically, then the tension will be out of phase with the length change, the amount depending on the extent to which the material resembles a fluid (i.e. is viscous) or a solid (i.e. is elastic) [Jeronimidis 1984]. This will be seen in the frequency analysis of the data in the following sections. The fact that the biological tissues contain water (about 70% by weight on average) contributes to their viscoelastic behavior [Jeronimidis 1984]. It is worth mentioning here that the reason the Krebs solution was used in the experiments was to avoid significant changes of the water content of the sample by maintaining a physiologically iso-osmotic environment.
Among experiments with 40 rabbits, although not all tracings of the same type of deformation are identical, they are of the same shape and trend. For example, the tension in the static tracing at 10% strain and 30% strain may have different magnitudes, and thus different plateau values, but the stress-relaxation phenomenon occurred in both tracings as shown in Figure 3.7. All tracings are analyzed in this chapter and the effect of age on the stiffness is reported at the end of the chapter. These data will also be compared, in Chapter Five, to parameters which describe the orientation of the elastic components of the tissue samples.
3.3.2. Data Conversion

To be consistent with the literature and to derive meaningful parameters to characterize the mechanical properties of the tissue samples, tension data were converted to engineering stress and the position data were converted to engineering strain. By definition [Hayden 1965], engineering stress and strain are expressed as:

Engineering stress: \( \sigma = \frac{T}{CSA_0} \), where \( T \) is tension and \( CSA_0 \) is the original cross-sectional area. Engineering strain: \( \varepsilon = \frac{L - L_0}{L_0} \), where \( L \) is the length of tissue sample and \( L_0 \) is its original length.

\( L_0 \) was measured at the beginning of the experiment, before any external force was applied to the sample. \( L \) was calculated as \( (L_0 + (P - P_0)) \), where \( P \) was the position signal and \( P_0 \) was the position signal when \( L_0 \) was measured.

Although by definition the engineering stress and strain are not the same as the true stress and strain, the two types of stress-strain curves are not different within the elastic limit. The difference appears around the fracture point when the sample breaks [Hayden 1965]. As specified previously, all samples were examined only within the elastic limit, which allowed the analysis to treat the engineering stress and strain as the stress and strain terms used in most literature.
3.3.3. Modeling of the Data

The main reason to model the data is that a better understanding of the tissue response is needed. In some situations, carrying out actual experiments may be very difficult. As stated in our hypothesis, the mechanical properties of the mucosal membrane ultimately will be used to quantify a mechanical load the membrane may impose on the airway smooth muscle. To achieve this goal, the actual load-bearing mechanics of the mucosal membrane needs to be studied in vivo during smooth muscle contraction. The ability to predict the mechanical response of this tissue is necessary for this calculation.

Due to the limitations of the equipment and the nature of the mucosal membrane, a large amount of noise exists in the data. Although the data are themselves sufficient, modeling is needed to overcome the obstacle imposed by the noise in the system to better understand the physical relations. Our choice of model was suggested by the tracings of static preloading. The same model must also fit the dynamic data (pulse test). The fitting of the model allows the calculations of time constants which then helps us to better understand the elements of the mechanical model (the springs and the dashpots). Modeling also allows the results of our study to be compared with other values reported in the literature.

In this study, the tissue samples were examined within the elastic limit which allowed standard linear theories to be applied for the modeling. Many studies have shown non-linearity of biological tissues [Jeronimidis 1984]. If a tissue sample is stretched beyond its elastic limit, non-linear behavior occurs [Hayden 1965]. The extensions that
causes the non-linearity may not be physiological, and modeling of the response requires
difficult to handle non-linear equations.

Simple lumped models (minimized number of parameters) were of particular
interest in this study. The advantage of having few parameters to describe the behavior of
the system sufficiently well should make it easier to correlate the response with
morphometrical findings.

Most biological tissues are viscoelastic [Fung 1994]. Three mechanical models are
the simplest and most often used to describe viscoelastic behavior. They are the Maxwell,
the Voigt, and the Kelvin models, all of which are composed of combinations of linear
springs and dashpots. Since the Maxwell model best describes the behavior of a
viscoelastic fluid, it is not considered to fit the data of the solid mucosal membrane.

3.3.3.1. In time domain

3.3.3.1.1. Voigt model

The Voigt model consists of a linear spring (spring constant $\mu$) and a dashpot
(viscosity $\eta$) combined in parallel (Figure 3.8), $u$ is the displacement.
The relationship between the force $F$ and the displacement $u$ is [Fung 1994]:

$$F(t) = \mu (1 - e^{-\tau/\eta}) u(t)$$  \hspace{1cm} (3.1)

In Figure 3.9 the generated tension tracing (Voigt model, with best-fit parameters $\frac{1}{\mu} = 0.0003$ mm$^2$/mg, $\tau = \frac{\eta}{\mu} = 1.4$ sec) is superimposed on the raw data. The predicted tracing was obtained using the computational program Voigt.bas (Appendix 3A). The model tracing does not show the stress relaxation [Fung 1994] which is apparent in the actual data.
When a sudden deformation is imposed on a material that behaves according to the Voigt model, the dashpot will not move instantaneously, which makes $u = 0$. Since there is no deformation on the spring, there is no tension change. As the deformation builds up when the dashpot starts moving, $u$ increases, and the spring takes a greater and greater share of the load. Therefore the tension increases gradually. When the spring eventually takes all the load, the dashpot no longer moves. The tension remains constant since the deformation is kept constant. There is no tension relaxation throughout the entire process.
3.3.3.1.2. Kelvin model

The Kelvin model is composed of the combination of linear springs with spring constant ($\mu_1$ for linear spring 1, $\mu_2$ for linear spring 2) and dashpot with coefficient of viscosity $\eta$ shown in Figure 3.10 [Fung 1994]. Their displacements are $u, u_1', and u_1$.

![Diagram of Kelvin model](image)

Figure 3.10 Kelvin model (Standard linear model)

\[ u = u_1 + u_1', \quad F = F_1 + F_2, \quad F_2 = \mu_2 u, \quad F_1 = \eta \frac{du_1}{dt} = \mu_1 u_1' \]  

(3.2)

\[ \tau_{\varepsilon} = \frac{\eta}{\mu_1}, \quad \tau_{\sigma} = \frac{\eta}{\mu_2} (1 + \frac{\mu_2}{\mu_1}), \quad E_R = \mu_2 \]  

(3.3)

If the force (or tension) and the displacement (or position) are expressed as functions of time $t$, then

\[ F(t) = E_R [1 - (1 - \frac{\tau_{\sigma}}{\tau_{\varepsilon}}) e^{-\frac{t}{\tau_{\varepsilon}}}] u(t) \]  

(3.4)
Using Eqn.3.4 and the time interval $t$ from the actual data, the tension and position signals can be generated by assuming values for $E_R$, $\tau_\varepsilon$, and $\tau_\sigma$ (See Appendix 3A for the computational program Kelvin.bas). Figure 3.11 illustrates the tracing of the generated tension signal according to a Kelvin model with the best-fit parameters

$$\frac{1}{E_R} = 0.001585 \text{ mm}^2/\text{mg}, \quad \tau_\sigma = 0.77 \text{ sec}, \quad \text{and} \quad \tau_\varepsilon = 0.24 \text{ sec}. \quad \text{This tracing was then superimposed on the actual data. The model tracing agreed well with the data tracing: both increased to a peak value then decreased until a plateau was reached. This indicated that the Kelvin model hypothesis predicts a response similar to that seen in static tests.}

**Figure 3.11 Tracing of the Static Preloading**

When a sudden deformation is applied to a Kelvin body, the dashpot does not move the moment the deformation is imposed. Due to the existence of spring 1, the
deformation is applied to both spring 1 and spring 2, which is indicated by an increase in tension $F = F_1 + F_2 = (\mu_1 + \mu_2)u$. As the dashpot gradually moves, $u_1'$ decreases, thus $F_2$ decreases (Figure 3.10). Since the deformation is kept at a constant value, $u$ does not change, and $F_1$ maintains a constant value. The total tension of the Kelvin body decreases, which is seen as the noted stress-relaxation. As the dashpot eventually stops moving, $u_1' = 0$. The total load is only carried by spring 2 (the parallel spring). Since the deformation is still kept constant, there is no more change in tension. This is shown by the tension reaching a plateau.

The static tracing suggested that the Kelvin model [Fung 1994] is the best lumped model (among the simplest models) to represent these data.

### 3.3.3.2. In frequency domain

The analysis in the time domain is limited. In order to understand the tissue response to mechanical deformations, the dynamics must be revealed. That is, how does the tissue respond to each frequency component of an input signal? This is called the frequency response [Ramirez 1985]. Sine wave oscillation has most often been used for this purpose [Fung 1994]. However, the results of pulse testing were used to model the frequency response of the rabbit's airway mucosal membrane in this study.

#### 3.3.3.2.1. The Advantages of Single Pulse Testing Over Sine Wave Oscillation

The sine wave technique can give dynamic response curves at amplitudes well below the nonlinear range [Wang et al. 1997]. However, the collection of data over a full frequency range is tedious and time consuming. To obtain enough points to define the
frequency response curve adequately, it is necessary to make a large number of sinusoidal tests at specific frequencies. The equipment also has limitations to its range of oscillatory frequencies. Furthermore, with the force transducer being very sensitive, and with the extension of the sample length being as small as the amplitude of the sine wave, the noise in the tension signal could be significant enough to cause large disturbances as shown in Figure 3.6.

The pulse testing method has been used in engineering for many years [Clement, 1963, Hougen 1964]. It can get around some of the weaknesses of the sine wave technique (Table 3.2). It is fast and simple to carry out, thus many tests can be performed in a short time and averaged to reduce the errors caused by noise. Since pulse testing is advantageous and reveals the same properties as the sine wave technique, only the results of the pulse tests have been used to model the frequency response in this project. The sine wave technique is used only to justify the results of the pulse testing.

Table 3.2 Comparison Between Sine Wave Technique and Pulse Testing

<table>
<thead>
<tr>
<th>1. Equipment for data production</th>
<th>Same, but cheaper and simpler equipment can be used for the pulse testing</th>
</tr>
</thead>
<tbody>
<tr>
<td>2. Time required for data collection</td>
<td>One pulse can give data over a range of frequencies while in the sinusoidal case, measurements must be made for each frequency</td>
</tr>
<tr>
<td>3. Time required for data analysis</td>
<td>Same for one pulse and for sine wave of one frequency, hence ratio of the analysis times roughly the same as the data collection times</td>
</tr>
<tr>
<td>4. Accuracy</td>
<td>Roughly the same, but pulse data must be culled for out-liers at known frequencies</td>
</tr>
</tbody>
</table>
3.3.3.2.2. The Fourier Transformation

Fourier transformation is a mathematical tool that transfers time domain data to the frequency domain. The Laplace transform is a special case of the Fourier transform since it is only concerned with the positive times. The Laplace transform of a function \( Y(t) \) is defined as:

\[
LT(Y(t)) = \int_{0}^{\infty} e^{-s} Y(t) dt
\]  

(3.5)

where \( \omega \) is the frequency.

After the operation, the signal \( Y(t) \) is decomposed into magnitude and phase angle at various frequencies. One example of the transformed pulse data using discrete form of Eqn.3.5 is shown in Figure 3.12a and b, in which the magnitudes of the transformed position and tension signals are plotted as functions of frequency.
The position signal applied to the tissue sample was a triangular pulse function. As expected [Hougen 1964], the maximum relative magnitude occurred at a frequency of zero (Figure 3.12a). The magnitude of the position signal then decreased with frequency especially after about 10 to 15 Hz. The tissue sample was examined within this frequency range, beyond which the signal became insignificant. The range of frequency in which the magnitude is significant is related to the pulse amplitude and pulse duration [Hougen 1964]. The smaller the pulse duration, or the higher the amplitude, the larger the frequency range. However, in this work the pulse duration was limited by the equipment. If the amplitude of the pulses were increased, tissue would have been overly stretched and could have produced non-linear responses.
The Fourier transformed response (tension) is shown in Figure 3.12b. Similar to the position signal, the magnitude of the transformed tension became insignificant beyond about 10 Hz.

The transformed results indicated that, limited by the equipment, the energy of the deformation imposed on the tissue samples was mainly distributed at frequencies below 10 Hz. As a result, the frequency response of the mucosal membrane could only be revealed up to about 10 Hz, or about 63 rad/sec. When the airway mucosal membrane is subjected to the usual physiological conditions such as breathing, the frequency of deformation is close to normal breathing frequencies. On average, human beings breathe about 16 times per minute (0.26 Hz), half the speed of rabbits. However, during coughing or other unusual movements of the airway, the frequency of the deformation could be up to 500 Hz.
(personal communication, Dr. Roger Kamm, MIT). In such cases, the tissue response could be extrapolated from that at the lower frequencies, realizing the possible errors which can be caused by such extrapolation. This reinforces the importance of developing a model to explain the experimental data.

3.3.3.2.3. Fitting the Data and Model Using the Transfer Function

From a theoretical point of view, consider two quantities \( x(t) \) and \( y(t) \). Let \( x(t) \) be the independent variable or forcing function and \( y(t) \) the dependent variable or the response function. Both are functions of time \( t \) and vary with frequency \( \omega \) (radians per second). The transfer function, which is a characteristic of the system, is defined [Stephanopoulos 1984] as the ratio of the Laplace transformed difference variables or, when time starts at zero, the Fourier transformed variables, i.e.,

\[
\text{Transfer Function} = \frac{\int e^{-j\omega t} y(t) dt}{\int e^{-j\omega t} x(t) dt} = \frac{LT(y(t))}{LT(x(t))} = f(\omega) \tag{3.6}
\]

3.3.3.2.3.1. The Transfer Function of a Voigt model

If the deformation of the tissue samples is imposed on a Voigt model (as illustrated in Figure 3.8), the response will be

\[
F(t) = \mu u(t) + \eta \frac{du}{dt} \tag{3.7}
\]

After taking Laplace transforms of both sides of the Eqn. 3.7,
\[ LT(F(t)) = \mu LT(u(t)) + \eta s LT(u(t)) \]  

(3.8)

where \( s(=j\omega) \) is the Laplace operator. Therefore,

\[
\frac{LT(u(t))}{LT(F(t))} = \frac{1}{\mu + \eta s} = \frac{1}{\mu} \left(1 + \frac{\eta}{\mu}\right)s
\]

(3.9)

This is the transfer function of a first order lag system, according to process control theory [Stephanopoulos 1984].

3.3.3.2.3.2. The Transfer Function of a Kelvin model

The Kelvin model (Figure 3.10) has the response [Fung 1994]:

\[ F(t) + \left(\frac{\eta}{\mu_1}\right) \frac{dF}{dt} = \mu_2 (u(t) + \eta \left(1 + \frac{\mu_2}{\mu_1}\right) \frac{du}{dt}) \]  

(3.10)

After transformation and rearranging the equation, the response is,

\[
\frac{LT(u(t))}{LT(F(t))} = \left[1 + \left(\frac{\eta}{\mu_1}\right)s\right] \left[\frac{1}{\mu_2} \frac{\frac{1}{\mu_2} + 1}{\mu_1 + \eta \left(\frac{1}{\mu_2} + \frac{1}{\mu_1}\right)s}\right]
\]

(3.11)

where \( \tau_\epsilon = \frac{\eta}{\mu_1} \), \( \tau_\sigma = \eta \left(\frac{1}{\mu_2} + \frac{1}{\mu_1}\right) \), \( \frac{\tau_\epsilon}{\tau_\sigma} = \frac{\mu_2}{\mu_2 + \mu_1} \)

(3.12)

Eqn. 3.11 suggests that the total response can be viewed as a combination of two systems. One is a first order lead element [Stephanopoulos 1984] with a time constant of \( \tau_\epsilon \), which is denoted as the first part of the equation. This response is multiplied by, or
put in series, with the other response. The second is a first order lag element with a time
countant of $\tau_a$, similar to a Voigt model. The overall response is zero order.

A computer program (Calbode.bas) is attached in Appendix 3A which can be used
to calculate the absolute gain and phase angle for any model that is a combination of lead
and lag elements.

3.3.3.2.3.3. The Transfer Function of the Experimental Data

The algorithm for calculating the transfer function of the experimental data is

[Stephanopoulos 1984]:

$$
\text{Transfer Function} = \frac{\sum_{k=1}^{n} \text{Strain}(k\Delta t)e^{-j\omega k\Delta t}}{\sum_{k=1}^{n} \text{Stress}(k\Delta t)e^{-j\omega k\Delta t}}
$$

where $k$ is a counter going from 1 to $n$, $n$ is the number of data points in each variable

$j = \sqrt{-1}$, $\omega = \text{frequency (rad/sec)}$, and $\Delta t$ is the sampling interval (sec).

Eqn. 3.13 is solved by substituting the recorded data and using Euler's formula,

$$
e^{-j\omega k\Delta t} = \cos(\omega k \Delta t) - j \sin(\omega k \Delta t)
$$

(3.14)

to produce a ratio of two complex numbers (i.e. $(\frac{d+cj}{g+hi})$) at each frequency. The
denominator is cleared by multiplying numerator and denominator by its conjugate
$(g - hj)$ to give one complex number $(a + bj)$. Thus, finally, the amplitude ratio or absolute gain is given by

$$|G(s)| = \sqrt{a^2 + b^2}$$  \hspace{1cm} (3.15)

and the phase angle by

$$\theta = -\tan^{-1}\left(\frac{b}{a}\right)$$  \hspace{1cm} (3.16)

For the computational program (Ga96ot1 bas) see Appendix 3B.

3.3.3.2.4. The Bode Diagram

The frequency response of a system can be analyzed by calculating its transfer function. But to visualize the response, Bode diagrams are more convenient.

Bode diagrams (named in honor of H.W. Bode) [Eckman 1962] consist of a pair of plots showing how the logarithm of the amplitude ratio and the phase angle vary with the log of the frequency in rad/sec. A Bode diagram by nature allows the addition of $\theta$ values at corresponding frequencies and multiplication of the $|G(s)|$ values by addition of $\log(|G(s)|)$ [Stephanopoulos 1984]. In doing so, combinations of orders can be easily obtained. Another convenience of using the Bode diagrams is that the model parameters can be found by curve fitting these results.

However, even if the time constants (the $\tau$'s) can be directly read from the Bode diagram, the effect of each spring and dashpot element cannot be visualized. This is
because the time constants result from both spring and dashpot. It is the combination of both elements that determines the frequency response.

3.3.3.2.4.1. The Bode Diagram of Tissue Samples

Since there was no frequency response originating from the equipment itself (Figure 2.8 Chapter Two), the Bode diagram of the tissue sample can be obtained directly from the captured experimental data (Eqn. 3.15 and Eqn. 3.16). According to the Bode diagram, the steady-state gain ($G_{ss}$) can be extrapolated from the absolute gain at low frequencies. It is independent of any model. However, the shape of the curves can give some idea of the model form, and thus a rough estimation of time constant(s) according to the assumed model.

An example of the Bode diagram of a tissue sample is shown in Figure 3.13.
The absolute gain was at a constant value until a frequency of about 0.6 rad/sec when the data points started to diverge. However there were more points falling below the initial value than there were above, which indicated a decreasing trend. On the other hand, the phase angle started from zero then gradually decreased. This is an indication that the stress and strain signals had a phase shift and that the amount of shift was increasing with the frequency. After a smooth and more dramatic decrease in values at about 0.8 rad/sec, the data points also diverged, with a mean of about zero. The divergence was caused by the noise present in the obtained data. In spite of the noise, trends exist in the data points.

3.3.3.2.4.2 Fitting the Bode Diagram of Tissue Sample with the Voigt Model

According to the Voigt model, the absolute gain decreases at frequencies greater than a certain value (break point). The Voigt model was fitted in Figure 3.14 a and b to the data shown in the example (Figure 3.13). The time constant of the Voigt model was calculated by a least squares fit of the data, that is, the time constant that caused the smallest sum-of-squared-differences between the data and the model was selected. The fitting was done using the program Ga96jy4.bas attached in Appendix 3C.
The absolute gain of the Voigt model (Figure 3.14a) also starts as a constant, then after a break point, decreases with a slope of (-1). The time constant for the Voigt model is $\tau$, \( \tau = \frac{1}{\omega} \) where $\omega$ is the frequency at which the break point occurs. Although up to the break point the model agreed with the data, there appeared to be a great discrepancy at higher frequencies.
The phase angle of the same Voigt model (Figure 3.14b) starts to diverge from the data at even lower frequencies. The model phase angle starts from zero and then decreases to approach -90 degrees at high frequencies. However, the phase angle data seemed to decrease from zero and then return towards zero.

Thus, the Voigt model does not appear to represent the data, neither in the time domain nor in the frequency domain.

3.3.3.2.4.3. Fitting the Bode Diagram of Tissue Sample with the Kelvin Model

The transfer function of a Kelvin model suggests that it is a first order lag element (equivalent to that of a Voigt model) in series with a first order lead element [Stephanopoulos 1984]. The Bode diagram of a Kelvin model is therefore the multiplication of the gain and the addition of the phase angle of the two elements.
Furthermore, the lead element (with a time constant $\tau_e$) appears at higher frequencies.

The time constant of the lag element $\tau_o$, is theoretically greater than $\tau_e$ (Eqn.3.12).

For graphical comparison, the Kelvin model was fitted, using the least squares approach, to the same data as the Voigt model and the results are shown in Figure 3.15a and b. A detailed description and analysis is presented in the following section.
The results indicated that the Kelvin model agrees better with the experimental data than does the Voigt model for this particular experiment. The model gain and the phase angle followed the same trend as seen in the data. In fact, this is true for all the data tested by using computer program Vkcomp.bas (see Appendix 3C).

The transfer function (Eqn. 3.13), which led to the calculations of gain, is defined as \( \frac{\text{Strain signal}}{\text{Stress signal}} \). The Young’s modulus (\( E = \frac{\text{Stress}}{\text{Strain}} \)) is a constant for homogeneous materials at steady-state. The gain on the Bode diagram also approaches a constant as the frequency approached zero (steady-state is reached when frequency = zero). Therefore the inverse of the absolute gain at frequencies close to zero gives the Young’s modulus. Since the tissue samples were not homogeneous materials as will be discussed in the next...
chapter, the term "stiffness" was used instead of the "Young's modulus". According to the Kelvin model (Figure 3.10), at low frequencies, the displacement of spring 1 \( u_1' \) equals zero. The inverse of the absolute gain at low frequencies gives the stiffness of the spring 2, which is a constant. At much higher frequencies when the absolute gain approaches another constant, the displacement of the dashpot \( u_1 = 0 \). The inverse of the absolute gain gives the stiffness of both springs.

When fitting the data with the Kelvin model, the bigger the difference between the calculated \( \tau_\sigma \) and \( \tau_\varepsilon \) values, the less accurate is the estimation of \( \tau_\varepsilon \). This is because the lead element which contains \( \tau_\varepsilon \) appears at higher frequencies that approach the maximum frequency range available for the normal operation of the equipment.

### 3.3.5.3. Determination of the Parameters of the Kelvin Model

The pulse data was used to investigate the frequency response of the mucosal membrane. A computational program (Ga96ot2.bas, Appendix 3B) was developed for the fitting of the Kelvin model to the experimental data. This program calculates the model parameters for the five sets of data (see Section 3.2.4.2.2.2.) taken at each preload, then averages the five sets of results to obtain the values reported as mean and standard deviation (appendix 3D4 to 3D9).

The noise at high frequencies is relatively large when compared with the signal. This could be due to the fact that at higher frequencies, as shown in Figure 3.12a and b, the pulse energy was low. A filter was used to reduce the noise. As shown in Figure 3.16, identical filters were used on both signals, even though the position signal was actually
free of noise. Thus each signal would be altered equivalently to maintain the original phase relation between the two signals.

Various types of digital filters [Otnes 1972] were tested (Filter.bas Appendix 3B). The most common filter, adjacent points averaging, was chosen. The choice of the number of points was based on how well the averaged points represent the actual data. If more than 10-15 points were used to calculate the average, the shape of the averaged pulse began to differ from the shape of the actual data. A five-point-average filter was found to be effective in handling the noise and was used throughout.

It was also noticed as in Figures 3.5 and 3.16, that there was a gap in the tension tracing prior to the actual pulse. This was the time period during which the operator initiated the pulse, therefore no recordings of the tension or position were made. In order
not to affect the proper use of the filter, this pause was first recognized by the time interval between data points. The data points before the end of the pause were discarded.

Once the pulse data had been transformed and converted to a gain and a phase angle at each frequency (Eqn. 3.15 and Eqn. 3.16), the next step was to fit the chosen model to these data.

The objective function to be minimized by the choice of the best Kelvin model parameters was

\[
SS(\omega) = \sum [(100(G_K(\omega) - G_D(\omega)))^2 + (\theta_K(\omega) - \theta_D(\omega))^2]
\]  

(3.17)

where, \(G_K(\omega)\) and \(\theta_K(\omega)\) are the absolute gain and the phase angle of the Kelvin model at frequency \(\omega\) for guessed parameters; \(G_D(\omega)\) and \(\theta_D(\omega)\) are the absolute gain and the phase angle of data at frequency \(\omega\). Because of the relative magnitudes of the gain and the phase angle, a weighting factor of 100 was used on the gain term to make them of a similar magnitude.

A hill-climbing minimization routine and a pattern search [Wilde 1964] (Simplex) routine were first used to minimize this function to find the model parameters. The first method would not converge because of the noise and the second method gave poor reproducibility. It was then realized that the noisy signal could produce what appeared to be a multiple minimum problem. It was, therefore, necessary to use a total grid search, which is slower, to determine the parameters which best fit the Kelvin model.
As can be seen in Figure 3.13, both the gain and phase angle became noisier as the frequency increased. For either Voigt or Kelvin model, any increase in gain, which is supposed to be a decreasing function, can only be caused by noise. Therefore, the frequency where the first increase in gain was detected in the computational program (Ga96ot2.bas) and both gain and phase angle above that frequency were not used for minimizing the objective function. The algorithm used by the program is shown in Figure 3.17.

---

Retrieve captured data
Convert data to stress and strain
Smooth data with digital filter
Fourier transform data into frequency domain
Calculate gain and phase angle from the transfer function
Detect and discard noise from the gain and the phase angle
Minimize the objective function to obtain model parameters

---

Figure 3.17 The algorithm of the fitting of the Kelvin model

---

The model fitting part of the program (Ga96ot2.bas) was validated using noise-free Kelvin models with known parameters. The absolute gain and phase angle were generated using these known parameters (Calbode.bas, Appendix 3A), which were then
fed to the fitting program (Testken.bas, Appendix 3B) to estimate the model parameters.

A comparison between the known and the estimated parameters is in Table 3.3.

Table 3.3 Comparison of the Known and the Estimated Parameters of Kelvin Model

<table>
<thead>
<tr>
<th>Known parameters</th>
<th>Estimated parameters</th>
<th>Estimated - Known</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gss</td>
<td>τ_ε</td>
</tr>
<tr>
<td></td>
<td>Gss</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.0005</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td>0.001</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>0.003</td>
<td>0.15</td>
</tr>
<tr>
<td>4</td>
<td>0.005</td>
<td>0.20</td>
</tr>
<tr>
<td>5</td>
<td>0.007</td>
<td>0.30</td>
</tr>
<tr>
<td>Mean of the difference between the estimated and known</td>
<td>0.00</td>
<td>0.009</td>
</tr>
<tr>
<td>SD of the difference between the estimated and known</td>
<td>0.00</td>
<td>0.008</td>
</tr>
</tbody>
</table>

_Gss is the steady-state gain; the unit of τ_ε and τ_σ is second._

The comparison indicated that the average error in estimating τ_ε is ± 5.4 % and that of τ_σ is ± 1.8 %. There was no difference in the estimated and the actual Gss. The difference between the estimated and the known parameters was, therefore, insignificant.

Since the Bode diagram of the data (Figure 3.13) was noisy compared with that of the perfect Kelvin model (or the known parameter Bode diagram), the fitting program (Ga96ot2.bas) was tested to estimate the error in the prediction of the model parameters when actual data were used.

97
By trial and error, a random noise signal was added to a noiseless Kelvin model (program: Boder.bas, Appendix 3B) to simulate that seen in the actual data. Figure 3.18 shows the Bode diagram of both the simulated and the actual data.

The similarity between the actual and the simulated data was further confirmed using the bandwidth [Seborg et al. 1989] of the noisy signals and the mean error calculated by comparing each to a noiseless model. The bandwidth calculated using program Autocord.bas (Appendix 3B) of one set of data is shown in Figure 3.19 a and b as an example. The noise of the simulated data has the same bandwidth as that of the actual data.
Figure 3.19a Autocorrelation of Experimental Data
Bandwidth of Noise Shown

Figure 3.19b Autocorrelation of Simulated Data
Bandwidth of Noise Shown
The Bode diagram of the actual data and that of simulated noisy data were both compared with a noise free model by calculating the sum of squares of differences over the whole frequency range (program: Errordta.bas, Appendix 3B). The mean and the standard deviation of the sum of squares of differences for each comparison is listed in Table 3.4.

Table 3.4 Comparison of the Actual Data /Kelvin and the Simulated Data /Kelvin Model

<table>
<thead>
<tr>
<th>Group</th>
<th>Absolute gain</th>
<th>Phase angle</th>
<th>Absolute gain</th>
<th>Phase angle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>7.01 E-5</td>
<td>2.73 degree</td>
<td>2.41 E-4</td>
<td>2.41 degree</td>
</tr>
<tr>
<td>SD</td>
<td>1.44 E-5</td>
<td>0.85 degree</td>
<td>7.14 E-4</td>
<td>0.71 degree</td>
</tr>
</tbody>
</table>

*Group A: Comparing actual data to a noise-free Kelvin model

*Group B: Comparing the simulated data with the Kelvin model.*

The noise in the simulated data was slightly larger than that in the actual data.

Using simulated noisy signals, a group of five solutions over a full spectrum of $\tau_\varepsilon$ and $\tau_\sigma$ was tested. The results are shown in Table 3.5.
### Table 3.5 Comparison of Known (in simulated data) and Estimated Parameters (Kelvin)

<table>
<thead>
<tr>
<th>Known parameters</th>
<th>Estimated parameters</th>
<th>Estimated - Known</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$G_{ss}$</td>
<td>$\tau_\varepsilon$</td>
</tr>
<tr>
<td>1</td>
<td>0.0005</td>
<td>0.08</td>
</tr>
<tr>
<td>2</td>
<td>0.001</td>
<td>0.10</td>
</tr>
<tr>
<td>3</td>
<td>0.003</td>
<td>0.15</td>
</tr>
<tr>
<td>4</td>
<td>0.005</td>
<td>0.20</td>
</tr>
<tr>
<td>5</td>
<td>0.007</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Mean of the difference between the estimated and known: 0.00, 0.018, 0.070.

SD of the difference between the estimated and known: 0.00, 0.012, 0.026.

The data used in the analysis (Table 3.5) were all produced with the same random noise generation equation in each case, which was the same as that used in Figure 3.18, and had the same bandwidth and noise distribution. It was found that the average error in $\tau_\varepsilon$ was $\pm 10.8\%$ with standard deviation in error of 0.012 seconds. The average error of $\tau_\sigma$ was $\pm 6.9\%$ with standard deviation in error of 0.026 seconds.

In summary, the Kelvin model was fitted to the experimental data as a lumped model. It is a good representation of the tissue behavior within the physiological frequency range. The estimated errors in the fitted model parameters are about $\pm 11\%$ in $\tau_\varepsilon$, $\pm 7\%$ in $\tau_\sigma$, and zero in the steady-state gain $G_{ss}$.
3.3.5.4. Sine Wave Dynamic Analysis

The sine wave data at each frequency were analyzed (program Fftlu557.bas, Appendix 3C) the same way as the pulse data were (Figure 3.17). The results were not conclusive because the dynamic response was masked by non-random noise. The non-random noise was likely caused by the stepwise movement of the linear table (see Chapter Two). Thus the samples had secondary responses, corresponding to each stepper motor movement as a step input (see Figure 2.9 and Figure 3.6).

The sine wave method itself is adequate but the noise appeared to be larger than the signal, possibly due to the very small force measured by the linear transducer (all signals were near its lower limit of measurement). Larger amplitudes were not possible with sine wave generation due to the limitations of the equipment (see Chapter Two). As a result the frequency analysis of sine wave data was not used in the fitting of models.

3.3.5.5. Step Function Analysis

In effect, the static preloading of the sample can be analyzed as a step function in time to determine the dynamic parameters. In such a test the time needed to reach steady-state was very long (15 - 20 minutes). During this time and also after, there was drift in the signal due to noise and equipment changes such as temperature fluctuations. It was therefore impossible to use this supposedly simple technique for the analyses of the model parameters.
3.4. Results

The steady-state stiffness of the rabbit airway mucosal membrane was calculated from the data of both the static preloading tests and the pulse tests. The time constants $\tau_e$ and $\tau_a$ were estimated by fitting the pulse data to the Kelvin model.

3.4.1. Steady-State Stiffness of the Airway Mucosal Membrane

3.4.1.1. Results of Static Experiments

10-15 minutes after each extension in the static preloading experiments, the tension approached a plateau (Figure 3.4). The plateau tension was converted to stress. Each stress was plotted against the corresponding strain. Figure 3.20 (a and b) gives examples of stress-strain relationships. The mean of repeated tests in both longitudinal and circumferential samples gave a linear fit with regression coefficient $r \geq 0.99$. The slope of the linear fit gave the steady-state stiffness of the tissue sample under static tensile loading. Better fits were obtained from the measurements of the longitudinal samples since the force readings of the longitudinal samples were larger than the circumferential samples. The deviations from the mean in the longitudinal data were also smaller than those in the circumferential data.
Figure 3.20a Steady-State Stress and Strain (Static Experiment)

Circumferential sample: 01/29/96

- 1st test
- 2nd repeat
- 3rd repeat
- Mean of all tests
- Regression of the Mean

Linear Regression of the Mean, R=0.994, p=0.006
Slope=Stress/Strain= 359.6 mg/mm²

Figure 3.20b Steady-State Stress and Strain (Static Experiment)

Longitudinal sample: 01/30/96

- 1st test
- 2nd repeat
- 3rd repeat
- Mean of all tests
- Regression of the Mean

Linear Regression of the Mean, R=0.998, p=0.002
Slope=Stress/Strain= 2975.2 mg/mm²
The unit of the stiffness was converted from mgf/mm² to the International Unit kPa and plotted against the age of the animals in Figure 3.21 (a and b). The values are listed in Appendix 3D. In Figure 3.21a, all measurements of the circumferential and longitudinal samples were presented without distinguishing the pairs of samples from the same animal. A linear relationship between the stiffness and the age factor was not apparent ($p \geq 0.3$).

To compare the stiffness in the circumferential and that in the longitudinal directions of the airway, the mean stiffness from each direction was calculated and plotted in Figure 3.21b. The standard deviations (SD) were used as error bars to indicate the large variation in the measurements. However, a t-test showed ($\alpha \leq 0.05$) that the mean of the longitudinal samples was significantly higher than that of the circumferential samples.
3.4.1.2. Results of Dynamic Experiments (Pulse Test)

Extrapolated from the absolute gain as the frequency approaches zero, the steady-state stiffness of the tissue samples was obtained from the analysis of the pulse data (see Appendix 3D). Unlike the results from the static experiments, the steady-state stiffness was calculated at each strain value. In Figure 3.22, the large values of SD (error bar) show that there is no statistical increase of the steady-state stiffness with strain. However, there appears to be a trend of increase in stiffness with strain for the mean values.
As shown in Figures 3.22 a, b, and c, there was no significant relationship between the steady-state stiffness (obtained from pulse tests) and the age of the animals.
Figure 3.22a Steady-State Stiffness at 10% Strain (Dynamic Experiments)

Figure 3.22b Steady-State Stiffness at 20% Strain (Dynamic Experiments)
3.4.1.3. Comparison of the Results of the Static and Dynamic Experiments

As pointed out by Fung [1994], the incremental moduli (equivalent to the stiffnesses measured at different strains) are not the same as the tangent of the static tensile loading curve. Differences in the measurements of the stiffness (pulse tests and static preloading) are expected. However, since both tests were performed on the same sample, the estimated stiffness should be similar. In fact, the two values agree well. Table 3.6 compares the static results with the pulse results at all three strain values. The agreement of the magnitudes supports the use of these methods for the estimation of the stiffness of the airway mucosal membrane.
Table 3.6 Steady-State Stiffness of Airway Mucosal Membrane (All Values in kPa)

<table>
<thead>
<tr>
<th></th>
<th>N=40</th>
<th>Mean</th>
<th>SD</th>
<th>SE</th>
<th>CE</th>
<th>95% C.I.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Circumferential (Static Experiment)</td>
<td></td>
<td>6.50</td>
<td>4.00</td>
<td>0.63</td>
<td>0.62</td>
<td>(5.22, 7.78)</td>
</tr>
<tr>
<td>Longitudinal (Static Experiment)</td>
<td></td>
<td>12.83</td>
<td>6.59</td>
<td>1.04</td>
<td>0.51</td>
<td>(10.73, 14.94)</td>
</tr>
<tr>
<td>Circumferential (Pulse, 10% preload)</td>
<td></td>
<td>4.28</td>
<td>3.13</td>
<td>0.49</td>
<td>0.73</td>
<td>(3.28, 5.28)</td>
</tr>
<tr>
<td>Longitudinal (Pulse, 10% preload)</td>
<td></td>
<td>9.94</td>
<td>5.61</td>
<td>0.89</td>
<td>0.56</td>
<td>(8.14, 11.73)</td>
</tr>
<tr>
<td>Circumferential (Pulse, 20% preload)</td>
<td></td>
<td>6.27</td>
<td>4.06</td>
<td>0.64</td>
<td>0.65</td>
<td>(4.97, 7.57)</td>
</tr>
<tr>
<td>Longitudinal (Pulse, 20% preload)</td>
<td></td>
<td>12.44</td>
<td>5.80</td>
<td>0.92</td>
<td>0.47</td>
<td>(10.58, 14.29)</td>
</tr>
<tr>
<td>Circumferential (Pulse, 30% preload)</td>
<td></td>
<td>7.38</td>
<td>4.71</td>
<td>0.74</td>
<td>0.64</td>
<td>(5.87, 8.88)</td>
</tr>
<tr>
<td>Longitudinal (Pulse, 30% preload)</td>
<td></td>
<td>14.12</td>
<td>6.81</td>
<td>1.08</td>
<td>0.48</td>
<td>(11.94, 16.30)</td>
</tr>
</tbody>
</table>

\[
SE = \frac{SD}{\sqrt{N}}, \quad CE = \frac{SD}{Mean}, \quad \text{and C.I.: confidence interval.}
\]

3.4.1.4. The Elastic Constants Found in the Literature

Numerous studies have been made to measure the elastic constant, or the Young’s modulus and the point of fracture of soft biological tissues, although little has been reported on the incremental stiffness. The Young’s modulus of ovine tracheal wall in circumferential direction was reported by Codd et al. [1994] to be about 20 kPa. Table 3.7 is a list of the Young’s moduli of some materials from the literature [Fung 1994, Yamada 1970].
Table 3.7 The Young's Moduli of Several Biological materials

<table>
<thead>
<tr>
<th>Material</th>
<th>Elastin</th>
<th>Collagen (along fiber)</th>
<th>Arterial tissue</th>
<th>Skin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Young's modulus (kPa)</td>
<td>600</td>
<td>1 x 10^6</td>
<td>290</td>
<td>740</td>
</tr>
</tbody>
</table>

A difference in the longitudinal and circumferential directions of tissue from various tubular organs was also reported [Yamada 1970], although only stress-strain curves are available. The tissue samples were elongated to the point where they broke (about 120 to 300% strain) and the linear portion was usually about up to 40-60% strain. Another study reported the stress vs. elongation curve of adult human tracheal membranous wall (including the mucosal membrane and the airway smooth muscle) [Yamada 1970]. The samples from both the longitudinal and circumferential directions were elongated to 100% strain (twice as long as the initial length). Their results showed that the stress in the longitudinal sample was higher than in the circumferential one at all strain values, the longitudinal curve increased dramatically after 40% strain (achieved a stress of 0.2 x 10^5 mg/mm^2) until the sample broke at 60% strain; the circumferential curve started to increase significantly at 60% strain (stress 0.1 x 10^5 mg/mm^2) and broke at 82% strain.

Studies have shown that the pulmonary resistance and the airway responsiveness declines with maturation [Tepper et al. 1994, 1995a and b]. It was suggested that these age related differences could be due to changes in airway smooth muscle function and/or to changes in the mechanical properties of the lung parenchyma or airway wall. Our results have shown that the mucosal membrane is stiffer in the longitudinal than in the
circumferential direction of the airway; however, there is no significant statistical change with age in either direction.

Okazawa et al. [1993] have shown that there could be a significant load on the ASM during shortening provided by the lung elastic recoil and/or other elastic components within the airway wall itself. They calculated [1992] the tension generated by the excised dog ASM, at optimal length, to be about 200 g/cm², which is equivalent to 19.62 kPa. They also calculated [1993] that when a canine lung lobe was held at a $P_L$ of 10 cmH₂O (when the ASM was at a length close to the optimal length), the additional stress on the ASM applied by the lung parenchyma recoil ($\Delta P_X$) was 6.3 cmH₂O, which is equivalent to 0.62 kPa. Assuming the measurements of the same quantity from different animals are of a similar magnitude, the difference between the stress generated by the ASM and the stress applied to the ASM by the lung parenchyma could be about 19 kPa. The magnitude of the stiffness of the airway mucosal membrane (4-14 kPa), as shown in Table 3.6, indicates that the mucosal membrane is capable of providing a significant load counter balancing the stress generated by the ASM.

3.4.2. Time Constants of the Airway Mucosal Membrane

The great advantage of the pulse test over the static experiment is not only that it is possible to estimate the stiffness more precisely, but also to measure sample’s time response, thereby revealing the dynamic frequency response of the tissue sample. The time constant of a material defines the speed with which a response can occur after a change
has been made. The smaller the time constant the more rapid the change. The following
discussion is based on the use of the Kelvin model to define the time constants.

3.4.2.1. \( \tau_e \) and \( \tau_\sigma \) of the Circumferential Samples

In Figure 3.23a, b, and c are plotted the estimated time constants \( \tau_e \) and \( \tau_\sigma \) at
three strain values for animals of different ages (listed in Appendix 3D). The age has no
statistically significant effect on the time constants. As expected from the Kelvin model
(Eqn. 3.3), the estimated \( \tau_e \) was lower than the \( \tau_\sigma \).

At each strain value, there was a large scatter in \( \tau_\sigma \) values. This is most likely due
to the fact that the tension signals of the circumferential sample were close to the lower
sensitivity limit of the force transducer, therefore a large amount of noise was masking the
actual signal. The estimation of \( \tau_e \) required information at higher frequencies, which was
not sufficiently provided due to the limitations of the equipment. The estimate of \( \tau_e \)
would be expected to be less accurate than that of \( \tau_\sigma \). The smoother appearance of the \( \tau_e \)
plot with age could be caused by artifacts, such as an arbitrary cut off frequency beyond
which the signal was considered as noise during the model fitting. The minimization of the
squared differences which was used to estimate \( \tau_e \) was not sensitive enough to recognize
the effect of variations in this parameter. At best, these values are estimates.
Figure 3.23a Estimated Time Constants (Kelvin model)

Circumferential samples: 10% strain

Time constant (second)

Age (month)

Figure 3.23b Estimated Time Constants (Kelvin model)

Circumferential samples: 20% strain

Time constant (second)

Age (month)
3.4.2.2. $\tau_\varepsilon$ and $\tau_\sigma$ of the Longitudinal Samples

The $\tau_\varepsilon$ and $\tau_\sigma$ of the longitudinal samples are presented in Figures 3.24 a, b, and c and are listed in Appendix 3D. As was the case for the circumferential samples, there was no significant relationship between the age factor and the time constants. There is less scatter in $\tau_\sigma$ compared to the circumferential samples. This could be because the longitudinal samples were stiffer than the circumferential ones, therefore the tension readings were larger and hence relatively less noisy. $\tau_\varepsilon$ is closer to the magnitude of $\tau_\sigma$, which means that the frequency information required by the estimation of $\tau_\varepsilon$ was close to that of $\tau_\sigma$. Therefore the estimated $\tau_\varepsilon$ had less artifact than the $\tau_\varepsilon$ estimation of the circumferential samples.
Figure 3.24a Estimated Time Constants (Kelvin model)

Longitudinal samples: 10% strain

Time constant (second)

Age (month)

Figure 3.24b Estimated Time Constants (Kelvin model)

Longitudinal samples: 20% strain

Time constant (second)

Age (month)
3.4.2.3. \( \tau_ε \) and \( \tau_σ \) for Different Strain Values

As shown in Figure 3.25, \( \tau_σ \) decreased with strain and there appeared to be an increase in \( \tau_ε \). The change in \( \tau_σ \), which could be described by a exponential decay equation was more dramatic than that in \( \tau_ε \), which appears linear.
3.4.2.4. Kelvin Model Parameters \((\mu_1, \mu_2, \text{ and } \eta)\)

Since the steady-state gain (mentioned in Section 3.3.3.2.4.3) is equal to \(\frac{1}{\mu_2}\), in other words, the steady-state stiffnesses plotted in Figures 3.22 through 3.22c are the spring constants (or, stiffness \(\mu_2\)) of the linear spring 2 of the Kelvin model (Figure 3.10). Furthermore, Eqn. 3.12 can be solved for the Kelvin model parameters as,

\[
\begin{align*}
\mu_2 &= \text{ steady-state stiffness} \\
\mu_1 &= \mu_2 \left(\frac{\tau_\sigma}{\tau_\varepsilon} - 1\right) \\
\eta &= \mu_1 \tau_\varepsilon
\end{align*}
\]
3.4.2.4.1. $\mu_1$, Measured from the Circumferential Samples

Figures 3.26 a, b, and c show the calculated spring constant (stiffness $\mu_1$) of the linear spring 1 of the Kelvin model (Figure 3.10) as a function of the age of the tested animals at three different preloads (also listed in Appendix 3D). There is no statistically significant relationship between the stiffness of the spring and the age of the animals.

![Figure 3.26a The Stiffness of the Linear Spring 1 (Kelvin Model)](image-url)
Figure 3.26b The Stiffness of the Linear Spring 1 (Kelvin Model)

Figure 3.26c The Stiffness of the Linear Spring 1 (Kelvin Model)
Comparing the stiffness of the two springs in the Kelvin model, as shown in Figure 3.27, the calculation showed that the spring "constant" changes with strain. The spring in series with the dashpot (Figure 3.10) appears to have a decreasing stiffness (\( \mu_1 \)) while the stiffness (\( \mu_2 \)) of the spring that is parallel to the dashpot increases. It is also noticed that the stiffness \( \mu_1 \) falls more rapidly from 10% to 20% strain than it does from 20% to 30%. \( \mu_1 \) is stiffer than \( \mu_2 \) at all three strain values.

![Figure 3.27 Mean Spring Constant (Kelvin Model) (Measured from Circumferential Samples)](image)

3.4.2.4.2. \( \mu_1 \) Measured from the Longitudinal Samples

Figures 3.28 a, b, and c show the calculated spring constant (stiffness \( \mu_1 \)) of the linear spring 1 of the Kelvin model (Figure 3.10) plotted against the age of the tested animals at three different preloads (also listed in Appendix 3D). Except for a few cases,
there is less scatter in these measurements than those from the circumferential samples. However, similar to the results of the circumferential samples, there is no statistically significant relationship between the stiffness of the spring and the age of the animals.

Figure 3.28a The Stiffness of the Linear Spring 1 (Kelvin Model)

- Longitudinal samples at 10% strain
Figure 3.28b The Stiffness of the Linear Spring 1 (Kelvin Model)

Figure 3.28c The Stiffness of the Linear Spring 1 (Kelvin Model)
A comparison of the mean value of the stiffnesses of the two springs in the Kelvin model, shown in Figure 3.29, demonstrates that the spring "constants" measured from the longitudinal samples also change with strain. Spring 1, in series with the dashpot (Figure 3.10), appears to have a decreasing stiffness while the stiffness of spring 2 increases slightly. Spring 1 is stiffer than spring 2 except at 30% strain. The decrease of the stiffness of spring 1 is about the same over both strain intervals (10% to 20% and 20% to 30% strain).

Figure 3.29 Mean Spring Constant (Kelvin Model) (Measured from Longitudinal Samples)
3.4.2.4.3. The Viscosity $\eta$ Measured from the Circumferential Samples

Figures 3.30 a, b, and c show the calculated viscosity of the dashpot (Figure 3.10) plotted against the age of the animals at three strain values (see also Appendix 3D). No significant relationship between the viscosity and the age of the animals was found.

![Figure 3.30a The Viscosity of the Dashpot (Kelvin Model)](image)
Figure 3.30b The Viscosity of the Dashpot (Kelvin Model)

Figure 3.30c The Viscosity of the Dashpot (Kelvin Model)
The mean value of the viscosity at each strain is plotted in Figure 3.31. There is a decrease from 10% to 20% strain, however, the viscosity seems to increase by a small amount from 20% to 30% strain. This trend in the viscosity is similar to the trend of $\mu_1$ because, mathematically, $\eta$ is roughly proportional to $\mu_1$ when there is little change in $\tau_e$ (Figure 3.25, Eqn. 3.20).

![Figure 3.31 Mean Viscosity of the Dashpot (Kelvin Model) (Circumferential Samples)]

3.4.2.4.4. The Viscosity $\eta$ Measured from the Longitudinal Samples

Figures 3.32 a, b, and c show the calculated viscosity of the dashpot (Figure 3.10) plotted against the age of the animals at three strain values (see also Appendix 3D). No significant relationship between the viscosity and the age of the animals was found.
Figure 3.32a The Viscosity of the Dashpot (Kelvin Model)

Figure 3.32b The Viscosity of the Dashpot (Kelvin Model)
The mean value of the viscosity at each strain is plotted in Figure 3.33. The viscosity decreases with strain. This trend in the viscosity is also similar to the trend of $\mu_1$. 
During the elongation of the mucosal membrane, if the samples behave as a Kelvin model, an increase in $\mu_2$ (Figure 3.22), a decrease in $\mu_1$ (Figure 3.27 and Figure 3.29) and a decrease in $\eta$ (Figure 3.31 and Figure 3.33) may occur, which cause changes in the time constants $\tau_e$ and $\tau_\sigma$.

### 3.4.3. Storage and Loss Moduli

The storage modulus and the loss modulus are referred to in some of the literature to describe the mechanical properties of materials. They are defined as the real part and the imaginary part of the complex modulus $G$ [Vincent 1982, Ferry 1967, Naimark 1992], which is equivalent to the “$a$” and “$b$” in Eqn. 3.15. Instead of reporting the values of “$a$” and “$b$”, the absolute gain and the phase angle were calculated from them in this study.
The storage and loss moduli can be calculated at each frequency using the values of the absolute gain (|\(G(s)\)|) and the phase angle (\(\theta\)) (see Eqn. 3.15 and Eqn. 3.16).

\[
\text{Storage modulus (} E_s \text{)} = \frac{1}{|G(s)|} \cdot \cos \theta \tag{3.21}
\]

\[
\text{Loss modulus (} E_L \text{)} = \frac{1}{|G(s)|} \cdot \sin \theta \tag{3.22}
\]

\(E_s\) is the elastic energy stored during the loading cycle and recovered when the load is removed. \(E_L\) is the energy dissipated during loading and unloading. This is represented by the stress vs. strain hysteresis loop which can only occur when the phase angle is not zero [Hayden 1965]. Thus with the airway mucosal membrane there appears to be no energy lost in vibrating the tissue at low or high frequencies. The range of frequencies over which this occurs is shown by the phase angle (see Figure 3.15b).

There is some confusion in the use of these terms since some authors have used other definitions for these quantities. For example, Mijailovich et al. [1993] have defined "hysteresivity" as the "loss tangent modulus". Therefore care must be taken in the interpretation of such data.
Chapter Four

Morphometrical Measurements

4.1. Introduction

The results of the physiological experiments (Chapter Three) showed that the rabbit mucosal membrane is stiffer in the longitudinal than in the circumferential direction. To explain this difference in the mechanical properties, the structure of the mucosal membrane in both directions was studied. The connective tissue matrix of the mucosal membrane, like that of other tissues, contains collagen fibers, elastic fibers, and ground substances such as proteoglycans [Hukins 1984]. All components may contribute to the mechanical properties of the tissue, however, in the morphometrical measurements it was found that the elastic fibers were mainly oriented in the longitudinal direction while the collagen fibers were in a meshwork. Since the mechanical properties of the samples were found to be different in the longitudinal and circumferential directions, only the morphometrical observations of the elastic fibers are dealt with in this project.

In this chapter the development of a method to quantify the volume fraction and the orientation of the elastic fibers in the tissue samples which have been physically tested will be described. The correlation between the mechanical properties and the morphometrical findings will be discussed in the next chapter.
4.2. Histological Processing for Morphometrical Measurement

4.2.1. Section Preparations

After the physiological experiments, the tissue samples were fixed in 10% formalin. Following the conventional procedures [Gordon et al. 1977], the fixed tissue samples were embedded in paraffin. The cross-sections (the planes that were in the circumferential and longitudinal directions of the airway) of 3-5 μm thickness were obtained. In order to reveal the morphology of the entire sample, the sections were obtained randomly throughout the length of the tissue sample. Furthermore, sections in the circumferential direction of the airway were obtained from the longitudinal samples and the sections in the longitudinal direction of the airway were obtained from the circumferential samples as shown in Figure 4.1.

![Diagram of sections for morphometrical measurements](image)

Figure 4.1 Sections for morphometrical measurements

133
4.2.2. Staining

Six sections from each sample were randomly chosen for staining. Following the classical procedures [Reid 1994], two were with Verhoeff elastic staining, which causes the elastic fibers to appear dark purple or black; two were with Van Gieson's staining, which colored the collagen fibers pink; one was with combined Verhoeff and Van Gieson staining; and the last with TBO stain for tissue cross sectional area measurement (see Chapter Three).

Some additional sections were also obtained and stained with standard HE staining for use with a confocal microscopy.

4.3. Morphometrical Equipment Setup

The confocal images were made by Mr. M. Weis, Department of Botany, University of British Columbia, using a Bio-Rad MRC-600 laser scanning confocal imaging system. The essential feature of a confocal microscope is that the illumination and detection are confined to the same spot in the specimen at any one time and that out-of-focus regions of the sample do not contribute to the image. Light (laser) from an aperture was reflected into the rear of the objective lens and was focused on the specimen. Light returning from the specimen, as a result of fluorescence, passed back through the lens and was focused on a second aperture. By scanning the light beam over a fixed specimen, a complete optical image of the specimen was produced.
The morphometrical equipment used for measuring the elastic fiber orientation was a BioQuant system, the same as that used to measure the cross-sectional area of tissue samples (see Chapter Three). The unit of measurement was millimeters and the magnification of the microscope eye piece was chosen as × 100 (oil) to display elastic fibers with the highest resolution available.

The equipment used for measuring the relative volume of elastic fibers has a setup similar to the BioQuant, except that instead of using the side eyepiece to project the image tracing on to the computer, a grid displayed on the computer screen was reflected to the microscopic view through a side eyepiece. The grid was generated using the software Gridder (Pulmonary Research Laboratory, UBC. St. Paul's Hospital) which facilitated the selection of the grid type, shape, and density.

4.4. Measuring the Elastic Fiber Orientation

The elastic fibers in the mucosal membrane are stained black or dark purple as shown in the photographs (Figure 4.5 b and c). The elastic fiber orientation is a statistical measure of the percentages of elastic fibers in the longitudinal and circumferential directions of the airway. This estimation was based on measurements of single fiber bundles (Figure 4.2). Since electron microscopy shows that the elastin core consists of fibers of 3-4 nm diameter and the microfibrils are of diameter 10-12 nm [Hukins 1984], single elastic fiber is not easily visible under a light microscope. It was assumed that the elastic fibers appear as bundles. Each elastic fiber bundle consists of several elastic fibers.
The Verhoeff stained sections from each sample were randomly chosen to be measured. To ensure unbiased measurements of samples from both circumferential and longitudinal directions, the description of all selected sections were covered and marked only with numbers. The selection of fibers for measurement on each section was systematically random, that is, the first bundle was randomly chosen, then every fifth fiber bundle horizontally across the microscopic view. Fifty fiber bundles were measured from each microscopic section. Four variables \((a_x, b_y, \ell, \delta)\) were measured from each fiber bundle.

Since the epithelial cells form a single layer on each section, it was used as an orientation reference. An x-y coordinate system was used, with the x axis parallel to the epithelial cell layer, to measure the fiber bundle orientation relative to the epithelial cell layer (Figure 4.2). The measured \(a_x\) and \(b_y\) are the maximum distances occupied by the fiber bundle in the x and y directions.
The length \( t \) of a selected fiber bundle was measured by tracing the longest distance across the bundle along its long axis. It was assumed that the thickness of each fiber bundle is constant throughout the length of the bundle. Two reference points were chosen on each fiber bundle such that the line connecting these two points was perpendicular to the long axis of the fiber bundle through the center of this line (Figure 4.2). The thickness \( \delta \) was measured as the distance between the two reference points.

**4.5. Measuring the Relative Volume of the Elastic Fibers**

The relative volume of the elastic fibers is the percentage of the tissue sample volume occupied by the elastic fibers. The magnification of the eye-piece of the microscope was \( \times 20 \) (limited by the size of the tissue sample). A cross-shaped grid was
chosen. The grid was reflected onto the microscopic view without distortion by adjusting the mirror attached to the side eye-piece. This was confirmed by the equal distance between grid points measured by a micrometer. The density of the grid was 775 points per field. This setting, together with the number of fields per section, produced a coefficient of error (CE) for target point counting [Gunderson et al. 1987] to be equal to or less than 10%, which was considered adequate. The higher the number of sections, the number of fields per section, and the grid density, the lower the CE and the more time consuming is the process.

The same sections used for estimating the elastic fiber orientations were measured for the volume fraction of the elastic fibers. Each section was divided into five fields. Each field was of rectangular shape including the complete thickness of the sample: from the epithelial cells to the outermost layer of the submucosa. The long axis of the field was perpendicular to the long axis of the cross section of the tissue sample. Following the conventional point counting method [Weibel 1979, Aherne 1982], the target points and the total points were counted. The grid points that hit the elastic fiber bundles with their centers are called the target points. The grid points that hit the tissue sample with their centers are called the total points.
4.6. Analysis

Unlike the analysis of the point counting result, there is no conventional protocol for estimating the elastic fiber orientation. The analysis presented here is a novel method based on theories of geometry and probability.

4.6.1. Elastic Fiber Orientation

All four variables measured from single fiber bundles were used to classify the elastic fibers according to their shape and relative orientation to the epithelial cell layer. The latter served as an indicator of the orientation of the tissue sample in the trachea. On the sections from the circumferential samples (Figure 4.1), the epithelial cell layer is along the longitudinal direction of the airway. The epithelial cell layer runs along the circumferential direction of the airway on the sections obtained from the longitudinal samples. Combining the types of elastic fibers thus classified from both circumferential and longitudinal samples of the same trachea, the percentage of each type gave an estimation of the elastic fiber orientation for the particular airway.
4.6.1.1. **Classification of the Elastic Fiber Bundles of Each Section**

As shown in Figure 4.3, there are four types of fiber bundles in circumferential (type L, T, D, A) and longitudinal (type C, T, D, A) samples. The definitions are:

L: straight fibers that are along the longitudinal direction of the airway.

C: straight fibers that are along the circumferential direction of the airway.

T: wavy fibers (have components in both longitudinal and circumferential directions of the airway).

A: fibers across the thickness of the mucosal membrane.

D: fibers which appear to be a dot, have to be further classified to contribute to the numbers in the L, C, or T classes.

**Figure 4.3 Classification of the elastic fiber bundles**

- **type D**  
  (length / diameter) <= 2

- **type T**  
  0.5 <= (a_x / b_y) <= 2

- **type C or L**  
  (a_x / b_y) > 2

- **type A**  
  (a_x / b_y) < 0.5
The parameters used as criteria for identifying each type of fibers (Figure 4.3) were set to allow for normal variations in the fiber dimensions. Slight alterations of the values of these parameters had no significant effect on the results of the classification.

4.6.1.2. Re-Classification of the Type D Fiber Bundles

As shown in Figure 4.4, the type D fibers (dots) of a circumferential sample correspond to type C or type T fibers in the longitudinal sample of the same airway. Similarly, the type D fibers of a longitudinal sample correspond to type L or type T fibers in the circumferential sample. Thus the number of type D fibers was split (statistically) into the number of longitudinally or circumferentially distributed fibers.

Figure 4.4 Estimation of the elastic fiber orientation

First, the ratio of type C and T fibers measured from the longitudinal sample of the particular trachea was calculated:
\[ \% \ C_{\text{longitudinal}} (C \& T) = \frac{\text{number of type } C}{\text{number of type } C + \text{number of type } T} \] (4.1)

\[ \% \ T_{\text{longitudinal}} (C \& T) = \frac{\text{number of type } T}{\text{number of type } C + \text{number of type } T} \] (4.2)

Similarly, the ratio of type L and T fibers in the circumferential sample of the same trachea was calculated:

\[ \% \ L_{\text{circumferential}} (L \& T) = \frac{\text{number of type } L}{\text{number of type } L + \text{number of type } T} \] (4.3)

\[ \% \ T_{\text{circumferential}} (L \& T) = \frac{\text{number of type } T}{\text{number of type } L + \text{number of type } T} \] (4.4)

According to the ratios calculated above, the number of type D fibers in a longitudinal sample \( D_{\text{longitudinal}} \) was re-classified into type L \( L_{\text{add}} \) and type T \( T_{\text{add-long}} \) fibers, using

\[ L_{\text{add}} = \% \ L_{\text{circumferential}} (L \& T) \times D_{\text{longitudinal}} \] (4.5)

\[ T_{\text{add-long}} = \% \ T_{\text{circumferential}} (L \& T) \times D_{\text{longitudinal}} \] (4.6)

Similarly, the number of type D fibers in the circumferential sample \( D_{\text{circumferential}} \) of the same airway was re-classified into type C \( C_{\text{add}} \) and type T \( T_{\text{add-circ}} \), using

\[ C_{\text{add}} = \% \ C_{\text{longitudinal}} (C \& T) \times D_{\text{circumferential}} \] (4.7)

\[ T_{\text{add-circ}} = \% \ T_{\text{longitudinal}} (C \& T) \times D_{\text{circumferential}} \] (4.8)
For example, if there are 20 type D, 5 type C, and 12 type T fiber bundles found in a longitudinal sample, and 15 type D, 25 type L, and 8 type T fiber bundles found in the circumferential sample of the same airway, then

\[ L_{add} = 20 \times \left( \frac{25}{25+8} \right) = 15.1 \approx 15 \quad \text{and} \quad T_{add-long} = 20 \times \left( \frac{8}{25+8} \right) = 4.8 \approx 5; \]

\[ C_{add} = 15 \times \left( \frac{5}{5+12} \right) = 4.4 \approx 4 \quad \text{and} \quad T_{add-circ} = 15 \times \left( \frac{12}{5+12} \right) = 10.5 \approx 11. \]

4.6.1.3. The Orientation of the Elastic Fibers in the Airway

After re-classification of type D fibers, the measurements from both the circumferential and longitudinal samples are linked. Adding up all types of fibers from the pair of samples (longitudinal and circumferential from the same airway), gives

\[ L_f = L_f^* + L_{add} \quad (4.9) \]

where \( L_f^* \) is the number of type L fibers, estimated from circumferential sections,

\[ C_f = C_f^* + C_{add} \quad (4.10) \]

where \( C_f^* \) is the number of type C fibers, estimated from longitudinal sections, and

\[ A_f = A_{longitudinal} + A_{circumferential} \quad (4.11) \]

\[ T_f = T_{longitudinal} + T_{circumferential} + T_{add-long} + T_{add-circ} \quad (4.12) \]

\[ Total = L_f + C_f + T_f + A_f \quad (4.13) \]
\[
L_{\text{percent}} = \frac{L_f}{Total} = \text{fraction of elastic fibers in longitudinal direction} \quad (4.14)
\]

\[
C_{\text{percent}} = \frac{C_f}{Total} = \text{fraction of elastic fibers in circumferential direction} \quad (4.15)
\]

\[L_{\text{percent}} \text{ and } C_{\text{percent}} \text{ represent a statistical measure of the fractions of the total fibers that are distributed in the longitudinal and circumferential directions of the airway respectively. Type T fibers may have a distribution in both the circumferential and longitudinal directions of the airway depending on the ratio of "a\_x" to "b\_y". Type A fibers are running along the thickness of the airway, that is, from the airway lumen to the boundary of the mucosal membrane and the trachea. Type A fibers distribute in neither circumferential nor longitudinal direction of the airway. The computational program Morpho1.FOR (Appendix 4A) was used to analyze the measured \( \ell, \delta, a_x, \) and \( b_y \) values of each selected fiber bundle in order to determine the fiber orientation. The program calculates the numbers of type L, C, T, and A fibers from the longitudinal and circumferential sections of the same airway. Since the same total number (50) of fibers was used from each section, adding the number of L, C, T, and A fibers from each section gives directly the average percentage of these values, that is, the \( L_{\text{percent}}, C_{\text{percent}}, T_{\text{percent}}, \) and \( A_{\text{percent}} \) of each measured airway.}

**4.6.2. The Relative Volume of the Elastic Fibers**

Summing the target points and the total points throughout the five fields on each section gives the number of total target points and the number of overall total points. The
ratio of the two gives the relative area fraction \((A_v)\) of the elastic fibers of the section. The thickness of the microscopic section is larger than the average diameter of the fiber bundles, this could have caused an artifact in the result. That is, the elastic fibers from different depths were projected onto one surface to be measured under the microscope, thus increasing the area fraction. Therefore, the area fraction should be corrected to give the volume fraction, a less biased parameter, to describe the abundance of the elastic fibers in the tissue sample.

It was assumed that the section was of even thickness everywhere and hence, that the area fraction was proportional to the value of the relative volume fraction of the elastic fibers [Aherne 1982, Weibel 1979]. Assuming that the systematic error due to section compression during histological processing is negligible, the volume fraction of the elastic fibers was calculated [Weibel 1979] as,

\[
V_v = A_v \times K_t
\]

(4.16)

where \(K_t\) is the correction factor.

The elastic fiber bundles were treated as long tubules of thickness \(\delta\) and length \(\ell\) (measurements described in the previous section). The microscopic sections are of thickness \(tt\). The correction factor \(K_t\) can be calculated [Weibel 1979] as

\[
K_t = \frac{\lambda}{\lambda + gg(\lambda + 0.5)}
\]

(4.17)

where the shape factor \(\lambda = \frac{\ell}{\delta}\) and \(gg = \frac{tt}{\delta}\).
By substituting the average of the measured $\ell$, $\delta$ (described in Section 4.4, Figure 4.2), and $\mu$ values, the correction factor $K_t$ was calculated. However, the exact value of the average $\ell$ was not found. This is because the fiber bundles do not distribute from one end of the sample to the other, instead, they appear to be short segments with various shapes depending on how the section was cut. The average $\ell$ measured from type C, L, and A fibers ranges between 8 and 10 $\mu$m. With $\bar{\delta} = 1.6$ $\mu$m and $\mu = 5$ $\mu$m, the correction factor $K_t$ is found to be between 0.225 and 0.229.

4.7. Results

Inspection of both the 2-D (Figure 4.5a) and the 3-D (Figure 4.6) images suggests that there is no specific orientation of the collagen fibers in the rabbit tracheal mucosal membrane. The structure is rather a meshy, interwoven all through the length of the trachea. Therefore the orientation of the collagen fibers was not quantitatively measured. The collagen fibers not only display a mesh organization, but were also very densely packed. Because of its isotropic distribution in the tissue, and also because the Van Gieson staining gives a pink color that sometimes, is difficult to be distinguished from the tissue background, the relative volume of the collagen fibers was also not measured. However, on the other hand, the Verhoeff staining gives a good contrast between the dark purple elastic fiber and the off-white tissue background. The 2-D images (Figure 4.5 b and c) suggest strongly that the elastic fibers are oriented along the longitudinal direction of the airway. Since it was found that the longitudinal samples were stiffer than the
circumferential samples, the morphological study was concentrated on a quantification of the elastic fiber distribution. The collagen mesh will no doubt contribute to the strength of the tissue equally in both directions and may also affect its dynamic behavior.
Photograph taken by author

Figure 4.5a 2-D images of the collagen fibers, under a light microscope, oil lens × 2000.
Figure 4.5 b 2-D images of the elastic fibers with the vertical axis in the longitudinal direction of an airway. Photograph taken under a light microscope, oil lens × 2000.
Figure 4.5c 2-D images of the elastic fibers (view of the cross section of an airway), under a light microscope, oil lens × 2000.
Figure 4.6 3-D images of the collagen fibers (Photograph taken by Mr. Michael Weis, Department of Botany, UBC, using a confocal microscope.)
4.7.1. The Elastic Fiber Orientation

4.7.1.1. Measurements of Single Fibers

Under the light microscope, it was seen that the elastic fiber bundles, embedded in the connective tissue matrix, were mainly distributed just beneath the epithelial cell layer. Although a majority of the fiber bundles had specific orientations, differently shaped fiber bundles were also present. This is partly due to the direction in which the microscopic sections were obtained relative to the orientation of the fiber bundles. For example, if a fiber bundle was oriented along the longitudinal direction of the airway, it would appear to be a line on a section cut parallel to the longitudinal direction of the airway and a dot on a section parallel to the circumferential direction of the airway. Fibers obliquely oriented along the airway may appear to be of oval or wavy shape. Therefore the measured length ($\ell$) may not represent the true length of the fiber bundle unless the section was cut from a direction perfectly parallel to the fiber. The length ($\ell$) only gives a dimension of the fiber bundle on a particular section to facilitate the estimation of the fiber orientation. On the other hand, however, it is reasonable to assume that the measured thickness ($\delta$) is close to the actual thickness, or diameter, of the fiber bundles. This is because $\delta$ is expected to be less affected by the relative direction of the fiber and microscopic sections. As shown in Figure 4.7 (also listed in Appendix 4B), the thicknesses of the measured fiber bundles were between 1.2 and 2 $\mu$m, with an average of about 1.6 $\mu$m. No significant relationship with the age of the tested animals was found.
4.7.1.2. Elastic Fiber Orientation

The estimated orientation of the elastic fiber bundles is expressed in $L_{\text{percent}}$, $C_{\text{percent}}$, $T_{\text{percent}}$, and $A_{\text{percent}}$ as shown in Figure 4.8a and b. Data are also listed in Appendix 4B. The results show that about 57\% of the elastic fibers were oriented in the longitudinal direction and about 8\% oriented in the circumferential direction of the airway. The fiber bundles that were oriented across the thickness of the mucosal membrane (type A) account for only 2\% while there were 33\% of the total fiber bundles having a twisted or wavy shape (type T), partly in the circumferential and partly in the longitudinal direction of the airway. No significant relationship between the fiber orientation and the age of the tested animals was found.

Figure 4.7 The Average Fiber Bundle Thickness
Figure 4.8a Elastic Fiber Orientation in the Airway

Figure 4.8b Elastic Fiber Orientation in the Airway (expressed as Mean and SD)
4.7.2. The Relative Volume of the Elastic Fibers

4.7.2.1. The Area Fraction of the Elastic Fibers ($A_v$)

The fraction of the cross-sectional area of samples covered by the elastic fibers was obtained by dividing the number of hit points by the number of total points (listed in Appendix 4B). The calculated $A_v$ values of each sample are shown in Figure 4.9a and b (also Appendix 4B).

Figure 4.9a Area Fraction of the Elastic Fiber Bundles
The measured area fraction from both circumferential and longitudinal samples agreed well. The result showed that about 22% of the cross-sectional area of the tested samples were seen covered by the elastic fiber bundles.

4.7.2.2. The Volume Fraction ($V_v$)

After correction by using factor $K_r$, the area fraction was converted to volume fraction. As shown in Figure 4.10a and b (also Appendix 4B), the volume fraction, calculated from both the circumferential and longitudinal directions, was about 5%. That is, 5% of the tissue samples was occupied by elastic fibers. There was no significant relationship found between the volume fraction and the age of the animals.
Figure 4.10a: Volume Fraction of the Elastic Fiber Bundles

Figure 4.10b: Volume Fraction of the Elastic Fiber Bundles (expressed as Mean and SD)
In Table 3.7 the Young's modulus for elastin was given as 600 kPa. On average the mucosal membrane was found to have a stiffness between 8 and 16 kPa in the longitudinal direction. Since the volume of the elastic fibers is 5% of the mucosal membrane and since 57% of the fibers are in the longitudinal direction, an apparent Young's modulus of the mucosal membrane, assuming it is purely elastic fibers, may be estimated to be between 280 and 640 kPa. In spite of the large variations, it would appear from this approximation that the tissue stiffness is mainly due to the elastic fibers.
Chapter Five

Correlations of Experimental and Morphometrical Results

5.1. Introduction

Structural characteristics may be used to explain the physical response of the tissue samples being tested. The goal of this chapter is to explore possible correlations between the morphometrical findings and the measured mechanical properties. It was found that the longitudinal samples were stiffer than the circumferential samples. However, this could not have been caused by the collagen fibers because under confocal microscopy, the collagen fibers displayed a random mesh. Hence their contribution to the elastic response longitudinally and circumferentially should be roughly equal. Therefore, the variables under consideration are the morphometrical measurements for the elastic fibers (Chapter Four) and the calculated elastic elements $\mu_2, \mu_1$, the viscous element $\eta$ along with the time constants $\tau_e$ and $\tau_a$ (Chapter Three) obtained by fitting the Kelvin model to the experimental data. Since this is a lumped model, these elastic elements can not be equated directly to any parts of the actual tissue.
5.2. General Considerations of Tests of Correlation

Since at 30% strain the magnitude of the tension is higher than it is at the 20% and 10% strain levels, these readings are farther from the sensitivity limit of the force transducer, and the noise is, hence, considered to be less significant relative to the signal. Therefore, more accurate information can be expected from tissue samples under 30% strain. The relevant correlation variable pairs used for the 30% data will be repeated with data under 20% and 10% strain to confirm the findings.

5.2.1. Linear Correlation and the Goodness of Fit

The mechanical properties of the mucosal membrane were plotted against the morphometrical observations. No other correlation, except for possible linear relationships, was suggested by the trend of the data points. A statistical analysis program (Microcal Origin Version 3.5) was used to perform linear regression. The significance of the linear relationship was indicated by the probability level $p$, indicating the chance that a linear correlation does not exist. The correlation coefficient $r$ indicates how closely the linear regression fits the data [Volk 1958]. The correlation coefficient is defined as

$$ r = \sqrt{1 - \frac{\sum_{i=1}^{n} (y_i - \bar{y}_{\text{fitted}})^2}{\sum_{i=1}^{n} (y_i - \bar{y}_i)^2}} $$

(5.1)

where $n$ is the number of data points.
In the following tables, a negative $r$ value is used to indicate a negative slope in the correlation.

The $r$ value was tabulated [Volk 1958] to correspond to various probability levels and degrees of freedom ($D.F. = n - 2$) based on the hypothesis that there is no correlation between the two variables involved. For example, the tabulated value of $r$ for 38 degrees of freedom at the 0.1 probability level is 0.260. This means that there is only a 0.1 chance of getting an $r$ value of 0.260 or larger when there is no correlation between the variables. If in calculating the correlation coefficient from 40 observations a value greater than 0.260 is obtained, then the hypothesis that there is no correlation may be rejected with only 0.1 chance of being wrong. In this study, the existence of a significant correlation was determined by a $p$ value $\leq 0.05$ [Hogg et al. 1987].

### 5.2.2. Selection of the Morphometrical Parameters

The morphometrical measurements include the volume fraction ($V_v$) of the elastic fibers measured from both circumferential ($V_{circ}$) and longitudinal samples ($V_{long}$) and the elastic fiber orientation $L_{percent}$, $C_{percent}$, and $T_{percent}$. Backward stepwise regression between these measurements and the mechanical parameters $\mu_2$, $\mu_1$, and $\eta$ was carried out to rate the importance of the morphometrical parameters to each mechanical variable. For example, for the $\mu_2$ value of the circumferential samples, the most significant morphometrical parameter was found to be $V_{circ}$, followed by $C_{percent}$. However, the stepwise regression output does not supply a measure of goodness of fit. Starting with the most significant parameters predicted by this method, linear regressions were made on all
the morphometrical parameters to determine whether a significant correlation exists. In the preliminary tests, no statistically significant correlation was found between $\mu_2$ and $C_{\text{percent}}$. The product ($V_{\text{circ}} \times C_{\text{percent}}$), however, was significantly correlated with $\mu_2$.

Similarly as shown in Table 5.2, other combinations such as ($V_{\text{long}} \times L_{\text{percent}}$), ($\frac{T_{\text{percent}}}{L_{\text{percent}}}$), and ($\frac{T_{\text{percent}}}{C_{\text{percent}}}$) were also tested.

Additional variables could also be calculated. For example, if it is assumed that single fiber bundles are cylinders with identical diameter and length, then they will have the same volume. The total volume of elastic fibers in the tissue sample ($TotV$), the total number of elastic fibers ($TotN$), the total number of fibers in the longitudinal direction ($TotL$) and in the circumferential direction of the airway ($TotC$), and the total number of the wavy fibers (type T) ($TotT$) can be calculated as follows (results are listed in Appendix 5A).

\[
\text{Sample volume} = \text{cross sectional area} \times \text{initial tissue sample length}
\]  
\[
TotV = \text{Sample volume} \times V_v
\]  
\[
\text{Single fiber volume} = \overline{\ell} \times \pi \times \left(\frac{\overline{\delta}}{2}\right)^2, \overline{\ell} = 9 \mu m, \overline{\delta} = 1.6 \mu m
\]  
\[
TotN = \frac{TotV}{\text{Single fiber volume}}
\]  
\[
TotL = TotN \times L_{\text{percent}}
\]  
\[
TotC = TotN \times C_{\text{percent}}
\]
However, when correlating the $\mu_2$, $\tau_2$, and $\tau_\phi$ data (measured from longitudinal samples at 30% strain) to the above calculated variables, no significant correlation was found.

This lack of correlation is not due to the difference in the magnitudes of the $x$ and $y$ variables. Although the $x$ variables are of the magnitude of $10^6$ while the $y$ variables are less than 20, scaling down the $x$ variables did not change the $p$ or $r$ values. Since these additionally calculated variables failed to be linked to the mechanical properties of the tissue, they were not selected in the systematic testing for correlation.

5.3. Tests of Correlations of the Experimental Data at 30% Strain

As discussed in the previous chapter, since the elastic fibers could make a significant contribution to the measured steady-state stiffness of the mucosal membrane, it is possible to arrive at the following hypotheses:

1. the steady-state stiffness ($\mu_2$) is proportional to the volume fraction of the elastic fibers,

2. $\mu_2$ of the membrane in the longitudinal (or circumferential) direction is proportional to the volume fraction of elastic fibers in the same direction of the airway,
3. the viscosity of the dashpot and the time constants (Kelvin model) are related to the fraction of type L and type T fibers.

To test these hypotheses, the measured mechanical properties were correlated to the corresponding morphometrical parameters.

5.3.1. Data from Longitudinal Samples

Data of the longitudinal samples at 30% strain were examined first. The regression analysis showed that the stiffness of spring 2 \( (\mu_2) \) (Figure 3.10) is related to the volume fraction of elastic fibers. Judging from the \( p \) value, the product of volume fraction and \( L_{\text{percent}} \) has a more significant effect on \( \mu_2 \), which can be seen from Figures 5.1 and 5.2. It is worth mentioning that \( \mu_2 \) is the steady-state stiffness of the tissue sample, hence is not limited by the assumption of a model such as the Kelvin model. The observed trend with the volume fraction of the elastic fiber indicates that with more total elastic fibers or, with more elastic fibers in the longitudinal direction of the airway, the mucosal membrane will exhibit a greater steady-state stiffness.
Figure 5.1 Correlation between Stiffness $\mu_2$ and Volume Fraction (Longitudinal Samples, at 30% Strain)

- Steady-state stiffness $\mu_2$ (kPa)
- Volume fraction of elastic fibers

Figure 5.2 Correlation between Stiffness $\mu_2$ and $(V_{\text{long}} \times L_{\text{percent}})$ (Longitudinal Samples, at 30% Strain)

- Steady-state stiffness $\mu_2$ (kPa)
- $V_{\text{long}} \times L_{\text{percent}}$
The dynamic parameters $\eta$, $\tau$, and $\tau_\sigma$ define the time dependency of change, hence the magnitude of the phase shift in dynamic tests and the rate of stress relaxation in static preloading. As shown in Figures 5.3, 5.4, and 5.5, both volume fraction and the orientation of elastic fibers affect the viscosity $\eta$.

Figure 5.3 Correlation between Viscosity $\eta$ and Volume Fraction (Longitudinal Samples, at 30% Strain)

Viscosity $\eta$ as a term may be misleading. It is the viscous element term in the force equation (Eqn. 3.2) or, more specifically, $F_{\text{viscous}} = \eta \frac{du}{dt}$ (see Figure 3.10). From Figures 5.3 and 5.5 it is seen that the larger the volume fraction and the product of volume fraction times $L_{\text{percent}}$, the larger the viscosity. This would indicate that there is a possible frictional element involved between the elastic fibers and possibly, some other tissue components (for example collagen and ground substance) in the mucosal membrane.
The fact that the viscosity also correlates to the fraction of elastic fibers in the longitudinal direction ($L_{\text{percent}}$) indicates that the frictional element among parallel fibers plays an even more important role in affecting viscosity. Therefore,

**Figure 5.4 Correlation between Viscosity $\eta$ and $L_{\text{percent}}$**
(Longitudinal Samples, at 30% Strain)

![Graph showing correlation between viscosity and $L_{\text{percent}}$](image)

$R = 0.349$

$P = 0.027$

$1 - P = 0.973$

combining the effect of the volume fraction of the elastic fiber and $L_{\text{percent}}$ caused a significant decrease in the $p$ value.
Figure 5.5 Correlation between Viscosity $\eta$ and $(V_{\text{long}} \times L_{\text{percent}})$
(Longitudinal Samples, at 30% Strain)

Another type of fiber orientation which correlates with the viscosity $\eta$ is the type T, or cross directional curly fibers. Both $T_{\text{percent}}$ (Figure 5.6) and the ratio of $T_{\text{percent}}$ to $L_{\text{percent}}$ (Figure 5.7) show linear correlations with the viscosity $\eta$. 
Combining the effect of $T_{\text{percent}}$ and $L_{\text{percent}}$ enhanced the decrease of viscosity $\eta$.

This is to be expected since $\eta$ has a positive slope with respect to $L_{\text{percent}}$ and a negative slope with respect to $T_{\text{percent}}$. 
The fact that $T_{\text{percent}}$ appears to affect viscosity suggests that the wavy fibers may be acting as binding coils of fibers which may impede the movement of the straight fibers, in this case, fibers in the longitudinal direction ($L_{\text{percent}}$).

The time constants $\tau_{\varepsilon}$ and $\tau_{\sigma}$ are overall dynamic parameters. As a reminder,

$$
\tau_{\varepsilon} = \frac{\eta}{\mu_1}, \quad \tau_{\sigma} = \frac{\eta}{\mu_2} \left(1 + \frac{\mu_2}{\mu_1}\right),
$$
as shown in Eqn. 3.3. They are related to both the stiffness and the viscous element of the tissue samples. They seem to be affected significantly by the elastic fiber orientation, namely $L_{\text{percent}}$ and $T_{\text{percent}}$. This can be seen in Figures 5.8, 5.9, 5.10, and 5.11.
Figure 5.8 Correlation between $\tau_e$ and $L_{\text{percent}}$
(Longitudinal Samples, at 30% Strain)

Figure 5.9 Correlation between $\tau_0$ and $L_{\text{percent}}$
(Longitudinal Samples, at 30% Strain)
Figure 5.10 Correlation between $\tau_c$ and $T_{\text{percent}}$
(Longitudinal Samples, at 30% Strain)

$R = 0.476$
$P = 0.011$
$1-P = 0.989$

Figure 5.11 Correlation between $\tau_\sigma$ and $T_{\text{percent}}$
(Longitudinal Samples, at 30% Strain)

$R = 0.399$
$P = 0.011$
$1-P = 0.989$
It appears that the more type L fibers the shorter the time constants. On the contrary, the more type T (wavy) fibers, the longer the time constants. This confirms the observation made previously that the wavy fibers (type T) may be slowing down the tissue sample's reaction to external forces. Since $T_{\text{percent}}$ and $L_{\text{percent}}$ give opposite slopes individually, the ratio of the two gives the most striking correlation with time constants as shown in Figures 5.12 and 5.13.

**Figure 5.12 Correlation between $\tau_c$ and ($T_{\text{percent}}/L_{\text{percent}}$)**

(Longitudinal Samples, at 30% strain)

![Graph showing correlation between $\tau_c$ and ($T_{\text{percent}}/L_{\text{percent}}$)](image-url)
The regression analysis suggested that there is good possibility, from 30% longitudinal data, that the hypotheses are correct. The correlations are summarized in Table 5.1.
Table 5.1 Correlations between the mechanical properties and morphometrical observations (Longitudinal sample at 30% strain)

<table>
<thead>
<tr>
<th>x variables</th>
<th>y variables</th>
<th>$\mu_2$</th>
<th>$\tau_\varepsilon$</th>
<th>$\tau_\sigma$</th>
<th>$\eta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{\text{long}}$</td>
<td>$r = 0.332$</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>$r = 0.328$</td>
</tr>
<tr>
<td></td>
<td>$p = 0.036$</td>
<td></td>
<td></td>
<td></td>
<td>$p = 0.039$</td>
</tr>
<tr>
<td>$V_{\text{long}} \cdot L_{\text{percent}}$</td>
<td>$r = 0.37$</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>$r = 0.422$</td>
</tr>
<tr>
<td></td>
<td>$p = 0.019$</td>
<td></td>
<td></td>
<td></td>
<td>$p = 0.007$</td>
</tr>
<tr>
<td>$L_{\text{percent}}$</td>
<td>---</td>
<td>$r = -0.411$</td>
<td>$r = -0.328$</td>
<td>$r = 0.349$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$p = 0.008$</td>
<td>$p = 0.039$</td>
<td>$p = 0.027$</td>
<td></td>
</tr>
<tr>
<td>$T_{\text{percent}}$</td>
<td>---</td>
<td>$r = 0.476$</td>
<td>$r = 0.399$</td>
<td>$r = -0.344$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$p = 0.002$</td>
<td>$p = 0.011$</td>
<td>$p = 0.03$</td>
<td></td>
</tr>
<tr>
<td>$T_{\text{percent}}/L_{\text{percent}}$</td>
<td>---</td>
<td>$r = 0.535$</td>
<td>$r = 0.422$</td>
<td>$r = -0.386$</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>$p = 0.0004$</td>
<td>$p = 0.007$</td>
<td>$p = 0.014$</td>
<td></td>
</tr>
</tbody>
</table>

5.3.2. Data from Circumferential Samples

The main difference between the results from the circumferential and longitudinal samples is that in circumferential direction $\mu_2$ did not show good correlation with volume fraction ($V_{\text{circ}}$). However, it is possible that there exists a correlation between $\mu_2$ and the product of volume fraction and $C_{\text{percent}}$ (with a $p$ value of 0.056). In addition, $\mu_1$ showed good correlation with volume fraction, as had been suspected for the longitudinal samples. $T_{\text{percent}}$ had a possible correlation with viscosity $\eta$ (with a $p$ value of 0.058).
Since it was found from both longitudinal and circumferential samples that there was a correlation between $\mu_2$ and the volume fraction of elastic fibers, the significance of a linear fit between the combined data for 80 data points was tested. The $p$ values for the longitudinal and circumferential samples taken individually were 0.036 and 0.084. For the combined data, the $p$ value decreased to 0.019. Both methods of analysis showed that statistically significant correlations exist between these parameters. However, other morphometrical measurements did not show simultaneous correlations with the same mechanical variables measured from both the circumferential and longitudinal samples, hence were not tested with the combined data.

There is a clear correlation between $\mu_1$ and the volume fraction of elastic fibers in the circumferential direction as shown in Figure 5.14. The equivalent correlation in the longitudinal direction was not significant, in fact, $\mu_1$ in the longitudinal direction at 30% strain did not correlate with any of the morphometrical factors.
Figure 5.14 Correlation between Stiffness $\mu_1$ and Volume Fraction (Circumferential Samples, at 30% Strain)

Stiffness $\mu_1$ (kPa)

Volume fraction of elastic fibers

$R = 0.323$
$P = 0.042$
$1-P = 0.958$
The viscosity increases with the volume fraction. This is shown in Figures 5.15. It is also possible that the product of volume fraction and $C_{\text{percent}}$ influences the viscosity (with a $p$ value of 0.057), which would agree with what was found from samples in the longitudinal direction of the airway.

$\eta$ and $\tau_e$ correlate with $T_{\text{percent}}$ and $\frac{T_{\text{percent}}}{L_{\text{percent}}}$. The fact that the time constants of the circumferential samples correlate with $L_{\text{percent}}$ and not $C_{\text{percent}}$ is interesting. This will be discussed in the summary section of this chapter.
The correlation between $\eta$ and $\frac{T_{\text{percent}}}{C_{\text{percent}}}$ was not attempted since $\eta$ is a function of $r_e$ and $\mu_1$, and no correlation was found, either between $r_e$ and $\frac{T_{\text{percent}}}{C_{\text{percent}}}$ or between $\mu_1$ and $\frac{T_{\text{percent}}}{C_{\text{percent}}}$.

As was the case with the longitudinal samples, type T fibers caused a decrease in viscosity. However, a $p$ value of 0.58 indicated that the correlation is only marginally significant according to the statistics. The viscosity was also affected by (with a $p$ value of 0.051) the combined influence of $T_{\text{percent}}$ and $L_{\text{percent}}$, similar to that seen in the longitudinal samples. The negative effect of the fraction of type T fibers on viscosity is observed from samples in both the circumferential and longitudinal directions of the airway. Although $L_{\text{percent}}$ by itself had no significant effect on $\eta$, combining the effect of $T_{\text{percent}}$ and $L_{\text{percent}}$ (namely $\frac{T_{\text{percent}}}{L_{\text{percent}}}$) gave a lower $p$ value than the effect of $T_{\text{percent}}$ only, suggesting that $L_{\text{percent}}$ may display an opposite effect from $T_{\text{percent}}$ on the viscosity, similar to that found in the longitudinal samples.

Changes in $r_e$ with elastic fiber orientation are shown in Figures 5.16, 5.17, and 5.18. Similar to what was seen in the longitudinal samples, $L_{\text{percent}}$ caused a decrease in the time constant while $T_{\text{percent}}$ caused an increase. It is interesting that $\frac{T_{\text{percent}}}{C_{\text{percent}}}$ did not give a
correlation with $\tau_e$ in the circumferential direction. It would appear that the main correlation is with $T_{\text{percent}}$ since $C_{\text{percent}}$ also did not correlate with $\tau_e$.

Figure 5.16 Correlation between $\tau_e$ and $L_{\text{percent}}$
(Circumferential Samples, at 30% Strain)
Figure 5.17 Correlation between $\tau_\varepsilon$ and $T_{\text{percent}}$
(Circumferential Samples, at 30% Strain)

Figure 5.18 Correlation between $\tau_\varepsilon$ and ($T_{\text{percent}}/L_{\text{percent}}$)
(Circumferential Samples, at 30% Strain)
The significant correlations found from circumferential samples at 30% strain are summarized in Table 5.2.

Table 5.2 Correlations between the mechanical properties and morphometrical observations (Circumferential sample at 30% strain)

<table>
<thead>
<tr>
<th>$x$ variables</th>
<th>$y$ variables</th>
<th>$\mu_i$</th>
<th>$\tau_g$</th>
<th>$\eta$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{circ}$</td>
<td></td>
<td>$r = 0.323$</td>
<td>---</td>
<td>$r = 0.329$</td>
</tr>
<tr>
<td></td>
<td></td>
<td>$p = 0.042$</td>
<td></td>
<td>$p = 0.038$</td>
</tr>
<tr>
<td>$L_{percent}$</td>
<td></td>
<td>---</td>
<td>$r = -0.384$</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$p = 0.014$</td>
<td></td>
</tr>
<tr>
<td>$T_{percent}$</td>
<td></td>
<td>---</td>
<td>$r = 0.49$</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$p = 0.001$</td>
<td></td>
</tr>
<tr>
<td>$T_{percent}/L_{percent}$</td>
<td></td>
<td>---</td>
<td>$r = 0.544$</td>
<td>---</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$p = 0.0003$</td>
<td></td>
</tr>
</tbody>
</table>

It is possible that the large amount of noise in the results from circumferential samples, due to a lower stiffness, has masked the existing correlations. However, the correlations that are significant agreed with the ones found from longitudinal data in both $r$ and $p$ values, which suggests that the existence of these correlations are not based on random effects. To confirm these findings, the original hypotheses were tested on 20% and 10% data which were nosier and hence less reliable.
5.4. Tests of Correlation of the Experimental Data at 20% Strain

To reduce the volume of graphs in the body of the thesis, the graphs of the possible correlations (with a \( p \) value \( \leq 0.1 \)) have been placed in Appendix 5B. The reason for choosing a larger \( p \) value for presentation is to allow for the larger noise effects from the data at the 20% and 10% strain. Significant correlations are those having a \( p \) value \( \leq 0.05 \).

Data at 30% strain showed several trends, the question now is whether they will repeat in the data obtained at different strains. Also, is it possible that other effects will become dominant in samples with lower strain?

5.4.1. Data from Longitudinal Samples

\( \mu_2 \) was, once again, affected by the volume fraction of elastic fibers (with a \( p \) value of 0.059) and the product of volume fraction and \( L_{\text{percent}} \). With respect to \( \mu_1 \), certain correlations that did not appear in the data at 30% strain appeared here, such as the effect of \( L_{\text{percent}} \) (with a \( p \) value of 0.052) and \( T_{\text{percent}} \) along with \( \frac{T_{\text{percent}}}{L_{\text{percent}}} \). This would be expected since \( \mu_1 \) is mathematically related to viscosity \( \eta \), and \( \eta \) is affected by these morphometrical parameters as seen in the data at 30% strain.

The viscosity \( \eta \) at 20% strain only displayed significant correlations with the volume fraction and the product of the volume fraction and \( L_{\text{percent}} \).
The time constants did not show any statistically significant correlation with morphometry of the elastic fibers. However, this is not to say that there is no correlation possible. \( \tau_\sigma \) had a correlation with volume fraction \( (p = 0.2) \) and with \((V_{\text{long}} \times L_{\text{percent}})\) \((p = 0.14)\). \( \tau_e \) is a variable that is more difficult to measure accurately than is \( \tau_\sigma \). These findings lead to speculation that the time constants may be more significantly affected by other tissue components such as collagen and the ground substance than by elastic fibers.

Other than these exceptions the 20% strain tests gave similar results to the 30% strain results and even gave reasonable correlations between the stiffness \( \mu_1 \) and morphometrical factors. The result is summarized in Table 5.3.

*Table 5.3 Correlations between the mechanical properties and morphometrical observations (Longitudinal samples at 20% strain)*

<table>
<thead>
<tr>
<th>( x ) variables</th>
<th>( y ) variables</th>
<th>( \mu_2 )</th>
<th>( \mu_1 )</th>
<th>( \eta )</th>
</tr>
</thead>
<tbody>
<tr>
<td>( V_{\text{long}} )</td>
<td>( V_{\text{long}} )</td>
<td>---</td>
<td>---</td>
<td>( r = 0.368 )</td>
</tr>
<tr>
<td>( V_{\text{long}} \times L_{\text{percent}} )</td>
<td>( V_{\text{long}} \times L_{\text{percent}} )</td>
<td>( r = 0.363 )</td>
<td>---</td>
<td>( r = 0.42 )</td>
</tr>
<tr>
<td>( T_{\text{percent}} )</td>
<td>( T_{\text{percent}} )</td>
<td>---</td>
<td>( r = 0.403 )</td>
<td>---</td>
</tr>
<tr>
<td>( T_{\text{percent}} \times L_{\text{percent}} )</td>
<td>( T_{\text{percent}} \times L_{\text{percent}} )</td>
<td>---</td>
<td>( r = 0.453 )</td>
<td>---</td>
</tr>
</tbody>
</table>
5.4.2. Data from Circumferential Samples

The graphical representations are given in Appendix 5B.

The correlations between the mechanical properties and the morphometrical findings agreed well with the data at 30% strain except for $\tau_\varepsilon$. Volume fraction appeared to have significant effects on both $\mu_2$ and $\mu_1$.

Similar to what was seen with the longitudinal samples, $\eta$ was affected by volume fraction, $C_{\text{percent}}$, as well as $T_{\text{percent}}$. Again, poor correlation was found between time constant $\tau_\varepsilon$ and the morphometrical measurements. $\tau_\sigma$, as for the 30% strain case, correlated well with the product ($V_{\text{long}} \times L_{\text{percent}}$, $L_{\text{percent}}$, and $\frac{T_{\text{percent}}}{L_{\text{percent}}}$) (with a $p$ value of 0.059), which indicates that it was affected by the fibers in the directions that are not parallel to the stress. The results are listed in Table 5.4.
Table 5.4 Correlations between the mechanical properties and morphometrical observations (Circumferential samples at 20% strain)

<table>
<thead>
<tr>
<th>X variables</th>
<th>Y variables</th>
<th>μ²</th>
<th>α</th>
<th>μ₁</th>
<th>η</th>
</tr>
</thead>
<tbody>
<tr>
<td>V_circ</td>
<td></td>
<td>0.315</td>
<td></td>
<td>0.349</td>
<td>0.346</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.048</td>
<td></td>
<td>0.027</td>
<td>0.029</td>
</tr>
<tr>
<td>V_long • L_percent</td>
<td></td>
<td></td>
<td>0.38</td>
<td></td>
<td>0.016</td>
</tr>
<tr>
<td>V_circ • C_percent</td>
<td></td>
<td>0.378</td>
<td></td>
<td>0.323</td>
<td>0.341</td>
</tr>
<tr>
<td>L_percent</td>
<td></td>
<td></td>
<td>0.313</td>
<td></td>
<td>0.049</td>
</tr>
<tr>
<td>T_percent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.34</td>
</tr>
<tr>
<td>T_percent/L_percent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.335</td>
</tr>
</tbody>
</table>

5.5. Tests of Correlation of the Experimental Data at 10% Strain

The data at 10% strain was also correlated with the morphometrical observations. The test results are summarized in Tables 5.5 and 5.6. The graphs are given in Appendix 5C. The results confirmed the findings for 20% and 30% strain. However, not all the correlations that were found significant in 20% and 30% data reappeared in the 10% data. This could be because of the large amount of noise that existed in the 10% data due to
relatively small elongation, hence the low force reading from the transducer, or, because some of the correlations were simply not clear. No contradictory result was found.
Table 5.5 Correlations between the mechanical properties and morphometrical observations (Longitudinal samples at 10% strain)

<table>
<thead>
<tr>
<th>$x$ variables</th>
<th>$y$ variables</th>
<th>$r$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$L_{\text{percent}}$</td>
<td>---</td>
<td>$r = 0.46$</td>
<td>---</td>
</tr>
<tr>
<td>$T_{\text{percent}}$</td>
<td>$r = 0.38$</td>
<td>$r = -0.458$</td>
<td>$p = 0.003$</td>
</tr>
<tr>
<td>$V_{\text{long} \cdot L_{\text{percent}}}$</td>
<td>---</td>
<td>$r = 0.42$</td>
<td>---</td>
</tr>
<tr>
<td>$T_{\text{percent}}/L_{\text{percent}}$</td>
<td>$r = 0.404$</td>
<td>$r = -0.49$</td>
<td>$p = 0.001$</td>
</tr>
</tbody>
</table>

Table 5.6 Correlations between the mechanical properties and morphometrical observations (Circumferential samples at 10% strain)

<table>
<thead>
<tr>
<th>$x$ variables</th>
<th>$y$ variables</th>
<th>$r$</th>
<th>$p$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{percent}}$</td>
<td>---</td>
<td>---</td>
<td>$r = 0.427$</td>
</tr>
<tr>
<td>$L_{\text{percent}}$</td>
<td>---</td>
<td>$r = -0.388$</td>
<td>---</td>
</tr>
<tr>
<td>$T_{\text{percent}}$</td>
<td>---</td>
<td>$r = 0.448$</td>
<td>$r = -0.374$</td>
</tr>
<tr>
<td>$V_{\text{circ} \cdot C_{\text{percent}}}$</td>
<td>$r = 0.332$</td>
<td>---</td>
<td>$r = 0.442$</td>
</tr>
<tr>
<td>$T_{\text{percent}}/L_{\text{percent}}$</td>
<td>---</td>
<td>$r = 0.455$</td>
<td>$r = -0.337$</td>
</tr>
</tbody>
</table>

188
5.6. Summary of Correlations

The parameters of the fitted Kelvin model were correlated with the morphometrical characteristics of the same tested tissue samples measured after the external force was removed. A small group of additional samples were used to test for possible changes in the morphometrical measurements caused by stretching. Each sample was cut in half, with one half relaxed and the other stretched to 30% strain before histological fixation. No change was found in the volume fraction of the elastic fibers with or without stretching and, very little change was seen in elastic fiber orientation, i.e. the percentage distribution among straight fibers (type L or type C) and wavy fibers (type T) varied within 10% which could be due to the natural variations of the tissue structure from one region to another of the sample.

By limiting the correlations of interest to those having a $p$ value less than 0.05, all correlations, summarized in Table 5.7, shown have a 95% probability of a linear relationship. Having made this limitation, it is now possible to speculate on the trends found at different strain values. However, due to the noisy nature of the data, the goal of the study is to find cause-effect relationships rather than specific slopes. As explained before, more weight was given to the 30% strain data because of the larger force available for measurement. The data at other strains were independently tested for correlations with the morphological factors. The trends found from data at 30% strain, as well as the slopes of the correlations, were in most cases confirmed. Meanwhile, some previously unseen correlations were also becoming evident. In this summary, these consistencies as well as
some inconsistencies are discussed. It is worth pointing out that even though the Kelvin model used for data analysis can not represent the complexity of the samples being tested, the correlations found between the model parameters and morphometrical observations do provide a better understanding of the tissue behavior.
Table 5.7 Summary of correlations between mechanical properties and morphometrical characteristics

<table>
<thead>
<tr>
<th>Mechanical parameters</th>
<th>Strain value</th>
<th>Correlations with morphometrical factors</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td><strong>Longitudinal samples</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>Circumferential samples</strong></td>
</tr>
<tr>
<td>Stiffness $\mu_2$</td>
<td>30% $V_{\text{long}} ; (V_{\text{long}} \times L_{\text{percent}} )$</td>
<td>$---$</td>
</tr>
<tr>
<td></td>
<td>20% $(V_{\text{long}} \times L_{\text{percent}} )$</td>
<td>$V_{\text{circ}} ; (V_{\text{circ}} \times C_{\text{percent}} )$</td>
</tr>
<tr>
<td></td>
<td>10% $---$</td>
<td>$(V_{\text{circ}} \times C_{\text{percent}} )$</td>
</tr>
<tr>
<td></td>
<td>30% $---$</td>
<td>$V_{\text{circ}}$</td>
</tr>
<tr>
<td>Stiffness $\mu_1$</td>
<td>20% $T_{\text{percent}} ; T_{\text{percent}} / L_{\text{percent}}$</td>
<td>$V_{\text{circ}} ; (V_{\text{circ}} \times C_{\text{percent}} )$</td>
</tr>
<tr>
<td></td>
<td>10% $---$</td>
<td>$---$</td>
</tr>
<tr>
<td>Viscosity $\eta$</td>
<td>30% $V_{\text{long}} ; (V_{\text{long}} \times L_{\text{percent}} ) ; L_{\text{percent}} ; T_{\text{percent}} ; T_{\text{percent}} / L_{\text{percent}}$</td>
<td>$V_{\text{circ}}$</td>
</tr>
<tr>
<td></td>
<td>20% $(V_{\text{long}} \times L_{\text{percent}} )$</td>
<td>$V_{\text{circ}} ; (V_{\text{circ}} \times C_{\text{percent}} ) ; T_{\text{percent}} ; T_{\text{percent}} / L_{\text{percent}}$</td>
</tr>
<tr>
<td></td>
<td>10% $(V_{\text{long}} \times L_{\text{percent}} ) ; L_{\text{percent}} ; T_{\text{percent}} ; T_{\text{percent}} / L_{\text{percent}}$</td>
<td>$C_{\text{percent}} ; (V_{\text{circ}} \times C_{\text{percent}} ) ; T_{\text{percent}} ; T_{\text{percent}} / L_{\text{percent}}$</td>
</tr>
<tr>
<td>Time</td>
<td>30% $L_{\text{percent}} ; T_{\text{percent}} ; T_{\text{percent}} / L_{\text{percent}}$</td>
<td>$---$</td>
</tr>
<tr>
<td>$\tau_\sigma$</td>
<td>20% $---$</td>
<td>$(V_{\text{long}} \times L_{\text{percent}} ) ; L_{\text{percent}}$</td>
</tr>
<tr>
<td></td>
<td>10% $---$</td>
<td>$---$</td>
</tr>
<tr>
<td>Time</td>
<td>30% $L_{\text{percent}} ; T_{\text{percent}} ; T_{\text{percent}} / L_{\text{percent}}$</td>
<td>$L_{\text{percent}} ; T_{\text{percent}} ; T_{\text{percent}} / L_{\text{percent}}$</td>
</tr>
<tr>
<td>$\tau_\epsilon$</td>
<td>20% $---$</td>
<td>$---$</td>
</tr>
<tr>
<td></td>
<td>10% $T_{\text{percent}} ; T_{\text{percent}} / L_{\text{percent}}$</td>
<td>$L_{\text{percent}} ; T_{\text{percent}} ; T_{\text{percent}} / L_{\text{percent}}$</td>
</tr>
</tbody>
</table>

---: no correlations found
As shown in Table 5.7, there seems to be a separation of the effect of the volume fraction of elastic fibers from the fiber orientation ($L_{\text{percent}}$, $C_{\text{percent}}$, and $T_{\text{percent}}$). The volume fraction of the elastic fibers mainly affects the stiffness of the tissue samples, namely $\mu_2$. The fiber orientation mainly affects the time factors such as the viscosity $\eta$ and hence the time constants $\tau_\varepsilon$ and $\tau_\sigma$. The correlation between the stiffness and the fiber orientation was not clear.

The hypothesis that the tissue stiffness is positively related to the volume fraction of the elastic fibers is proven to be true. The effect is enhanced when the volume fraction is multiplied by $L_{\text{percent}}$ for longitudinal samples, and $C_{\text{percent}}$ for circumferential samples. This is expected, since the more elastic fibers found in a certain direction of the airway, the stiffer will be the tissue in that direction.

The dynamic parameter $\eta$ is affected mainly by the fiber orientation, which can be enhanced by the effect of volume fraction. The amount of type T fibers (wavy fibers) plays an important role in determining the viscosity. The more the wavy fibers, or the larger the ratio of $\frac{T_{\text{percent}}}{L_{\text{percent}}}$, the lower is the viscosity. It has been speculated [Mijailovich et al. 1993] that the energy dissipation of a system on deformation could occur at surfaces of direct fiber-fiber sliding contact. This could suggest that the more type T fibers, the fewer are the straight fibers available (given a fixed total number of fibers) to directly slide against one another; therefore the lower will be the energy dissipation which is related to the viscosity of composite soft materials.
The correlations indicate that the time constants are mainly affected by the fiber orientation, if only the elastic fibers are studied. Time constants are indicators of how fast the tissue sample reacts to external forces. The more straight fibers of type L or type C there are, the shorter are the time constants in the longitudinal and circumferential samples respectively. On the other hand, the more wavy (type T) fibers there are, the longer the time constants. However, $\tau_\sigma$ of the circumferential samples was also found to have a dependence on the volume fraction of the elastic fibers, most likely due to the correlation between the stiffness $\mu_2$ and the volume fraction. All the arguments ignore the unknown effect of the movement of the collagen mesh.

One of the more difficult to explain results of these linear correlations is the signs of the slope of the time elements ($\eta$, $\tau_\varepsilon$, and $\tau_\sigma$) with respect to $T_{\text{percent}}$ or $\frac{T_{\text{percent}}}{L_{\text{percent}}}$. Considering only the results from longitudinal samples under 30% strain given in Figures 5.6, 5.7, 5.10, 5.11, 5.12, and 5.13, the slope of viscosity $\eta$ versus $T_{\text{percent}}$ or $\frac{T_{\text{percent}}}{L_{\text{percent}}}$ is negative, while the slopes of $\tau_\varepsilon$ and $\tau_\sigma$ versus the same terms are positive. Since $\tau_\varepsilon$ and $\tau_\sigma$ are proportional to viscosity (Eqn. 3.3), it would be expected that they would have slopes of the same sign as that of the viscosity. As seen in the defining equations the time constants are also functions of the tissue stiffness $\mu_1$ and/or $\mu_2$. The change of the tissue stiffness with $T_{\text{percent}}$ or $\frac{T_{\text{percent}}}{L_{\text{percent}}}$ is not clear. It is therefore expected that the stiffness

193
decreases with an increase in the number of wavy fibers for the case of tissue at 30% strain.

In a recent study [Mijailovich et al. 1993], the energy dissipation of soft tissues was explained by the friction between the interstitium and the fibers parallel to the movement. In the morphological factors used here,

\[ L_{\text{percent}} + T_{\text{percent}} = 1.0 - A_{\text{percent}} - C_{\text{percent}} \approx \text{constant} \]  \hspace{1cm} (5.9)

since the fractions of type C and type A fibers are small and nearly constant. Therefore, if the fraction of type T fibers increases, the fraction of type L fibers, as a result, must decrease. The fewer parallel fibers, the less friction during sliding, hence the lower the viscosity or the less the energy dissipation.

One point of interest, which is best seen in Table 5.7, is that, with respect to the circumferential samples, the time factors (\( \eta \), \( \tau_x \), and \( \tau_y \)) are correlated with \( L_{\text{percent}} \) rather than \( C_{\text{percent}} \). This is most likely due to the fact that the number of type L fibers is predominant in the airway. If the folding of the mucosal membrane in the airways during bronchoconstriction is to be explained, the fact that there are a greater number of straight, longitudinally distributed fibers than other differently oriented fibers leads to the need for the tissue to produce folds towards the center of the airway lumen. This is because folding the interstitia requires less energy than folding the actual elastic fibers since the fibers are stiffer. The analogy could be similar to that of folding a Chinese bamboo blind, it requires less energy to fold it in the direction parallel to the bamboo sticks than perpendicular to them.
It should be mentioned here that expectations did not always agree with the results. For example, it was found in the data at 20% strain that the stiffness $\mu_1$ has a relationship with $T_{\text{percent}}$ and $L_{\text{percent}}$. This was not expected, but can be explained since $\mu_1$ is mathematically related to the time elements.

The viscous dissipation, as a result of the frictional drag due to the relative motion among tissue components, was considered by some authors as the most important factor governing the tissue viscoelastic properties [Mow et al. 1980, Mijailovich et al. 1993]. However, their explanations of the mechanism was not based on data relating tissue mechanical properties to the morphological characteristics of the samples. The results of this study show correlations between the measured physical properties and the structural characteristics of biological tissues, thus bring the existing speculations one step further towards a better understanding of the viscoelastic properties of soft tissues. The method of capturing the morphometrical characteristics of the tissue sample is novel, yet simple and may be used to explain the mechanical properties of other tissues.
Chapter Six

Conclusions

6.1. Summary

A stress-strain tester for fine biological tissues was designed and constructed to eliminate equipment responses in the frequency range of interest. No response corrections [Wang et al. 1997] were necessary in its use. The sensitivity of the equipment was 10 mg force and 0.25 μm linear movement which is precise enough to measure even tissues with low stiffness. The application of the latest computer software for Windows in the interface design provides sophisticated yet user-friendly operation. The experimental data can be graphed instantaneously on the computer screen and captured with high sampling frequency in digital form.

Pulse testing of biological tissues to obtain dynamic responses is a new method in the field of biomechanics. This method is accurate and efficient in obtaining the response of the tissue samples over a wide spectrum of frequencies.

The results from pulse tests were mathematically fitted to a Kelvin model revealing the viscoelastic properties of the mucosal membrane. The Kelvin model was found to best explain the shape of both the stress relaxation (from static preloading) and the Bode diagram (from pulse tests). The steady-state stiffness measured from both static tensile and
single pulse tests, as well as the parameters of the fitted Kelvin model (that is, the viscous component of the dashpot, and the time constants) were reported as the mechanical properties of the tracheal mucosal membrane of rabbits. It was found that:

(a) the static stiffness and $\mu_2$ are of a similar magnitude;

(b) the mucosal membrane is stiffer in the longitudinal than in the circumferential direction of the airway;

(c) the age of the tested 40 animals (between 2 and 35 months) did not show a statistically significant effect on the measured mechanical properties.

The collagen fibers in the mucosal membrane were seen to form a random mesh, whereas there was a definite dimensionality to the orientation of the elastic fibers. A morphometrical definition of the orientation of elastic fibers in the tissue samples was developed for the first time in this study. The elastic fibers in the airway were classified into four types according to their geometrical orientation in the airway. The measurements on all tested tissue samples showed that about 57% of the fibers are straight and run in the longitudinal direction of the airway; 8% of the fibers are straight but run in the circumferential direction of the airway; 33% of the fibers are wavy having components in both the longitudinal and circumferential directions; and the rest of the fibers run across the thickness of the membrane (from airway lumen to the cartilage ring). The method of measuring the fiber orientation developed in this study is novel and requires only a simple image analysis system. The volume fraction of the elastic fibers in the tissue was also
quantified using the classical point counting technique. The age of the tested animals influenced neither the volume fraction nor the orientation of the elastic fibers.

The main findings of the correlations between the measured Kelvin model parameters and the morphometrical factors are listed in Table 5.7. Of special interest are the following findings:

(a) the steady-state stiffness correlated with the volume fraction of elastic fibers, the effect of the fiber orientation on the tissue stiffness is unclear;

(b) the elastic fiber orientation appears to affect the viscous element $\eta$ and the time constants, which are indicators of the rate at which the tissue reacts to external forces;

(c) with a larger fraction of wavy fibers, the viscosity decreased and the time constants increased;

(d) the effect of the fiber orientation can also be enhanced by the volume fraction of the elastic fibers.

6.2. Significant Contributions

- Developed an ultra sensitive piece of digital equipment for high accuracy tests (both static and dynamic) on biological tissues.
• Introduced the use and the mathematics of pulse testing of dynamic systems to the field of biomechanical engineering.

• Showed that the Kelvin model represents the mechanical behavior of the rabbit airway mucosal membrane.

• Showed that the stiffness of the tissue samples at steady-state measured with static and dynamic techniques are of the similar magnitude.

• Showed that for the rabbit mucosal membrane tested, age (from 2 to 35 months) had no effect on the mechanical properties.

• Defined a new morphologic approach to estimate the elastic fiber orientation.

• Found a definite correlation between the morphometrical measurements of the elastic fibers and the mechanical behavior of the mucosal membrane.

• The viscoelastic properties of the mucosal membrane in tension indicated that it is capable of providing elastic loads to the airway smooth muscle because of the magnitude of the steady-state stiffness and possibly, the time required for the membrane to react to the external forces.

• The revealed elastic fiber orientation supports the observation of the direction in which the folding occurs, that is, the folding of the membrane is on the circumferential plane towards the center of the lumen.

• The strength of the folding of the mucosal membrane can be estimated when the tissue behavior in compression is available in the future.
6.3. Recommendations for Future Work

- The pulse testing method can be improved by altering the equipment so that the pulse shape and duration can be altered to give more information about the frequency response.

- The technique of enzyme digestion can be considered [Moretto et al. 1994]. If the elimination of the elastic fibers caused a change in the mechanical properties, then the correlations between the morphological and mechanical measurements could be further confirmed.

- The equipment can also be altered to test the mechanical properties of tissues in compression.

- The method used in this study can be applied to the testing of other types of biological tissues such as dermis.
Nomenclature

Latin letters

a, d, g  
real part of a complex number

A  
amplitude of a sine wave

A_{r}  
number of type A fibers

A_{\text{percent}}  
fraction of elastic fibers across the thickness of the sample

A_{v}  
relative area fraction

a_{x}  
maximum dimension of elastic fiber in the direction parallel to the epithelial cell layer

b, c, h  
imaginary part of a complex number

b_{y}  
maximum dimension of elastic fiber in the direction perpendicular to the epithelial cell layer

C_{r}  
number of type C fibers estimated from longitudinal sections

C_{r}^{*}  
number of type C fibers estimated from both longitudinal and circumferential sections of the same airway

C_{\text{percent}}  
fraction of straight elastic fibers in the circumferential direction of the airway

CE  
coefficient of error

E  
Young’s modulus

E_{L}  
loss modulus

E_{S}  
storage modulus

E_{R}  
elastic constant (or, stiffness)

f( )  
function
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>$F_1, F_2, F(t)$</td>
<td>force, or force as a function of time</td>
</tr>
<tr>
<td>$g$</td>
<td>correction factor for the thickness of the sections</td>
</tr>
<tr>
<td>$</td>
<td>G(s)</td>
</tr>
<tr>
<td>$G_{ss}$</td>
<td>steady-state absolute gain</td>
</tr>
<tr>
<td>$G_k$</td>
<td>absolute gain of the Kelvin model</td>
</tr>
<tr>
<td>$G_d$</td>
<td>absolute gain of data</td>
</tr>
<tr>
<td>$i, k, n$</td>
<td>counter</td>
</tr>
<tr>
<td>$j$</td>
<td>$\sqrt{-1}$</td>
</tr>
<tr>
<td>$K_i$</td>
<td>correction factor for the thickness of the sections</td>
</tr>
<tr>
<td>$\ell$</td>
<td>length of an elastic fiber bundle</td>
</tr>
<tr>
<td>$\bar{\ell}$</td>
<td>average length of elastic fiber bundles</td>
</tr>
<tr>
<td>$L$</td>
<td>length of tissue sample</td>
</tr>
<tr>
<td>$L_0$</td>
<td>initial length before deformation</td>
</tr>
<tr>
<td>$LT(\cdot)$</td>
<td>LaPlace transform</td>
</tr>
<tr>
<td>$L_f^*$</td>
<td>number of type L fibers estimated from circumferential sections</td>
</tr>
<tr>
<td>$L_f$</td>
<td>number of type L fibers estimated from both longitudinal and circumferential sections of the same airway</td>
</tr>
<tr>
<td>$L_{百分比}$</td>
<td>fraction of straight elastic fibers in the longitudinal direction of the airway</td>
</tr>
<tr>
<td>$p$</td>
<td>probability level</td>
</tr>
<tr>
<td>$P$</td>
<td>position signal</td>
</tr>
<tr>
<td>$P_0$</td>
<td>initial position (position signal when $L$ is at $L_0$)</td>
</tr>
</tbody>
</table>
\( P_i \)  
internal perimeter

\( P_L \)  
pressure applied to airway smooth muscle by lung elastic recoil

\( r \)  
correlation coefficient

\( s \)  
LaPlace operator, \( s = j\omega \)

\( SD \)  
standard deviation

\( SE \)  
standard error

\( SS(\cdot) \)  
objective function

\( t \)  
time

\( tt \)  
microscopic section thickness

\( T \)  
Tension signal

\( T_f \)  
number of type T fibers

\( T_{\text{percent}} \)  
fraction of the wavy elastic fibers

\( u, u_1, u(t) \)  
displacement of spring as a function of time

\( u_1 \)  
displacement of dashpot

\( V_v \)  
relative volume fraction

\( V_{\text{circ}} \)  
relative volume fraction measured from circumferential samples

\( V_{\text{long}} \)  
relative volume fraction measured from longitudinal samples

\( x \)  
displacement of the motor

\( x(t), y(t), Y(t) \)  
variables in time domain

\( y_i \)  
data points of the dependent variable in actual data

\( \bar{y}_i \)  
average value of the dependent variable in actual data

\( y_{\text{fitted}} \)  
data points of the dependent variable in the fitted linear regression
Greek letters

δ thickness of an elastic fiber bundle

δ̄ average thickness of an elastic fiber bundle

ΔPₓ transmural pressure

Δt sampling interval

ε engineering strain

η viscosity

θ phase angle

θₖ phase angle of Kelvin model

θₜ phase angle of data

λ shape factor

µ spring constant

µ₁ spring constant of spring 1 of the Kelvin model

µ₂ spring constant of spring 2 of the Kelvin model

σ engineering stress

τ, τₑ, τₚ time constants

ω frequency (rad sec⁻¹)
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASM</td>
<td>Airway Smooth Muscle</td>
</tr>
<tr>
<td>C.I.</td>
<td>Confident Interval</td>
</tr>
<tr>
<td>CSA</td>
<td>Cross Sectional Area</td>
</tr>
<tr>
<td>CSA₀</td>
<td>initial Cross Sectional Area</td>
</tr>
<tr>
<td>D.F.</td>
<td>Degree of Freedom</td>
</tr>
<tr>
<td>Eqn.</td>
<td>Equation</td>
</tr>
<tr>
<td>LHS</td>
<td>Left Hand Side</td>
</tr>
<tr>
<td>RHS</td>
<td>Right Hand Side</td>
</tr>
<tr>
<td>TBO</td>
<td>Toluidene Blue O staining agent</td>
</tr>
<tr>
<td>Tot₆C</td>
<td>total number of straight elastic fibers in the circumferential direction of the airway</td>
</tr>
<tr>
<td>Tot₆L</td>
<td>total number of straight elastic fibers in the longitudinal direction of the airway</td>
</tr>
<tr>
<td>Tot₆N</td>
<td>total number of elastic fibers</td>
</tr>
<tr>
<td>Tot₆T</td>
<td>total number of wavy elastic fibers</td>
</tr>
<tr>
<td>Tot₆V</td>
<td>total volume of elastic fibers</td>
</tr>
</tbody>
</table>
References


210


Appendix 1A: Folding of the Mucosal Membrane

Photograph is a copy (with permission) from the Pulmonary Research Laboratory, UBC.

Figure 1a.1. The mucosal folds shown in a peripheral airway. The airway smooth muscle ring and the lung parenchyma is also visible. Light microscope, x 40.
Appendix 2A: Machine Parameters

KULITE SEMICONDUCTOR

KULITE File Number: 24305

Test Report

<table>
<thead>
<tr>
<th>Type/Model</th>
<th>BG-10 Gram</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serial Number</td>
<td>G30-22</td>
</tr>
<tr>
<td>Rated Pressure or Load</td>
<td>10 g</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>10.1 mv/g</td>
</tr>
<tr>
<td>Rated Excitation</td>
<td>10 vdc</td>
</tr>
<tr>
<td>Zero balance</td>
<td>-1.7 mv</td>
</tr>
<tr>
<td>Input Impedance</td>
<td>2720 Ω</td>
</tr>
<tr>
<td>Output Impedance</td>
<td>1770 Ω</td>
</tr>
<tr>
<td>Isolation</td>
<td>∞ megohms at 50 Volts D.C.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Wiring Connections</th>
<th>Color</th>
<th>Pin</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ Excitation</td>
<td>Red</td>
<td>√</td>
</tr>
<tr>
<td>- Excitation</td>
<td>Black</td>
<td>√</td>
</tr>
<tr>
<td>+ Output</td>
<td>Green</td>
<td>√</td>
</tr>
<tr>
<td>- Output</td>
<td>White</td>
<td>√</td>
</tr>
</tbody>
</table>
2. Photograph of the equipment (Figure 2app.1). From top to bottom: the stepper motor, the linear table, the force transducer, the tissue bath, and the tissue bath stand.

Photograph taken by Mr. Stuart Greene (Pulmonary Research Laboratory UBC)
3. Mechanical drawings of the mechanical support of the equipment (Figure 2a. M1-9).
3/8\textsuperscript{th} STAINLESS STEEL PLATE
THREE 18' X 18' X 1' CONCRETE SLABS
NINE TENNIS BALLS
MILD STEEL CHANNEL

2' X 2' MILD STEEL TUBE

LEVEL ADJUSTERS

Figure 2a. M-1 ISOLATION TABLE

PLAN VIEW
Figure 2a. M-2 LINEAR TABLE

Dimensions mm

- MOTOR
- TABLE
- 55.0
- 50.8
- 50.8
- 127.0
- 101.6
- 101.6
- 44.45
- 31.75
Figure 2a. M-6 FORCE TRANSDUCER HOLDER
Figure 2a. M-7 FORCE TRANSDUCER LOCK
Figure 2a. M-8 TISSUE BATH
Figure 2a. M-9 TISSUE BATH BASE PLATE
Appendix 2B: Programs for Equipment Design

1. Photograph of the Controller Manual Move dialog box of the computer software

(Figure 2app. 2)

Photograph taken by author
2. Photograph of the Dynamic Configuration dialog box of the computer software (Figure 2app. 3)

Photograph taken by author
3. Chap2a.bas: Program for calculating time interval and time available for motor to switch direction during sine wave generation

3 REM CHAP2A.BAS
5 REM PRELIMINARY ALGORITHM
7 REM CALCULATE THE TIME INTERVAL FOR SINE WAVE GENERATION

10 DIM T(2000)
20 REM TIME FOR STEP
30 REM INPUT " STEP SIZE (MICRON)"; SS
35 SS=.25
40 REM INPUT "FREQUENCY (Hz)"; F
45 F=100
50 INPUT "AMPLITUDE (MICRON)"; A

60 T(0)=0
70 N=A/SS
80 PRINT "TOTAL TRAVEL TIME INTERVAL TOTAL TIME"
90 FOR J=1 TO INT(N)
100 X=J*SS/A
110 IF X=1 THEN X=.999999
120 T(J)=1/(6.28*F)*ATN(X/SQR(-X*X+1))
130 ST=T(J)-T(J-1)
140 Z=SS*J
150 PRINT USING "####.## ,####.##### ,######.####"; Z; ST; T(J)
160 NEXT J

170 END
4. Das1600.cfg: Interface Board configuration file

<table>
<thead>
<tr>
<th>Board</th>
<th>0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Name</td>
<td>DAS1201</td>
</tr>
<tr>
<td>Address</td>
<td>&amp;H330</td>
</tr>
<tr>
<td>ClockSel</td>
<td>10MHz</td>
</tr>
<tr>
<td>WaitState</td>
<td>NO</td>
</tr>
<tr>
<td>ADChanConfig</td>
<td>Differential</td>
</tr>
<tr>
<td>DMAChannel</td>
<td>3</td>
</tr>
<tr>
<td>IntLevel</td>
<td>7</td>
</tr>
<tr>
<td>PortA</td>
<td>Input</td>
</tr>
<tr>
<td>PortB</td>
<td>Input</td>
</tr>
<tr>
<td>PortCL</td>
<td>Output</td>
</tr>
<tr>
<td>PortCH</td>
<td>Output</td>
</tr>
<tr>
<td>NumOfEXP16</td>
<td>0</td>
</tr>
<tr>
<td>NumOfEXPGP</td>
<td>0</td>
</tr>
<tr>
<td>NumOfSSH</td>
<td>0</td>
</tr>
<tr>
<td>ADGain</td>
<td>100</td>
</tr>
</tbody>
</table>
5. Supplementary program (Wdas.ini)

[CalibPos] Maxt=0 Min=9000 Count1=27221 Count2=20881 Measured1=779260 Measured2=776090 Slope=0.500 Intercept=765650 Max=11000

[CalibTension] Min=0 Max=7573 Count1=1970 Count2=2082 Measured1=0 Measured2=500 Slope=4.464 Intercept=-8795

[Static] SampleFreq=1.00 SampleCount=200 Graphics=0

[Dynamic] WaveFormFreq=1.00 WaveFormAmp=117.00 SampleFreq=800.00 SampleCount=1000 SmartDownload=0

[Com] Port=2 Baud=2400 Parity=N Data=8 Stop=1 Handshake=NONE

[ManualMove] Direction=0 Speed=100.0 Distance=900.00

[Controller] TimeCountsPerSec=2000000 MicronsPerStep=0.500

[Continuous] SampleFreq=50.00 GraphMode=0

[Misc] ViewDataCalib=1 GraphicsEnabled=-1 TestCodeEnabled=0

[PulseMove] Height=780.0 Rate=975.0 Duration=1.7 Overshoot=0.0 MinPause=0.10
6. Wdas.exe: Operating program (form design and code)

The main Window of the Program: (photograph taken by author).
Sub DisplayViewData (count As Long, dt As Long, position As Long, tension As Long)
    If ViewCalibData = 0 Then
        panelData1.Caption = Str$(count)
        panelData2.Caption = Str$(dt)
        panelData3.Caption = Str$(position)
        panelData4.Caption = Str$(tension)
    Else
        panelData1.Caption = Str$(count)
        panelData2.Caption = Str$(dt)
        panelData3.Caption = Format$(CalibPosition(position), "0.00")
        panelData4.Caption = Format$(CalibTension(tension), "0.0")
    End If
End Sub

Sub DrawCurrentData (position As Long, tension As Long)
    Dim Y As Integer
    Dim X As Integer
    If DrawModeXY = False Then
        X = xGraph
        ' Draw a line to blank the previous data
        DrawBox.Line (X, 0)-(X, DrawBox.ScaleHeight), DrawBox.BackColor
        ' Draw the data points
        Y = CInt((DrawPosEqn.m * position) + DrawPosEqn.b)
        DrawBox.PSet (X, Y), RGB(0, 255, 255)
        Y = CInt((DrawTnsEqn.m * tension) + DrawTnsEqn.b)
        DrawBox.PSet (X, Y), RGB(255, 255, 0)
        ' Increment the x position
        X = X + 1
        If X > DrawBox.ScaleWidth Then
            X = DrawBoxLeftMargin
        End If
        ' Draw a line to indicate the border
        DrawBox.Line (X, 0)-(X, DrawBox.ScaleHeight), RGB(255, 0, 0)
        xGraph = X
    Else
        ' Draw the data points
        Y = CInt((DrawPosEqn.m * position) + DrawPosEqn.b)
\[ X = \text{Clnt}((\text{DrawTnsEqn.m} \times \text{tension}) + \text{DrawTnsEqn.b}) \]
\[ \text{DrawBox.PSet} (X, Y), \text{RGB}(0, 255, 0) \]
End If
End Sub

Sub mnuCaptureDynamic_Click()
formDyna.Show 0
End Sub

Sub mnuCaptureStatic_Click()
formStatic.Show 0
End Sub

Sub RunDataAcqCont (frequency As Double)
Dim rc As Variant
Dim curPos As Long
Dim curFrc As Long
Dim curTime As Long
Dim startTime As Long
Dim lastTime As Long
Dim lastDispTime As Long
Dim ti As TIMERINFO
Dim period As Long
Dim avgTension As Long
Dim avgCount As Integer

' Size of TIMERINFO structure in bytes
ti.dwSize = 12

StartFlag = 1
btnContStart.Enabled = False
btnDynaStart.Enabled = False
btnStaticStart.Enabled = False

If frequency <> 0 Then
    period = 1000 / frequency
Else
    period = 0
End If

' Capture data until done or the start flag is turned off

While StartFlag = 1
    rc = TimerCount(ti)
    If (ti.dwmsSinceStart - lastTime) >= period Then
        ' Save the current time
        lastTime = ti.dwmsSinceStart

        ' Save the elapsed time
        curTime = lastTime - startTime

        ' Turn on output bit to sample and hold register on controller
        DASDigitalOutput (255)

        ' Read sample and hold register for position reading
        curPos = DASDigitalInput()

        ' Read analog input for force reading
        curFrc = DASAnalogInput()

        ' Turn off out bit to sample and hold register
        DASDigitalOutput (0)

        avgCount = avgCount + 1
        avgTension = avgTension + curFrc
        If (ti.dwmsSinceStart - lastDispTime) >= 500 Then
            avgTension = avgTension / avgCount
            DisplayViewData 0, curTime, curPos, avgTension
            lastDispTime = ti.dwmsSinceStart
            avgTension = 0
            avgCount = 0
        End If
        DrawCurrentData curPos, curFrc
    End If
    DoEvents
Wend

btnContStart.Enabled = True
btnDynaStart.Enabled = True
btnStaticStart.Enabled = True
End Sub

Sub RunDynamic (frequency As Double, count As Long)
    Dim i As Long
    Dim rc As Variant
    Dim startTime As Long
    Dim lastTime As Long
    Dim ti As TIMERINFO
    Dim period As Long

    ' Size of TIMERINFO structure in bytes

236
ti.dwSize = 12

btnContStart.Enabled = False
btnDynaStart.Enabled = False
btnStaticStart.Enabled = False

If frequency <> 0 Then
    period = 1000 / frequency
Else
    period = 0
End If

' Get the start time
rc = TimerCount(ti)
startTime = ti.dwmsSinceStart
lastTime = ti.dwmsSinceStart

' Capture data until done or the start flag is turned off
i = 1
While i <= count
    rc = TimerCount(ti)
    If (ti.dwmsSinceStart - lastTime) >= period Then
        ' Save the current time
        lastTime = ti.dwmsSinceStart

        ' Save the elapsed time
        DATime(i) = lastTime - startTime

        ' Turn on output bit to sample and hold register on controller
        DASDigitalOutput(255)

        ' Read sample and hold register for position reading
        DAPos(i) = DASDigitalInput()

        ' Read analog input for force reading
        DAFrc(i) = DASAnalogInput()

        ' Turn off out bit to sample and hold register
        DASDigitalOutput(0)
    End If
    i = i + 1
End If

Wend

If i > 1 Then
    DACount = i - 1
Else
    DACount = 0
End If
btnContStart.Enabled = True
btnDynaStart.Enabled = True
btnStaticStart.Enabled = True
End Sub

Sub RunStatic (frequency As Double, count As Long)
    Dim i As Long
    Dim rc As Variant
    Dim startTime As Long
    Dim lastTime As Long
    Dim lastDispTime As Long
    Dim ti As TIMERINFO
    Dim period As Long

    ' Size of TIMERINFO structure in bytes
    ti.dwSize = 12

    StartFlag = 1
    btnContStart.Enabled = False
    btnDynaStart.Enabled = False
    btnStaticStart.Enabled = False

    i = 1
    If frequency <> 0 Then
        period = 1000 / frequency
    Else
        period = 0
    End If

    ' Get the start time
    rc = TimerCount(ti)
    startTime = ti.dwmsSinceStart
    lastTime = ti.dwmsSinceStart
    lastDispTime = ti.dwmsSinceStart

    ' Capture data until done or the start flag is turned off
    While i <= count And StartFlag = 1
        rc = TimerCount(ti)
        If (ti.dwmsSinceStart - lastTime) >= period Then
            ' Save the current time
            lastTime = ti.dwmsSinceStart

            ' Save the elapsed time
            DATime(i) = lastTime - startTime

            ' Turn on output bit to sample and hold register on controller
            DASDigitalOutput (255)
    End While
End Sub
'Read sample and hold register for position reading
DAPos(i) = DASDigitalInput()

'Read analog input for force reading
DAFrc(i) = DASAnalogInput()

'Turn off out bit to sample and hold register
DASDigitalOutput(0)

'Update the data panels if frequency is slow enough
If ViewGraphicsEnabled <> 0 Then
    If (ti.dwmsSinceStart - lastDispTime) >= 250 Then
        DisplayViewData i, DATime(i), DAPos(i), DAFrc(i)
        lastDispTime = ti.dwmsSinceStart
    End If
    panelProgress.FloodPercent = (i * 100) / count
    DrawCurrentData DAPos(i), DAFrc(i)
End If

    i = i + 1
End If
DoEvents
Wend

If i > 1 Then
    DACount = i - 1
    DisplayViewData i - 1, DATime(i - 1), DAPos(i - 1), DAFrc(i - 1)
Else
    DACount = 0
End If

If i > 1 Then
    'Update the data panels
    DisplayViewData i - 1, DATime(i - 1), DAPos(i - 1), DAFrc(i - 1)
    panelProgress.FloodPercent = ((i - 1) * 100) / count
End If

btnContStart.Enabled = True
btnDynaStart.Enabled = True
btnStaticStart.Enabled = True
StartFlag = 0
End Sub

Sub SaveCalibFile (Filename As String)
    Dim rc As Variant

    'Write the [CalibPos] section of the ini file
    rc = WritePrivateProfileString("CalibPos", "Count1", Format$(CalibPosCount1, "0"), Filename)
End Sub
rc = WritePrivateProfileString("CalibPos", "Count2", Format$(CalibPosCount2, "0"), Filename)
rc = WritePrivateProfileString("CalibPos", "Measured1", Format$(CalibPosMeasured1, "0"), Filename)
rc = WritePrivateProfileString("CalibPos", "Measured2", Format$(CalibPosMeasured2, "0"), Filename)
rc = WritePrivateProfileString("CalibPos", "Slope", Format$(CalibPosSlope, "0.000"), Filename)
rc = WritePrivateProfileString("CalibPos", "Intercept", Format$(CalibPosIntercept, "0"), Filename)

' Write the [CalibTension] section of the ini file
rc = WritePrivateProfileString("CalibTension", "Max", Format$(CalibTensionMax, "0"), Filename)
rc = WritePrivateProfileString("CalibTension", "Count 1", Format$(CalibTensionCount1, "0"), Filename)
rc = WritePrivateProfileString("CalibTension", "Count2", Format$(CalibTensionCount2, "0"), Filename)
rc = WritePrivateProfileString("CalibTension", "Measured1", Format$(CalibTensionMeasured1, "0"), Filename)
rc = WritePrivateProfileString("CalibTension", "Measured2", Format$(CalibTensionMeasured2, "0"), Filename)
rc = WritePrivateProfileString("CalibTension", "Slope", Format$(CalibTensionSlope, "0.000"), Filename)
rc = WritePrivateProfileString("CalibTension", "Intercept", Format$(CalibTensionIntercept, "0"), Filename)
End Sub

Sub SaveDataFile (Filename As String)
Dim i As Integer
Dim calPosition As Double
Dim calTension As Double
Dim timeAcc As Double

' Save data to a file
Open Filename For Output As #1 ' Open file.
For i = 1 To DACount
    calPosition = CalibPosition(DAPos(i))
    calTension = CalibTension(DAFrc(i))
    timeAcc = DATime(i)
    Print #1, Format$(i, "0") + ", " + Format$(timeAcc / 1000, "0.000") + ", " + Format$(DAPos(i), "0") + ", " + Format$(DAFrc(i), "0") + ", " + Format$(calPosition, "0.0") + ", " + Format$(calTension, "0.0")
Next
Close ' Close all files.
End Sub

Sub TestDataAcqCont (frequency As Double)
Dim i As Long
Dim rc As Variant
Dim curPos As Long
Dim curFrc As Long
Dim curTime As Long
Dim startTime As Long
Dim lastTime As Long
Dim ti As TIMERINFO
Dim period As Long

' Size of TIMERINFO structure in bytes
ti.dwSize = 12

StartFlag = 1
btnContStart.Enabled = False
btnDynaStart.Enabled = False
btnStaticStart.Enabled = False

If frequency <> 0 Then
    period = 1000 / frequency
Else
    period = 0
End If

' Get the start time
rc = TimerCount(ti)
startTime = ti.dwmsSinceStart
lastTime = ti.dwmsSinceStart

' Capture data until done or the start flag is turned off
i = 1
While StartFlag = 1
    rc = TimerCount(ti)
    If (ti.dwmsSinceStart - lastTime) >= period Then
        ' Save the current time
        lastTime = ti.dwmsSinceStart

    ' Save the elapsed time
    curTime = lastTime - startTime

    ' Read sample and hold register for position reading
    curPos = CLng(32767 * Sin(i * DegToRad)) + 32767

    ' Read analog input for force reading
    curFrc = CLng(1024 * Sin(2 * i * DegToRad)) + 3072

    DisplayViewData i, curTime, curPos, curFrc
    DrawCurrentData curPos, curFrc
    i = i + 1
End If
    DoEvents
Wend

btnContStart.Enabled = True
btnDynaStart.Enabled = True
btnStaticStart.Enabled = True

End Sub

Sub TestDataAcquisition (frequency As Double, count As Long)
    Dim i As Long
    Dim rc As Variant
    Dim startTime As Long
    Dim lastTime As Long
    Dim ti As TIMERINFO
    Dim period As Long

    ' Size of TIMERINFO structure in bytes
ti.dwSize = 12

    StartFlag = 1
    btnDynaStart.Enabled = False
    btnStaticStart.Enabled = False

    i = 1
    If frequency <> 0 Then
        period = 1000 / frequency
    Else
        period = 0
    End If

    ' Get the start time
    rc = TimerCount(ti)
    startTime = ti.dwmsSinceStart
    lastTime = ti.dwmsSinceStart

    ' Capture data until done or the start flag is turned off
    While i <= count And StartFlag = 1
        rc = TimerCount(ti)
        If (ti.dwmsSinceStart - lastTime) >= period Then
            ' Save the current time
            lastTime = ti.dwmsSinceStart

            ' Save the elapsed time
            DATime(i) = lastTime - startTime

            ' Read sample and hold register for position reading
            DAPos(i) = CLng(32767 * Sin(i * DegToRad)) + 32767
        End If
    Wend
End Sub
' Read analog input for force reading
DAFrc(i) = CLng(1024 * Sin(2 * i * DegToRad)) + 3072

' Update the data panels if frequency is slow enough
If ViewGraphicsEnabled <> 0 Then
    DisplayViewData i, DATime(i), DAPos(i), DAFrc(i)
    panelProgress.FloodPercent = (i * 100) / count
    DrawCurrentData DAPos(i), DAFrc(i)
End If

    i = i + 1
End If
DoEvents
Wend

If i > 1 Then
    DACount = i - 1
Else
    DACount = 0
End If

If i > 1 Then
    ' Update the data panels
    DisplayViewData i - 1, DATime(i - 1), DAPos(i - 1), DAFrc(i - 1)
    panelProgress.FloodPercent = ((i - 1) * 100) / count
End If

btnDynaStart.Enabled = True
btnStaticStart.Enabled = True
StartFlag = 0
End Sub

Sub btnContStart_Click ()
    ' Display a message to indicate no status display
    panelStatusText.Caption = "Continuous capture running"

    DrawBoxInitialize

    If TestCodeEnabled = 0 Then
        RunDataAcqCont ContSampleFreq
    Else
        TestDataAcqCont ContSampleFreq
    End If

    ' Display a message to indicate no status display
    panelStatusText.Caption = "Data acquisition off"
End Sub

Sub btnDynaStart_Click ()
' Display a message to indicate no status display
panelStatusText.Caption = "Dynamic capture running"

DrawBoxInitialize

If TestCodeEnabled = 0 Then
    RunDynamic DynaSampleFreq, DynaSampleCount
Else
    TestDataAcquisition DynaSampleFreq, DynaSampleCount
End If

' Display a message to indicate no status display
panelStatusText.Caption = "Data acquisition off"

End Sub

Sub btnDynaStop_Click()
    If StartFlag = 1 Then
        StartFlag = 0
    End If
End Sub

Sub btnOscStart_Click()
    Dim rc As Integer
    ' Send dynamic oscillation command to controller
    If DynaSmartDownload = 0 Or (WaveFormAmpLast <> DynaWaveFormAmp) Or
       (WaveFormFreqLast <> DynaWaveFormFreq) Then
        Screen.MousePointer = 11 ' Set mouse pointer to hourglass
        If CtrlSetWaveFormO = True And WaveFormCount > 0 Then
            WaveFormAmpLast = DynaWaveFormAmp
            WaveFormFreqLast = DynaWaveFormFreq
        End If
        Screen.MousePointer = 0 ' Restore mouse pointer to normal
    End If
    CtrlStartOscillation
    btnOscStart.Enabled = False
End Sub

Sub btnOscStop_Click()
    ' Send the controller the stop oscillation command
    CtrlHaltOscillation
    btnOscStart.Enabled = True
End Sub

Sub btnStaticStart_Click()
    ' Display a message to indicate no status display
    panelStatusText.Caption = "Static capture running"

    DrawBoxInitialize
If TestCodeEnabled = 0 Then
    RunStatic StatSampleFreq, StatSampleCount
Else
    TestDataAcquisition StatSampleFreq, StatSampleCount
End If

' Display a message to indicate no status display
panelStatusText.Caption = "Data acquisition off"
End Sub

Sub DrawBox_Paint ()
' Clear the graphics before redrawing
DrawBox.Cls
End Sub

Sub DrawBox_Resize ()
    DrawBoxInitialize
End Sub

Sub Form_Load ()
' Read the initialization file
ReadIniFile

' Initialize the DAS1201 board
DASInitialize

' Initialize the communications device
Comm1.Settings = Str$(ComBaud) + "," + ComParity + "," + Str$(ComData) + "," + Str$(ComStop)
Comm1.CommPort = ComPort
Comm1.PortOpen = True

' Set the state of the menu items
If ViewCalibData <> 0 Then
    mnuViewCalib.Checked = True
Else
    mnuViewCalib.Checked = False
End If
If ViewGraphicsEnabled <> 0 Then
    mnuViewGraphics.Checked = True
Else
    mnuViewGraphics.Checked = False
End If

DrawModeXY = False
mnuViewXYMode.Checked = False

DrawPosZoom = 1
DrawTnsZoom = 1
textScale1.Caption = Format(DrawPosZoom, "0")
textScale2.Caption = Format(DrawTnsZoom, "0")

DrawMinPosition = MinPosCount
DrawMinTension = MinTnsCount
If ViewCalibData = 0 Then
    textOffset1.Caption = Format(DrawMinPosition, "0")
    textOffset2.Caption = Format(DrawMinTension, "0")
Else
    textOffset1.Caption = Format(CalibPosition(DrawMinPosition), "0")
    textOffset2.Caption = Format(CalibTension(DrawMinTension), "0")
End If
End Sub

Sub Form_QueryUnload (Cancel As Integer, UnloadMode As Integer)
    WriteIniFile
End Sub

Sub Form_Resize ()
    ' Resize the main panel to fit in the form
    MainPanel.Top = ControlPanel.Top + ControlPanel.Height
    MainPanel.Left = 0
    MainPanel.Width = Form1.ScaleWidth
    If Form1.ScaleHeight > (StatusPanel.Height + ControlPanel.Height) Then
        MainPanel.Height = Form1.ScaleHeight - StatusPanel.Height - ControlPanel.Height
    End If

    ' Resize the draw box to fit in the panel
    DrawBox.Top = 100
    DrawBox.Height = MainPanel.Height - 200
    If MainPanel.Width > (DrawBox.Left + 100) Then
        DrawBox.Width = MainPanel.Width - DrawBox.Left - 100
    End If
End Sub

Sub Form_Unload (Cancel As Integer)
    WriteIniFile
    DASClose
End Sub

Sub mnuAbout_Click ()
    formAboutBox.Show 0
End Sub

Sub mnuCalibDisp_Click ()
    formPosCalib.Show 0
    DrawBoxInitialize
End Sub
Sub mnuCalibTension_Click()
    formTensionCalib.Show 0
End Sub

Sub mnuConfigCont_Click()
    formCont.Show 0
End Sub

Sub mnuConfigDynamic_Click()
    formDyna.Show 0
End Sub

Sub mnuConfigStatic_Click()
    formStatic.Show 0
End Sub

Sub mnuFileExit_Click()
    WriteIniFile
    End
End Sub

Sub mnuFileSave_Click()
    If LCase$(Right$(dataFileName, 4)) = ".cal" Then
        ' Open the calib file and save calib data
        SaveCalibFile (dataFileName)
    Else
        ' Open the file and save the captured data
        SaveDataFile (dataFileName)
    End If
End Sub

Sub mnuFileSaveAs_Click()
    ' CancelError is True
    On Error GoTo ErrHandler

    ' Set filters
    CMDialog1.Filter = "All Files (*.*)|*.*|Config Files(*.cal)*.cal"

    ' Set default filter
    CMDialog1.FilterIndex = 1

    ' Display the Save As dialog box
    CMDialog1.Action = 2

    ' Save the file name
    dataFileName = CMDialog1.Filename

    If LCase$(Right$(dataFileName, 4)) = ".cal" Then
        ' Open the calibration file and save calibration data

    End If
End Sub
SaveCalibFile (dataFileName)
Else
' Open the file and save the captured data
SaveDataFile (dataFileName)
End If

ErrHandler:
' User pressed the cancel button
Exit Sub
End Sub

Sub mnuUtilMove_Click ()
	formMove.Show 0
End Sub

Sub mnuUtilPulseMove_Click ()
	formPulseMove.Show 0
End Sub

Sub mnuUtilZeroPos_Click ()
	CtrlZeroPosition
End Sub

Sub mnuViewCalib_Click ()
' Check this menu item if it is unchecked
If mnuViewCalib.Checked = False Then
	mnuViewCalib.Checked = True
	ViewCalibData = 1
Else
	mnuViewCalib.Checked = False
	ViewCalibData = 0
End If
	DrawBoxInitialize
End Sub

Sub mnuViewGraphics_Click ()
If ViewGraphicsEnabled Then
	ViewGraphicsEnabled = False
	mnuViewGraphics.Checked = False
Else
	ViewGraphicsEnabled = True
	mnuViewGraphics.Checked = True
End If
	DrawBoxInitialize
End Sub

Sub mnuViewXYMode_Click ()
If DrawModeXY Then
	DrawModeXY = False
mnuViewXYMode.Checked = False
Else
    DrawModeXY = True
    mnuViewXYMode.Checked = True
End If
DrawBoxInitialize
End Sub

Sub mnuWavePreview_Click ()
    If WaveFormCount = 0 Then
        MsgBox "No data available for graphing!", 0, "Graph Data Error"
    Else
        formGraph.Show 0
    End If
End Sub

Sub spinOffset1_SpinDown ()
    DrawMinPosition = DrawMinPosition - PosSpinIncrement
    If DrawMinPosition < MinPosCount Then
        DrawMinPosition = MinPosCount
    End If
    If DrawMinPosition > MaxPosCount Then
        DrawMinPosition = MaxPosCount
    End If
    If ViewCalibData = 0 Then
        textOffset1.Caption = Format(DrawMinPosition, "0")
    Else
        textOffset1.Caption = Format(CalibPosition(DrawMinPosition), "0")
    End If
    DrawBoxInitialize
End Sub

Sub spinOffset1_SpinUp ()
    DrawMinPosition = DrawMinPosition + PosSpinIncrement
    If DrawMinPosition < MinPosCount Then
        DrawMinPosition = MinPosCount
    End If
    If DrawMinPosition > MaxPosCount Then
        DrawMinPosition = MaxPosCount
    End If
    If ViewCalibData = 0 Then
        textOffset1.Caption = Format(DrawMinPosition, "0")
    Else
        textOffset1.Caption = Format(CalibPosition(DrawMinPosition), "0")
    End If
    DrawBoxInitialize
End Sub

Sub spinOffset2_SpinDown ()
DrawMinTension = DrawMinTension - TnsSpinIncrement
If DrawMinTension < MinTnsCount Then
    DrawMinTension = MinTnsCount
End If
If DrawMinTension > MaxTnsCount Then
    DrawMinTension = MaxTnsCount
End If
If ViewCalibData = 0 Then
    textOffset2.Caption = Format(DrawMinTension, "0")
Else
    textOffset2.Caption = Format(CalibTension(DrawMinTension), "0")
End If
DrawBoxInitialize
End Sub

Sub spinOffset2_SpinUp()
    DrawMinTension = DrawMinTension + TnsSpinIncrement
    If DrawMinTension < MinTnsCount Then
        DrawMinTension = MinTnsCount
    End If
    If DrawMinTension > MaxTnsCount Then
        DrawMinTension = MaxTnsCount
    End If
    If ViewCalibData = 0 Then
        textOffset2.Caption = Format(DrawMinTension, "0")
    Else
        textOffset2.Caption = Format(CalibTension(DrawMinTension), "0")
    End If
    DrawBoxInitialize
End Sub

Sub spinScale1_SpinDown()
    DrawPosZoom = DrawPosZoom - 1
    If DrawPosZoom < 1 Then
        DrawPosZoom = 1
    End If
    If DrawPosZoom > 99 Then
        DrawPosZoom = 99
    End If
    textScale1.Caption = Format(DrawPosZoom, "0")
    DrawBoxInitialize
End Sub

Sub spinScale1_SpinUp()
    DrawPosZoom = DrawPosZoom + 1
    If DrawPosZoom < 1 Then
        DrawPosZoom = 1
    End If
    If DrawPosZoom > 99 Then

250
Sub spinScale2_SpinDown ()
    DrawTnsZoom = DrawTnsZoom - 1
    If DrawTnsZoom < 1 Then
        DrawTnsZoom = 1
    End If
    If DrawTnsZoom > 99 Then
        DrawTnsZoom = 99
    End If
    textScale2.Caption = Format(DrawTnsZoom, "0")
    DrawBoxInitialize
End Sub

Sub spinScale2_SpinUp ()
    DrawTnsZoom = DrawTnsZoom + 1
    If DrawTnsZoom < 1 Then
        DrawTnsZoom = 1
    End If
    If DrawTnsZoom > 99 Then
        DrawTnsZoom = 99
    End If
    textScale2.Caption = Format(DrawTnsZoom, "0")
    DrawBoxInitialize
End Sub

Sub TimerPulse_Timer ()
    Dim direction As Integer
    Dim steps As Integer

    ' Turn off the timer
    TimerPulse.Enabled = False

    If Pulse.overshootFlag > 0 Then
        ' Turn off the overshoot flag
        Pulse.overshootFlag = 0

        ' Set the move parameters for the return of the overshoot
        direction = Pulse.direction
        steps = Pulse.overshootSteps
    Else
        ' Set the direction to the reverse of the last move
        If Pulse.direction <> 0 Then
            direction = 0
        Else

            251
direction = 1
End If

' If this pulse is supposed to overshoot then adjust steps
If Pulse.overshoot > 0 And Pulse.rate <> 0 Then
    ' Set the flag to indicate an overshoot
    Pulse.overshootFlag = 1

    ' Set the steps equal to the pulse height plus the overshoot
    steps = Pulse.steps + Pulse.overshootSteps
Else
    ' Set the steps equal to the height of the pulse
    steps = Pulse.steps
End If
End If

' Do the move as determined above
CtrlMove direction, Pulse.tcPerStep, steps

If Pulse.overshoot > 0 And Pulse.rate <> 0 Then
    ' Set the timer interval
    TimerPulse.Interval = CInt((Pulse.rampTime + (Pulse.overshoot / Pulse.rate) +
        Pulse.minPause) * 1000)
    TimerPulse.Enabled = True
End If
End Sub
FORMCONT.FRM

OPTION EXPLICIT

Sub btnClose_Click()
    ' Unload the form
    Unload formCont
End Sub

Sub btnOk_Click()
    ' Save all the data from the edit boxes
    ContSampleFreq = Val(editFrequency.Text)
    ' Unload the form
    Unload formCont
End Sub

Sub Form_Load()
    ' Stuff the data into the edit boxes
    editFrequency.Text = Format$(ContSampleFreq, "0.00")
End Sub
OPTION EXPLICIT ' FORCE VARIABLE DECLARATION.

Sub btnCancel_Click ()
' Unload the form
Unload formDyna
End Sub

Sub btnOk_Click ()
Dim i As Integer
Dim k As Double
Dim period As Double
Dim amplitude As Double
Dim numCount As Integer
Dim elapsedTime As Long
Dim elapsedTimeLast As Long

' Save all the data from the edit boxes
DynaSampleFreq = Val(editAcqFreq.Text)
DynaSampleCount = Val(editAcqCount.Text)
DynaWaveFormFreq = Val(editWavFreq.Text)
DynaWaveFormAmp = Val(editWavAmp.Text)
DynaSmartDownload = btnSmartDownload.Value

' Initialize calculation variables
amplitude = DynaWaveFormAmp / 2 ' Calculate Peak to Peak amplitude in microns
period = TimeCountsPerSec / DynaWaveFormFreq ' Calculate the period in units of 500 ns
k = period / (4 * HalfPi) ' Calculate multiplier

numCount = CInt(amplitude / MicronsPerStep)
elapsedTimeLast = 0
For i = 1 To numCount
    elapsedTime = Arcsin(i / numCount) * k
    WaveFormData(i) = elapsedTime - elapsedTimeLast
    elapsedTimeLast = elapsedTime
Next
WaveFormCount = numCount

' Save data to a file
Open "WFDATA.DAT" For Output As # 1 ' Open file.
For i = 1 To WaveFormCount
    Print #1, Format$(i * MicronsPerStep, "0.00") + "," + Format$(WaveFormData(i), "0") + ",
" + FormatHex(WaveFormData(i))
Next
Close ' Close all files.

' Unload the form
Unload formDyna
End Sub

Sub Form_Load()
    ' Stuff the data into the edit boxes
    editAcqFreq.Text = Format$(DynaSampleFreq, "0.00")
    editAcqCount.Text = Format$(DynaSampleCount, "0")
    editWavFreq.Text = Format$(DynaWaveFormFreq, "0.00")
    editWavAmp.Text = Format$(DynaWaveFormAmp, "0.00")
    btnSmartDownload.Value = DynaSmartDownload
End Sub
Sub Form_Load()
    Dim i As Integer
    Dim elapsedTime As Long

    Graph1.AutoInc = 0
    Graph1.NumPoints = WaveFormCount * 2
    Graph1.NumSets = 1
    Graph1.ThisPoint = 1
    Graph1.SymbolData = 0

    elapsedTime = 0
    For i = 1 To WaveFormCount
        Graph1.ThisPoint = i
        Graph1.GraphData = i * 2.5
        elapsedTime = elapsedTime + WaveFormData(i)
        Graph1.XPosData = elapsedTime
    Next

    For i = 1 To WaveFormCount
        Graph1.ThisPoint = i + WaveFormCount
        Graph1.GraphData = (WaveFormCount * 2.5) - (i * 2.5)
        elapsedTime = elapsedTime + WaveFormData(WaveFormCount + 1 - i)
        Graph1.XPosData = elapsedTime
    Next

    Graph1.DrawMode = 2
End Sub

Sub Form_Resize()
    Graph1.Top = 0
    Graph1.Left = 0
    Graph1.Width = formGraph.ScaleWidth
    Graph1.Height = formGraph.ScaleHeight
End Sub
Sub btnClose_Click()
    ' Read the move steps from the edit box
    MoveSpeed = Val(editSpeed.Text)

    ' Determine the direction of the move
    If btnUp.Value = True Then
        MoveDirection = 1
    Else
        MoveDirection = 0
    End If

    Unload formMove
End Sub

Sub btnMove_Click()
    Dim tcPerStep As Integer
    Dim steps As Integer

    ' Read the move steps from the edit box
    MoveSpeed = Val(editSpeed.Text)
    tcPerStep = (MicronsPerStep * TimeCountsPerSec) / MoveSpeed

    ' Read the move steps from the edit box
    MoveDistance = Val(editSteps.Text)
    steps = CInt(MoveDistance / MicronsPerStep)

    ' Determine the direction of the move
    If btnUp.Value = True Then
        MoveDirection = 1
    Else
        MoveDirection = 0
    End If

    ' Send the position command to the controller
    CtrlMove MoveDirection, tcPerStep, steps
End Sub

Sub btnStop_Click()
    ' Send the stop move command to controller
    CtrlStop
End Sub

Sub Form_Load()
' Initialize the form controls
editSpeed.Text = Format$(MoveSpeed, "0.0")
editSteps.Text = Format$(MoveDistance, "0.00")
If MoveDirection = 0 Then
    btnUp.Value = False
    btnDn.Value = True
Else
    btnUp.Value = True
    btnDn.Value = False
End If

End Sub
Sub btnCalibrate_Click()
    Dim dy As Double
    Dim dx As Double

    ' Read the edit boxes
    CalibPosCount1 = Val(editCount1.Text)
    CalibPosCount2 = Val(editCount2.Text)
    CalibPosMeasured1 = Val(editMeasured1.Text)
    CalibPosMeasured2 = Val(editMeasured2.Text)

    ' Calculate the slope and intercept
    dx = CalibPosCount2 - CalibPosCount1
    dy = CalibPosMeasured2 - CalibPosMeasured1
    If dx <> 0 Then
        CalibPosSlope = dy / dx
        CalibPosIntercept = CalibPosMeasured1 - (CalibPosSlope * CalibPosCount1)
    Else
        MsgBox "Calibration could not be performed because of a divide by zero", 0, "Calibration Error"
        CalibPosSlope = 1
        CalibPosIntercept = 0
    End If

    staticSlope.Caption = Format$(CalibPosSlope, "0.000")
    staticIntercept.Caption = Format$(CalibPosIntercept, "0")
End Sub

Sub btnClose_Click()
    Unload formPosCalib
    DrawBoxInitialize
End Sub

Sub Form_Load()
    ' Initialize the edit boxes
    editCount1.Text = Format$(CalibPosCount1, "0")
    editCount2.Text = Format$(CalibPosCount2, "0")
    editMeasured1.Text = Format$(CalibPosMeasured1, "0")
    editMeasured2.Text = Format$(CalibPosMeasured2, "0")

    ' Initialize the static text
    staticSlope.Caption = Format$(CalibPosSlope, "0.000")
    staticIntercept.Caption = Format$(CalibPosIntercept, "0")
End Sub
Sub btnClose_Click ()
    ' Read the move steps from the edit box
    Pulse.rate = Val(editPulseRate.Text)

    ' Read the move steps from the edit box
    Pulse.height = Val(editPulseHeight.Text)

    ' Read the duration of the pulse
    Pulse.duration = Val(editPulseDuration.Text)

    ' Read the duration of the pulse overshoot
    Pulse.overshoot = Val(editPulseOvershoot.Text)

    Unload formPulseMove
End Sub

Sub btnMove_Click ()
    ' Read the move steps from the edit box
    Pulse.rate = Val(editPulseRate.Text)
    ' Read the move steps from the edit box
    Pulse.height = Val(editPulseHeight.Text)
    ' Read the duration of the pulse
    Pulse.duration = Val(editPulseDuration.Text)
    ' Read the duration of the pulse overshoot
    Pulse.overshoot = Val(editPulseOvershoot.Text)

    ' Calculate the overshoot if any
    If Pulse.overshoot > 0 Then
        Pulse.overshootSteps = CInt(Pulse.overshoot / MicronsPerStep)
    Else
        Pulse.overshootSteps = 0
    End If

    ' Determine the direction of the pulse move
    If Pulse.height < 0 Then
        ' If the pulse height is less then 0 then the direction is up
        Pulse.direction = 1
        Pulse.steps = CInt(-Pulse.height / MicronsPerStep) + Pulse.overshootSteps
    Else
        ' If the pulse height is greater then or equal to 0 then the direction is down
        Pulse.direction = 0
        Pulse.steps = CInt(Pulse.height / MicronsPerStep) + Pulse.overshootSteps
    End If

    If Pulse.rate > 0 Then
        ' Calculate the time counts per step for the move rate
Pulse.tcPerStep = (MicronsPerStep * TimeCountsPerSec) / Pulse.rate

' Determine the amount of time it takes to reach the pulse height
Pulse.rampTime = Abs(Pulse.height / Pulse.rate)

' Determine the amount of time for the overshoot if any
If Pulse.overshoot > 0 Then
    Pulse.overshootTime = Pulse.overshoot / Pulse.rate
    If Pulse.duration < ((Pulse.rampTime * 2) + (Pulse.minPause * 2) + (Pulse.overshootTime * 2)) Then
        Pulse.duration = (Pulse.rampTime * 2) + (Pulse.minPause * 2) + (Pulse.overshootTime * 2)
        editPulseDuration.Text = Format$(Pulse.duration, "0.0")
    End If
Else
    Pulse.overshootTime = 0
    If Pulse.duration < ((Pulse.rampTime * 2) + Pulse.minPause) Then
        Pulse.duration = (Pulse.rampTime * 2) + Pulse.minPause
        editPulseDuration.Text = Format$(Pulse.duration, "0.0")
    End If
End If

' Turn off the pulse overshoot flag for now
Pulse.overshootFlag = 0

' Set the timer interval
Form1.TimerPulse.Interval = CInt((Pulse.duration - Pulse.rampTime) * 1000)
Form1.TimerPulse.Enabled = True

' Send the position command to the controller
CtrlMove Pulse.direction, Pulse.tcPerStep, Pulse.steps
End If
End Sub

Sub btnStop_Click()
' Send the stop move command to controller
CtrlStop
End Sub

Sub Form_Load()
' Initialize the form controls
editPulseHeight.Text = Format$(Pulse.height, "0.0")
editPulseDuration.Text = Format$(Pulse.duration, "0.0")
editPulseRate.Text = Format$(Pulse.rate, "0.0")
editPulseOvershoot.Text = Format$(Pulse.overshoot, "0.0")
End Sub
FORMSTAT.FRM
OPTION EXPLICIT ' FORCE VARIABLE DECLARATION.

Sub btnClose_Click ()
' Unload the form
Unload formStatic
End Sub

Sub btnOk_Click ()
' Save all the data from the edit boxes
StatSampleFreq = Val(editFrequency.Text)
StatSampleCount = Val(editSamples.Text)

' Unload the form
Unload formStatic
End Sub

Sub Form_Load ()
' Stuff the data into the edit boxes
editFrequency.Text = Format$(StatSampleFreq, "0.00")
editSamples.Text = Format$(StatSampleCount, "0")
End Sub
Sub btnCalibrate_Click()
    Dim dy As Double
    Dim dx As Double
    Dim maxTension As Double

    ' Read the edit boxes
    maxTension = Val(editMaxTension.Text)
    CalibTensionCount1 = Val(editCount1.Text)
    CalibTensionCount2 = Val(editCount2.Text)
    CalibTensionMeasured1 = Val(editMeasured1.Text)
    CalibTensionMeasured2 = Val(editMeasured2.Text)

    ' Calculate the slope and intercept
    dy = CalibTensionMeasured2 - CalibTensionMeasured1
    dx = CalibTensionCount2 - CalibTensionCount1
    If dx <> 0 Then
        CalibTensionSlope = dy / dx
        CalibTensionIntercept = CalibTensionMeasured1 - (CalibTensionSlope * CalibTensionCount1)
    Else
        MsgBox "Calibration could not be performed because of a divide by zero", 0, "Calibration Error"
        CalibTensionSlope = 1
        CalibTensionIntercept = 0
    End If

    CalibTensionMax = (maxTension - CalibTensionIntercept) / CalibTensionSlope
    staticSlope.Caption = Format$(CalibTensionSlope, "0.000")
    staticIntercept.Caption = Format$(CalibTensionIntercept, "0")
End Sub

Sub btnClose_Click()
    Unload formTensionCalib
    DrawBoxInitialize
End Sub

Sub Form_Load()
    ' Initialize the edit boxes
    editMaxTension.Text = Format$(CalibTension(CalibTensionMax), "0")
    editCount1.Text = Format$(CalibTensionCount1, "0")
    editCount2.Text = Format$(CalibTensionCount2, "0")
    editMeasured1.Text = Format$(CalibTensionMeasured1, "0")
    editMeasured2.Text = Format$(CalibTensionMeasured2, "0")

    ' Initialize the static text
staticSlope.Caption = Format$(CalibTensionSlope, "0.000")
staticIntercept.Caption = Format$(CalibTensionIntercept, "0")
End Sub
DEFS.TXT

' Declarations for TOOLHELP.DLL API functions
Type TIMERINFO
    dwSize As Long
    dwmsSinceStart As Long
    dwmsThisVm As Long
End Type

Declare Function TimerCount Lib "toolhelp.dll" (lpti As TIMERINFO) As Integer
GLOBAL.BAS

Option Explicit ' Force variable declaration.

Global Const Pi = 3.14159265359
Global Const HalfPi = 1.570796326795
Global Const DegToRad = .01745329251994
Global Const MaxDACount = 32766
Global Const MaxWaveFormCount = 1024
Global Const MaxPosCount = 65535
Global Const MinPosCount = 0
Global Const MaxTnsCount = 4095
Global Const MinTnsCount = 2048
Global Const DrawBoxLeftMargin = 60
Global Const DrawBoxRightMargin = 20
Global Const DrawBoxBottomMargin = 20
Global Const DrawBoxTopMargin = 20
Global Const PosSpinIncrement = 1024
Global Const TnsSpinIncrement = 128

' Configuration file names for DAS1201 board
Global Const adConfigFileName = "das1600.cfg"
Global Const diConfigFileName = "das1600.cfg"
Global Const doConfigFileName = "das1600.cfg"

Type LineEqn
    m As Double
    b As Integer
End Type

Type DAREcType
    dt As Long
    pos As Long
    frc As Long
End Type

Type PulseMoveType
    height As Double
    rate As Double
    duration As Double
    minPause As Double
    rampTime As Double
    direction As Integer
    tcPerStep As Integer
    steps As Integer
    overshoot As Double
    overshootSteps As Integer
    overshootFlag As Integer
    overshootTime As Double
End Type
End Type

Global Pulse As PulseMoveType
Global DACount As Long
Global DTabe(MaxDACount) As DAREcType
Global DTme(MaxDACount) As Long
Global DPos(MaxDACount) As Long
Global DFrc(MaxDACount) As Long

' Static data acquisition global variables
Global StatSampleFreq As Double
Global StatSampleCount As Long
Global StatGraphics As Integer

' Dynamic data acquisition global variables
Global DynWaveFormFreq As Double
Global DynWaveFormAmp As Integer
Global DynSampleFreq As Double
Global DynSampleCount As Long
Global DynGraphics As Integer
Global DynSmartDownload As Integer
Global WaveFormFreqLast As Double
Global WaveFormAmpLast As Double

' Continuous data acquisition global variables
Global ContSampleFreq As Double
Global ContGraphXY As Integer

' Communications global variables
Global ComPort As Integer
Global ComBaud As Integer
Global ComParity As String
Global ComData As Integer
Global ComStop As Integer
Global ComHandshake As String

' Oscillation waveform global variables
Global WaveFormCount As Integer
Global WaveFormData(MaxWaveFormCount) As Long

' Position calibration global variables
Global CalibPosCount1 As Long
Global CalibPosCount2 As Long
Global CalibPosMeasured1 As Double
Global CalibPosMeasured2 As Double
Global CalibPosSlope As Double
Global CalibPosIntercept As Double

' Tension calibration global variables
Global CalibTensionMax As Long
Global CalibTensionCount1 As Long
Global CalibTensionCount2 As Long
Global CalibTensionMeasured1 As Double
Global CalibTensionMeasured2 As Double
Global CalibTensionSlope As Double
Global CalibTensionIntercept As Double

' Manual move global variables
Global MoveDirection As Integer
Global MoveSpeed As Double
Global MoveDistance As Double

' Miscellaneous global variables
Global TimeCountsPerSec As Long
Global MicronsPerStep As Double
Global dataFileName As String
Global dataFileDir As String
Global DAMaxLoad As Integer
Global DAMaxPos As Integer
Global DAMinPos As Integer
Global DASInitialized As Integer
Global ViewCalibData As Integer
Global ViewGraphicsEnabled As Integer
Global StartFlag As Integer
Global TestCodeEnabled As Integer
Global xGraph As Integer
Global DrawPosEqn As LineEqn
Global DrawTnsEqn As LineEqn
Global DrawModeXY As Integer
Global DrawPosZoom As Integer
Global DrawTnsZoom As Integer
Global DrawMinTension As Long
Global DrawMinPosition As Long

Sub ReadIniFile()
    Dim tempStr As String
    Dim fileName As String
    Dim rc As Integer

    fileName = "wdas.ini"
    tempStr = Space$(32)

    ' Read the [File] section of the ini file
    rc = GetPrivateProfileString("File", "LastFile", ",", tempStr, 32, fileName)
    dataFileName = tempStr
    rc = GetPrivateProfileString("File", "LastDir", ",", tempStr, 32, fileName)

End Sub
dataFileDir = tempStr

' Read the [CalibPos] section of the ini file
rc = GetPrivateProfileString("CalibPos", "Count1", "100", tempStr, 32, fileName)
CalibPosCount1 = Val(tempStr)
rc = GetPrivateProfileString("CalibPos", "Count2", "200", tempStr, 32, fileName)
CalibPosCount2 = Val(tempStr)
rc = GetPrivateProfileString("CalibPos", "Measured1", "100", tempStr, 32, fileName)
CalibPosMeasured1 = Val(tempStr)
rc = GetPrivateProfileString("CalibPos", "Measured2", "100", tempStr, 32, fileName)
CalibPosMeasured2 = Val(tempStr)
rc = GetPrivateProfileString("CalibPos", "Slope", "1.0", tempStr, 32, fileName)
CalibPosSlope = Val(tempStr)
rc = GetPrivateProfileString("CalibPos", "Intercept", "0", tempStr, 32, fileName)
CalibPosIntercept = Val(tempStr)

' Read the [CalibTension] section of the ini file
rc = GetPrivateProfileString("CalibTension", "Max", "0", tempStr, 32, fileName)
CalibTensionMax = Val(tempStr)
rc = GetPrivateProfileString("CalibTension", "Count1", "100", tempStr, 32, fileName)
CalibTensionCount1 = Val(tempStr)
rc = GetPrivateProfileString("CalibTension", "Count2", "200", tempStr, 32, fileName)
CalibTensionCount2 = Val(tempStr)
rc = GetPrivateProfileString("CalibTension", "Measured1", "100", tempStr, 32, fileName)
CalibTensionMeasured1 = Val(tempStr)
rc = GetPrivateProfileString("CalibTension", "Measured2", "100", tempStr, 32, fileName)
CalibTensionMeasured2 = Val(tempStr)
rc = GetPrivateProfileString("CalibTension", "Slope", "1.0", tempStr, 32, fileName)
CalibTensionSlope = Val(tempStr)
rc = GetPrivateProfileString("CalibTension", "Intercept", "0", tempStr, 32, fileName)
CalibTensionIntercept = Val(tempStr)

' Read the [Continuous] section of the ini file
rc = GetPrivateProfileString("Continuous", "SampleFreq", "10", tempStr, 32, fileName)
ContSampleFreq = Val(tempStr)
rc = GetPrivateProfileString("Continuous", "GraphMode", "0", tempStr, 32, fileName)
ContGraphXY = Val(tempStr)

' Read the [Static] section of the ini file
rc = GetPrivateProfileString("Static", "SampleFreq", "10", tempStr, 32, fileName)
StatSampleFreq = Val(tempStr)
rc = GetPrivateProfileString("Static", "SampleCount", "1000", tempStr, 32, fileName)
StatSampleCount = Val(tempStr)
rc = GetPrivateProfileString("Static", "Graphics", "OFF", tempStr, 32, fileName)
StatGraphics = Val(tempStr)

' Read the [Dynamic] section of the ini file
rc = GetPrivateProfileString("Dynamic", "WaveFormFreq", "10", tempStr, 32, fileName)
DynaWaveFormFreq = Val(tempStr)
rc = GetPrivateProfileString("Dynamic", "WaveFormAmp", "1024", tempStr, 32, fileName)
DynaWaveFormAmp = Val(tempStr)
rc = GetPrivateProfileString("Dynamic", "SampleFreq", "50", tempStr, 32, fileName)
DynaSampleFreq = Val(tempStr)
rc = GetPrivateProfileString("Dynamic", "SampleCount", "1000", tempStr, 32, fileName)
DynaSampleCount = Val(tempStr)
rc = GetPrivateProfileString("Dynamic", "SmartDownload", "1", tempStr, 32, fileName)
DynaSmartDownload = Val(tempStr)

' Read the [Com] section of the ini file
rc = GetPrivateProfileString("Com", "Port", "2", tempStr, 32, fileName)
ComPort = Val(tempStr)
rc = GetPrivateProfileString("Com", "Baud", "9600", tempStr, 32, fileName)
ComBaud = Val(tempStr)
rc = GetPrivateProfileString("Com", "Parity", "N", tempStr, 32, fileName)
ComParity = tempStr
rc = GetPrivateProfileString("Com", "Data", "8", tempStr, 32, fileName)
ComData = Val(tempStr)
rc = GetPrivateProfileString("Com", "Stop", "1", tempStr, 32, fileName)
ComStop = Val(tempStr)
rc = GetPrivateProfileString("Com", "Handshake", "NONE", tempStr, 32, fileName)
ComHandshake = tempStr

' Read the [ManualMove] section of the ini file
rc = GetPrivateProfileString("ManualMove", "Direction", "0", tempStr, 32, fileName)
MoveDirection = Val(tempStr)
MoveSpeed = Val(tempStr)
rc = GetPrivateProfileString("ManualMove", "Distance", "1000", tempStr, 32, fileName)
MoveDistance = Val(tempStr)

' Read the [PulseMove] section of the ini file
rc = GetPrivateProfileString("PulseMove", "Height", "0", tempStr, 32, fileName)
Pulse.height = Val(tempStr)
rc = GetPrivateProfileString("PulseMove", "Rate", "0", tempStr, 32, fileName)
Pulse.rate = Val(tempStr)
rc = GetPrivateProfileString("PulseMove", "Duration", "0", tempStr, 32, fileName)
Pulse.duration = Val(tempStr)
rc = GetPrivateProfileString("PulseMove", "MinPause", "0.1", tempStr, 32, fileName)
Pulse.minPause = Val(tempStr)
rc = GetPrivateProfileString("PulseMove", "Overshoot", "0", tempStr, 32, fileName)
Pulse.overshoot = Val(tempStr)

' Read the [Controller] section of the ini file
rc = GetPrivateProfileString("Controller", "TimeCountsPerSec", "2000000", tempStr, 32, fileName)
TimeCountsPerSec = Val(tempStr)
rc = GetPrivateProfileString("Controller", "MicronsPerStep", "2.5", tempStr, 32, fileName)
MicronsPerStep = Val(tempStr)

270
' Read the [Misc] section of the ini file
rc = GetPrivateProfileString("Misc", "ViewDataCalib", "0", tempStr, 32, fileName)
ViewCalibData = Val(tempStr)
rc = GetPrivateProfileString("Misc", "GraphicsEnabled", "0", tempStr, 32, fileName)
ViewGraphicsEnabled = Val(tempStr)
rc = GetPrivateProfileString("Misc", "TestCodeEnabled", "0", tempStr, 32, fileName)
TestCodeEnabled = Val(tempStr)
End Sub

Sub WriteIniFile()
    Dim tempStr As String
    Dim fileName As String
    Dim rc As Integer

    fileName = "wdas.ini"
    tempStr = Space$(32)

    ' Write the [File] section of the ini file
    rc = WritePrivateProfileString("File", "LastFile", dataFileName, fileName)
    rc = WritePrivateProfileString("File", "LastDir", dataFileDir, fileName)

    ' Write the [CalibPos] section of the ini file
    rc = WritePrivateProfileString("CalibPos", "Count1", Format$(CalibPosCount1, "0"), fileName)
    rc = WritePrivateProfileString("CalibPos", "Count2", Format$(CalibPosCount2, "0"), fileName)
    rc = WritePrivateProfileString("CalibPos", "Measured1", Format$(CalibPosMeasured1, "0"), fileName)
    rc = WritePrivateProfileString("CalibPos", "Measured2", Format$(CalibPosMeasured2, "0"), fileName)
    rc = WritePrivateProfileString("CalibPos", "Slope", Format$(CalibPosSlope, "0.000"), fileName)
    rc = WritePrivateProfileString("CalibPos", "Intercept", Format$(CalibPosIntercept, "0"), fileName)

    ' Write the [CalibTension] section of the ini file
    rc = WritePrivateProfileString("CalibTension", "Max", Format$(CalibTensionMax, "0"), fileName)
    rc = WritePrivateProfileString("CalibTension", "Count1", Format$(CalibTensionCount1, "0"), fileName)
    rc = WritePrivateProfileString("CalibTension", "Count2", Format$(CalibTensionCount2, "0"), fileName)
    rc = WritePrivateProfileString("CalibTension", "Measured1", Format$(CalibTensionMeasured1, "0"), fileName)
    rc = WritePrivateProfileString("CalibTension", "Measured2", Format$(CalibTensionMeasured2, "0"), fileName)
End Sub
rc = WritePrivateProfileString("CalibTension", "Slope", Format$(CalibTensionSlope, "0.000"), fileName)
rc = WritePrivateProfileString("CalibTension", "Intercept", Format$(CalibTensionIntercept, "0"), fileName)

' Read the [Continuous] section of the ini file
rc = WritePrivateProfileString("Continuous", "SampleFreq", Format$(ContSampleFreq, "0.00"), fileName)
rc = WritePrivateProfileString("Continuous", "GraphMode", Format$(ContGraphXY, "0"), fileName)

' Write the [Static] section of the ini file
rc = WritePrivateProfileString("Static", "SampleFreq", Format$(StatSampleFreq, "0.00"), fileName)
rc = WritePrivateProfileString("Static", "SampleCount", Format$(StatSampleCount, "0"), fileName)
rc = WritePrivateProfileString("Static", "Graphics", Format$(StatGraphics, "0"), fileName)

' Write the [Dynamic] section of the ini file
rc = WritePrivateProfileString("Dynamic", "WaveFormFreq", Format$(DynaWaveFormFreq, "0.00"), fileName)
rc = WritePrivateProfileString("Dynamic", "WaveFormAmp", Format$(DynaWaveFormAmp, "0.00"), fileName)
rc = WritePrivateProfileString("Dynamic", "SampleFreq", Format$(DynaSampleFreq, "0.00"), fileName)
rc = WritePrivateProfileString("Dynamic", "SampleCount", Format$(DynaSampleCount, "0"), fileName)
rc = WritePrivateProfileString("Dynamic", "SmartDownload", Format$(DynaSmartDownload, "0"), fileName)

' Write the [Com] section of the ini file
rc = WritePrivateProfileString("Com", "Port", Format$(ComPort, "0"), fileName)
rc = WritePrivateProfileString("Com", "Baud", Format$(ComBaud, "0"), fileName)
rc = WritePrivateProfileString("Com", "Parity", ComParity, fileName)
rc = WritePrivateProfileString("Com", "Data", Format$(ComData, "0"), fileName)
rc = WritePrivateProfileString("Com", "Stop", Format$(ComStop, "0"), fileName)
rc = WritePrivateProfileString("Com", "Handshake", ComHandshake, fileName)

' Write the [ManualMove] section of the ini file
rc = WritePrivateProfileString("ManualMove", "Direction", Format$(MoveDirection, "0"), fileName)
rc = WritePrivateProfileString("ManualMove", "Speed", Format$(MoveSpeed, "0.0"), fileName)
rc = WritePrivateProfileString("ManualMove", "Distance", Format$(MoveDistance, "0.00"), fileName)

' Write the [PulseMove] section of the ini file
rc = WritePrivateProfileString("PulseMove", "Height", Format$(Pulse.height, "0.0"), fileName)
rc = WritePrivateProfileString("PulseMove", "Rate", Format$(Pulse-rate, "0.0"), fileName)
rc = WritePrivateProfileString("PulseMove", "Duration", Format$(Pulse.duration, "0.0"), fileName)
rc = WritePrivateProfileString("PulseMove", "MinPause", Format$(Pulse.minPause, "0.00"), fileName)
rc = WritePrivateProfileString("PulseMove", "Overshoot", Format$(Pulse.overshoot, "0.0"), fileName)

' Write the [Controller] section of the ini file
rc = WritePrivateProfileString("Controller", "TimeCountsPerSec", Format$(TimeCountsPerSec, "0"), fileName)
rc = WritePrivateProfileString("Controller", "MicronsPerStep", Format$(MicronsPerStep, "0.000"), fileName)

' Write the [Misc] section of the ini file
rc = WritePrivateProfileString("Misc", "ViewDataCalib", Format$(ViewCalibData, "0"), fileName)
rc = WritePrivateProfileString("Misc", "GraphicsEnabled", Format$(ViewGraphicsEnabled, "0"), fileName)
End Sub
UTIL.BAS
Option Explicit ' Force variable declaration.
Global Const vScrollMax = 50
Global Const hScrollMax = 50

Function Arcsin (x As Double) As Double
    If x = 1 Then
        Arcsin = 1.570796326795
    Else
        Arcsin = Atn(x / Sqr(1 - (x * x)))
    End If
End Function

Function CalibPosition (count As Long) As Double
    CalibPosition = (CalibPosSlope * count) + CalibPosIntercept
End Function

Function CalibTension (count As Long) As Double
    CalibTension = (CalibTensionSlope * count) + CalibTensionIntercept
End Function

Function CommWait (count As Integer) As Integer
    Dim tempStr As String
    Dim delay As Integer

    ' Do
    ' Loop Until Form 1. Comm 1. InBufferCount >= count
    delay = 10000
    Do While Form 1. Comm 1. InBufferCount < count
        If delay > 0 Then
            delay = delay - 1
        Else
            MsgBox "Communications timeout", MB_ICONSTOP
            CommWait = False
            Exit Function
        End If
    Loop

    tempStr = Form 1. Comm 1. Input
    CommWait = True
End Function

Sub CtrlHaltOscillation ()
    ' Halt the controller
    Form 1. Comm 1. Output = "H"
End Sub

Sub CtrlMove (direction As Integer, speed As Integer, steps As Integer)
' Clear the output buffer first
Form1.Comm1.OutBufferCount = 0

' Set the direction
If direction = 0 Then
    Form1.Comm1.Output = "-"
Else
    Form1.Comm1.Output = "+
End If

' Validate limits on speed
If speed < 64 Then
    speed = 64
End If

' Set the move rate
Form1.Comm1.Output = "R" + FormatHex(CLng(speed))

' Set the number of steps
Form1.Comm1.Output = "S" + FormatHex(CLng(steps))

' Send the start move command
Form1.Comm1.Output = "G"
End Sub

Function CtrlSetWaveForm () As Integer
    Dim i As Integer
    Dim waveData As Long

    If WaveFormCount > 0 Then
        ' Clear the output buffer first
        Form1.Comm1.OutBufferCount = 0

        ' Send the calculated waveform to the controller
        Form1.Comm1.Output = "L"
        If CommWait(l) = False Then
            CtrlSetWaveForm = False
            Exit Function
        End If

        ' Output all of the waveform delta time values
        For i = 1 To WaveFormCount
            ' Modify data to suit the requirements of the controller
            If WaveFormData(i) > 3 Then
                waveData = WaveFormData(i) - 3
            Else
                waveData = 1
            End If

    End If
' Output the waveform data in hex to the comm port
Form1.Comm1.Output = "," + FormatHex(waveData)

' Wait for 5 data bytes from the comm port
If CommWait(5) = False Then
    CtrlSetWaveForm = False
    Exit Function
End If

' Update the percent done display
Form1.panelProgress.FloodPercent = (i * 100) / WaveFormCount
Next

' Send the end of waveform terminator
Form1.Comm1.Output = "Q"
If CommWait(1) = False Then
    CtrlSetWaveForm = False
    Exit Function
End If
End If
CtrlSetWaveForm = True
End Function

Sub CtrlStartOscillation ()
    ' Start the controller oscillation cycle
    Form1.Comm1.Output = "O"
End Sub

Sub CtrlStop ()
    ' Send the Stop command to the controller
    Form1.Comm1.Output = "X"
End Sub

Sub CtrlZeroPosition ()
    ' Send the Zero Position command to the controller
    Form1.Comm1.Output = "Z"
End Sub

Function DASAnalogInput () As Long
    ' Jump to the error handler if we get an error
    On Error GoTo DASAErrrorHandler
    Form1.DAS1600AD1.ADStartStop = 1 ' Initiates the operation
    ' Read the data and divide by 16 to decode data
    DASAnalogInput = Form1.DAS1600AD1.ADSingleRead / 16

    ' Exit the subroutine
    Exit Function
End Function
DASAIErrorHandler: 
    Forml.panelStatusText.Caption = "Error: Analog Input"
    Exit Function
End Function

Sub DASClose ()
    ' Jump to the error handler if we get an error
    On Error GoTo DASCloseErrorHandler
    ' Close the DAS board driver
    Form1.DAS1600AD1.ADDevClose = 1
    Form1.DAS1600DI1.DIDevClose = 0
    Form1.DAS1600DO1.DODevClose = 0
    ' Exit the subroutine
    Exit Sub
End Sub

DASCloseErrorHandler:
    Exit Sub
End Sub

Function DASDigitalInput () As Long
    Dim inData As Long
    ' Jump to the error handler if we get an error
    On Error GoTo DASDIErrorHandler
    Form1.DAS1600DI1.DIStrartStop = 1  ' Initiates the operation
    inData = Form1.DAS1600DI1.DISingleRead  ' Read the data
    DASDigitalInput = inData Mod 65536
    ' Exit the subroutine
    Exit Function
End Function

DASDIErrorHandler:
    Form1.panelStatusText.Caption = "Error: Digital Input"
    Exit Function
End Function

Sub DASDigitalOutput (outData As Long)
    ' Jump to the error handler if we get an error
    On Error GoTo DASDOErrorHandler
    Form1.DAS1600DO1.DOSingleWrite = outData  ' Set output to a 1
    Form1.DAS1600DO1.DOStartStop = 1  ' Initiates the operation
    ' Exit the subroutine
    Exit Sub
End Sub
DASDOErrorHandler:
   Form1.panelStatusText.Caption = "Error: Digital Output"
   Exit Sub
End Sub

Sub DASInitialize ()
   ' Jump to the end of the subroutine if we get an error
   On Error GoTo ErrorHandler

   ' Initialize the Analog Input Control
   Form1.DAS1600AD1.ADBoardNum = 0 ' Selects board number 0
   Form1.DAS1600AD1.ADConfigFile = adConfigFileName ' Sets the config file
   Form1.DAS1600AD1.ADDevOpen = 1 ' Initializes the board
   Form1.DAS1600AD1.ADMode = 0 ' Selects single mode analog input
   Form1.DAS1600AD1.ADStartChannel = 1 ' Select start channel 0
   Form1.DAS1600AD1.ADStopChannel = 1 ' Select stop channel 0

   ' Initialize the Digital Input Control
   Form1.DAS1600DI1.DIBoardNum = 0 ' Selects board number 0
   Form1.DAS1600DI1.DIConfigFile = diConfigFileName ' Sets the config file
   Form1.DAS1600DI1.DIDevOpen = 0 ' Board already initialized
   Form1.DAS1600DI1.DIMode = 0 ' Selects single mode digital input

   ' Initialize the Digital Output Control
   Form1.DAS1600DO1.DOBoardNum = 0 ' Selects board number 0
   Form1.DAS1600DO1.DOConfigFile = doConfigFileName ' Sets the config file
   Form1.DAS1600DO1.DODevOpen = 0 ' Initializes the board
   Form1.DAS1600DO1.DOMode = 0 ' Selects single mode digital output

   ' Data acquisition controls are initialized
   DASInitialized = True

   ' Exit the subroutine - everything is initialized
   Exit Sub

ErrorHandler:
   MsgBox "Error: Could not initialize the DAS1201", MB_OK + MB_ICONEXCLAMATION,
   "DAS1201 Error"
   ' Error occurred initializing card
   DASInitialized = False
   Exit Sub
End Sub

Sub DrawBoxInitialize ()
   Dim dyBox As Double
   Dim dxBox As Double
   Dim dyPos As Double
   Dim dyTns As Double
   Dim cyLabel As Integer

Dim cxLabel As Integer
Dim strLabel As String
Dim xLeft As Integer
Dim xRight As Integer
Dim yTop As Integer
Dim yBottom As Integer

' Check the zoom factors
If DrawPosZoom <= 0 Then
    DrawPosZoom = 1
End If
If DrawTnsZoom <= 0 Then
    DrawTnsZoom = 1
End If

' Calculate the height of the box
dyBox = (Form1.DrawBox.ScaleHeight - 1) - (DrawBoxBottomMargin + DrawBoxTopMargin)
dxBox = (Form1.DrawBox.ScaleWidth - 1) - (DrawBoxLeftMargin + DrawBoxRightMargin)
dyPos = (MaxPosCount - MinPosCount) / DrawPosZoom
dyTns = (MaxTnsCount - MinTnsCount) / DrawTnsZoom

' Check limits on ranges
If dyPos = 0 Then
    dyPos = 65535
End If
If dyTns = 0 Then
    dyTns = 2048
End If

' Calculate the line equation of the position data
DrawPosEqn.m = -(dyBox / dyPos)
DrawPosEqn.b = dyBox - (DrawPosEqn.m * DrawMinPosition) + DrawBoxTopMargin

' Calculate the line equation of the tension data
If DrawModeXY = True Then
    DrawTnsEqn.m = dxBox / dyTns
    DrawTnsEqn.b = DrawBoxLeftMargin - (DrawTnsEqn.m * DrawMinTension)
Else
    DrawTnsEqn.m = -(dyBox / dyTns)
    DrawTnsEqn.b = dyBox - (DrawTnsEqn.m * DrawMinTension) + DrawBoxTopMargin
End If

' Clear the graphics before redrawing
Form1.DrawBox.Cls

' Set the x position of the graph to zero
xGraph = DrawBoxLeftMargin

' Draw the vertical scale
xLeft = DrawBoxLeftMargin - 1
yTop = DrawBoxTopMargin
yBottom = Form1.DrawBox.ScaleHeight - DrawBoxBottomMargin
Form1.DrawBox.Line (xLeft, yTop)-(xLeft, yBottom), RGB(255, 0, 255)
Form1.DrawBox.Line (xLeft, yTop)-(xLeft - 5, yTop), RGB(255, 0, 255)
Form1.DrawBox.Line (xLeft, yBottom)-(xLeft - 5, yBottom), RGB(255, 0, 255)

If DrawModeXY = True Then
    ' Draw the horizontal scale if xy mode
    yTop = yBottom
    xRight = Form1.DrawBox.ScaleWidth - DrawBoxRightMargin
    Form1.DrawBox.Line (xLeft, yBottom)-(xRight, yBottom), RGB(255, 0, 255)
    Form1.DrawBox.Line (xLeft, yBottom)-(xLeft, yBottom + 5), RGB(255, 0, 255)
    Form1.DrawBox.Line (xRight, yBottom)-(xRight, yBottom + 5), RGB(255, 0, 255)
End If

' Print the maximum position label on the scale
Form1.DrawBox.ForeColor = RGB(0, 255, 255)
If ViewCalibData <> 0 Then
    strLabel = Format$(CalibPosition(DrawMinPosition + dyPos), "0")
Else
    strLabel = Format$(DrawMinPosition + dyPos, "0")
End If
cyLabel = Form1.DrawBox.TextHeight(strLabel)
cxLabel = Form1.DrawBox.TextWidth(strLabel)
Form1.DrawBox.CurrentX = DrawBoxLeftMargin - cxLabel - 10
Form1.DrawBox.CurrentY = DrawBoxTopMargin - cyLabel
Form1.DrawBox.Print strLabel

' Print the minimum position label on the scale
If ViewCalibData <> 0 Then
    strLabel = Format$(CalibPosition(DrawMinPosition), "0")
Else
    strLabel = Format$(DrawMinPosition, "0")
End If
cxLabel = Form1.DrawBox.TextWidth(strLabel)
Form1.DrawBox.CurrentX = DrawBoxLeftMargin - cxLabel - 10
Form1.DrawBox.CurrentY = Form1.DrawBox.ScaleHeight - DrawBoxTopMargin - cyLabel
Form1.DrawBox.Print strLabel

' Print the maximum tension label on the scale
Form1.DrawBox.ForeColor = RGB(255, 255, 0)
If ViewCalibData <> 0 Then
    strLabel = Format$(CalibTension(DrawMinTension + dyTns), "0")
Else
    strLabel = Format$(DrawMinTension + dyTns, "0")
End If
cxLabel = Form1.DrawBox.TextWidth(strLabel)
Form1.DrawBox.CurrentX = DrawBoxLeftMargin - cxLabel - 10
Form1.DrawBox.CurrentY = Form1.DrawBox.ScaleHeight - DrawBoxTopMargin - cyLabel
Form1.DrawBox.Print strLabel
Form1.DrawBox.CurrentX = Form1.DrawBox.ScaleWidth - DrawBoxRightMargin - (cxLabel / 2)
Form1.DrawBox.CurrentY = Form1.DrawBox.ScaleHeight - DrawBoxTopMargin + 5
Else
Form1.DrawBox.CurrentX = DrawBoxLeftMargin - cxLabel - 10
Form1.DrawBox.CurrentY = DrawBoxTopMargin
End If
Form1.DrawBox.Print strLabel

' Print the minimum tension label on the scale
If ViewCalibData <> 0 Then
strLabel = Format$(CalibTension(DrawMinTension), "0")
Else
strLabel = Format$(DrawMinTension, "0")
End If
cxLabel = Form1.DrawBox.TextWidth(strLabel)
If DrawModeXY = True Then
Form1.DrawBox.CurrentX = DrawBoxLeftMargin - (cxLabel / 2)
Form1.DrawBox.CurrentY = Form1.DrawBox.ScaleHeight - DrawBoxTopMargin + 5
Else
Form1.DrawBox.CurrentX = DrawBoxLeftMargin - cxLabel - 10
Form1.DrawBox.CurrentY = Form1.DrawBox.ScaleHeight - DrawBoxTopMargin
End If
Form1.DrawBox.Print strLabel
End Sub

Function FormatHex (hexData As Long)
Dim rc As Variant
Dim hexString As String

' Check limits on hexData
If hexData > 65535 Then
hexData = 65535
End If
' Format the number as hex with leading zeros
If hexData <= 4095 Then
If hexData <= 255 Then
If hexData <= 15 Then
FormatHex = "000" + Hex$(hexData)
Else
FormatHex = "00" + Hex$(hexData)
End If
Else
FormatHex = "0" + Hex$(hexData)
End If
Else
FormatHex = Hex$(hexData)
End If
End Function
Appendix 2C: Equipment Performance

1. Example of records on calibrations and linearity of the force transducer

Figure 2a1. Calibration curve of the force transducer (with weights)

Linear regression for measurement:
\[ Y = -0.66105 + 1.01183 \times X \]
\[ R = 0.99969 \]
2. Position calibration

<table>
<thead>
<tr>
<th>Command to the motor (µm)</th>
<th>Computer reading (µm)</th>
<th>Optical micrometer reading (cm)</th>
<th>Difference in reading (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>50</td>
<td>50</td>
<td>0.004</td>
<td>10</td>
</tr>
<tr>
<td>100</td>
<td>100</td>
<td>0.011</td>
<td>-10</td>
</tr>
<tr>
<td>150</td>
<td>150</td>
<td>0.014</td>
<td>10</td>
</tr>
<tr>
<td>200</td>
<td>200</td>
<td>0.021</td>
<td>-10</td>
</tr>
<tr>
<td>250</td>
<td>250</td>
<td>0.025</td>
<td>0</td>
</tr>
<tr>
<td>300</td>
<td>300</td>
<td>0.028</td>
<td>20</td>
</tr>
<tr>
<td>350</td>
<td>350</td>
<td>0.035</td>
<td>0</td>
</tr>
<tr>
<td>400</td>
<td>400</td>
<td>0.039</td>
<td>10</td>
</tr>
<tr>
<td>450</td>
<td>450</td>
<td>0.046</td>
<td>-10</td>
</tr>
<tr>
<td>500</td>
<td>500</td>
<td>0.050</td>
<td>0</td>
</tr>
<tr>
<td>1000</td>
<td>1000</td>
<td>0.101</td>
<td>-10</td>
</tr>
</tbody>
</table>

Average of the difference is 0.909µm, SD=10.444µm
3. Tracings of the tests with a standard spring

Figure 2a2 Static Pre-loading of Standard Spring

\[ wt = -21.20409 + 24.31141 \text{ (strain)} \quad R=0.99306 \]
4. Tracings of dynamic testing of the standard spring

Figure 2a3 Sinusoidal Oscillation of Standard Spring

- Tension (mg)
- Position (μm)
- Frequency = 0.2 Hz, Amplitude = 3% of actual length

Tracing after smoothing.
Figure 2a4 Single pulse test of Standard Spring

(p5, 03/01/96)

Pulse height = 15% of actual length
Appendix 3A: Programs for Model Development

1. program: Voigt.bas, to generate F(t) according to a Voigt model

5 REM PROGRAM TO SIMULATE THE RESPONSE OF A VOIGT MODEL: VOIGT.BAS
10 REM take time and position signals

30 INPFILE1$ = "C:\MAY28\STAT1.TXT": OUTFILE1$ = "C:\L\KSTST2.TXT"
40 INPFILE2$ = "C:\MAY28DYN\CIRC\DYN5.TXT": OUTFILE2$ = "C:\L\KSINE5.TXT"
50 INPFILE3$ = "C:\MAY28\CP4.TXT": OUTFILE3$ = "C:\L\KPLS4.TXT"

60 OPEN INPFILE1$ FOR INPUT AS #1
70 I = 1
75 WHILE NOT EOF(I)
80 INPUT #1, AA, T(I), CC, DD, U(I), FF
85 PRINT I, T(I), U(I)
90 I = I + 1
100 WEND
110 CLOSE #1

120 GSS = .0003: TAU = 1.4

125 REM generate tension signals

130 OPEN OUTFILE1$ FOR OUTPUT AS #1
140 FOR N = 1 TO 200
150 F(N) = (1/GSS) * (1 - EXP(-T(N) / TAU)) * U(N)
160 WRITE #1, T(N), U(N), F(N)
170 NEXT N
180 CLOSE #1

190 END
2. program: Kelvin.bas, to generate F(t) according to a Kelvin model

5 REM PROGRAM TO SIMULATE THE RESPONSE OF A KELVIN MODEL: KELVIN.BAS
10 REM take time and position signals

30 INPFILE1$ = "C:\MAY28\STAT1.TXT": OUTFILE1$ = "C:\\KSTST1.TXT"
40 INPFILE2$ = "C:\MAY28\DYN\CIRC\DYN5.TXT": OUTFILE2$ = "C:\\KSINE5.TXT"
50 INPFILE3$ = "C:\MAY28\CP4.TXT": OUTFILE3$ = "C:\\KPLS4.TXT"

60 OPEN INPFILE1$ FOR INPUT AS #1
70 I = 1
75 WHILE NOT EOF(1)
80 INPUT #1, AA, T(I), CC, DD, U(I), FF
85 PRINT I, T(I), U(I)
90 I = I + 1
100 WEND
110 CLOSE #1

115 IP = 772804: IT = 196: IL = 4260: CSA = .577
120 GSS = .001585: TG = .77: TE = .24

125 REM generate tension signals

130 OPEN OUTFILE1$ FOR OUTPUT AS #1
140 FOR N = 1 TO 200
150 F(N) = ((CSA / IL) / GSS) * (1 - (1 - (TG / TE)) * EXP(-T(N) * .04 / TE)) * (U(N) - IP) + IT - 5
160 WRITE #1, T(N), U(N), F(N)
170 NEXT N
180 CLOSE #1

190 END
3. Program: Calbode.bas, to generate Gain and phase angle according to any
transfer function

5 REM GENERATE BODE DATA---CALBODE.BAS
10 REM frequency analysis of control system
14 PY = 0
15 OPEN "B:KELVIN1.TXT" FOR OUTPUT AS #1
17 REM #(tableName)
20 'INPUT "Transport Lag?"; TL
30 'INPUT "Gain Kc"; K
40 'INPUT "Starting Frequency (rad/sec)"; SF
50 'INPUT "Maximum Frequency (rad/sec)"; WF
60 'INPUT "Step Factor between 0.1 and 1?"; U
70 PRINT "************NUMERATOR***************"
80 K = .0005: SF = .001: WF = 100: U = .01
90 II = 0
100 PRINT "EXPONENT", "COEFFICIENT"
110 FOR J = 0 TO R
120 PRINT
130 PRINT (R - J)
140 INPUT " "; C(R - J)
150 NEXT J
160 PRINT
170 PRINT "************DENOMINATOR***************"
180 FOR J = 0 TO N
190 PRINT
200 PRINT
210 PRINT (N - J)
220 INPUT " "; D(N - J)
230 NEXT J
240 PRINT
250 PRINT
260 PRINT "Frequency", "Phase Angle", "Gain", "Gain"
270 PRINT "Frequency", "Phase Angle", "Gain", "Gain"
280 PRINT "rad/sec", "degrees", "ratio", "decibels"
290 PRINT "rad/sec", "degrees", "ratio", "decibels"
300 PRINT
310 PRINT
320 PRINT
330 PRINT
340 W = SF: TL = 0
350 GOTO 410
360 WO = SF: II = II + 1: W = WO + U * II * W
370 A = 0
380 B = 0
390 X = 0

289
400 Y = 0
410 FOR I = 2 TO R STEP 4
420 A = A - C(I) * W^I
430 NEXT I
440 FOR I = 0 TO R STEP 4
450 A = A + C(I) * W^I
460 NEXT I
470 FOR I = 3 TO R STEP 4
480 B = B - C(I) * W^I
490 NEXT I
500 FOR I = 1 TO R STEP 4
510 B = B + C(I) * W^I
520 NEXT I
530 FOR I = 0 TO N STEP 4
540 X = X + D(I) * W^I
550 NEXT I
560 FOR I = 1 TO N STEP 4
570 Y = Y + D(I) * W^I
580 NEXT I
590 FOR I = 3 TO N STEP 4
600 Y = Y - D(I) * W^I
610 NEXT I
620 FOR I = 2 TO N STEP 4
630 X = X - D(I) * W^I
640 NEXT I
650 IF R < 0 THEN 1020
660 IF R > 0 THEN 730
670 G = K / SQR(X * X + Y * Y)
680 P = Y
690 Q = X
700 GOSUB 890
710 PH = -PA - W * TL
720 GOTO 820
730 G = K * SQR(A * A + B * B) / SQR(X * X + Y * Y)
740 P = Y
750 Q = X
760 GOSUB 890
770 PY = PA
780 P = B
790 Q = A
800 GOSUB 890
810 PH = PA - PY - W * TL
820 PP = PH * 57.29578
830 DB = 8.68433 * LOG(G)
840 PRINT
850 PRINT W, PP, G, DB
860 WRITE #1, W, G, PP
870 IF (W - WF) > 0 THEN 1020
880 GOTO 360
890 IF P > 0 THEN 930
900 IF Q = 0 THEN 1020
910 IF Q < 0 THEN 960
920 IF Q > 0 THEN 980
930 IF Q = 0 THEN 1020
940 IF Q < 0 THEN 960
950 IF Q > 0 THEN 1000
960 PA = 3.1417 + ATN(P / Q)
970 RETURN
980 PA = 6.2834 + ATN(P / Q)
990 RETURN
1000 PA = ATN(P / Q)
1010 RETURN
1020 END
% Elastic
% This program takes in Stress and Strain data and calculates
% the material constants.
% echo off;
clear
SR = 500; % Sampling rate in #/sec
load c:\may28\cp4.txt
S = cp4(:,6);
Sn = cp4(:,5);

[NP,a] = size(S); % NP = length of vector
X2=1:N
step = SR/(NP);
X = (0.0:step:(NP-1)*step)';
S2 = S;
Sn2 = Sn;

%plot(X,Sn,'ro',X,Sn2,'w-')
%axis([0 500 -0.15 0.15])

Fs = fft(S);
Fsn = fft(Sn);
G = Fsn./Fs;
Fsabs = abs(Fs);
Fsnabs = abs(Fsn);
save c:\l\cp4.out
Fs=cp4(:,1);
Fsn=cp4(:,2);

v = [0.25 40 0 10000]; % Change here for X axis
axis(v)
plot(X,Fsabs,'w')
pause % Strike any key to see flow plot
v = [0.02 60 100 10000]; % Change here for X axis

4. program: Elasticp.m, to calculate FFT of experimental data
axis(v)
plot(X,Fsnabs,'w')

pause % Strike any key to see flow plot

[m,N] = max(Fsn(2:600));
N = N+1;

step = step
F1 = X(N)
G1 = abs(G(N))
Ang1 = (180/3.14159)*atan(imag(G(N))/real(G(N)))
Appendix 3B: Programs for Fitting Data to Kelvin Model

1. program: Filter.bas: testing for different types of filters to smooth noisy data.

1 REM FILTER.BAS
5 REM THIS PROGRAM TESTS DIFFERENT FILTERS TO SMOOTH DATA
40 REM READ CAPTURED FIRST HAND DATA; V(I)=POSITION; U(I)=TENSION
60 OPEN INPFILE$ FOR INPUT AS #1
70 I = 1
75 WHILE NOT EOF(1)
80 INPUT #1, QQ, T(I), Q1, Q2, V(I), U(I)
95 I = I + 1
100 WEND
110 CLOSE #1
120 REM SPECIFY NUMBER OF SETS OF POINTS IN DATA
130 K = 800: REM NUMBER OF POINTS
140 REM (1) GENERAL AVERAGING FILTER
150 SMOOTH = 5: REM NUMBER OF POINTS TO USE TO OBTAIN AVERAGE
170 FOR I = START TO (K - SMOOTH + 1)
180 SU = 0; SV = 0
190 FOR J = 0 TO SMOOTH - 1: SU = SU + U(I + J): SV = SV + V(I + J)
220 NEXT J
230 U(I) = SU / SMOOTH: V(I) = SV / SMOOTH
250 NEXT I
260 REM (2) FIRST ORDER FILTER, MUST COMMENT OUT LINE 140-250
270 Q = .60653066#: U(0) = 0: V(0) = 0
280 FOR I = 1 TO K
290 U(I) = U(I) / Q + (1 - 1 / Q) * U(I - 1): V(I) = V(I) / Q + (1 - 1 / Q) * V(I - 1)
310 NEXT I
320 REM (3) BUTTERWORTH FILTER, MUST COMMENT OUT LINE 140-310
325 USUM = 0: VSUM = 0
330 FOR I = 1 TO K
340 USUM = USUM + U(I): VSUM = VSUM + V(I)
350 NEXT I
360 UMEAN = USUM / K: VMEAN = VSUM / K
370 FOR I = 1 TO K
380 U(I) = U(I) - UMEAN: V(I) = V(I) - VMEAN
390 NEXT I
400 UU(I) = UMEAN: UU(2) = UMEAN
410 FOR I = 3 TO K
420 UU(I) = U(I) + U(I - 1) + U(I - 2) - UU(I - 1) - UU(I - 2)
430 NEXT I
440 VV(I) = VMEAN: VV(2) = VMEAN
450 FOR I = 3 TO K
460 VV(I) = V(I) + V(I - 1) + V(I - 2) - VV(I - 1) - VV(I - 2)
470 NEXT I
480 END
2. program: Ga96ot2.bas: final program to fit data to Kelvin model.

1 REM FOURIER TRANSFORM OF A PULSE RESPONSE. GA96OT2.BAS OCTOBER 18, 1996.
2 REM OMEGA RANGE SCANNED, FIT KELVIN MODEL
3 REM DATA SMOOTHED BY AVERAGING, TAU ESTIMATED BY "LEAST SQUARES" METHOD
7 CLS
8 TIMER1 = TIMER
9 INPFILES = "C:\APRIL16\CP1.TXT"
10 OUTFILES = "A:\APRIL16\CPKB.TXT"
12 CHECKS = "A:\APRIL16\CPKBCHK.TXT"
13 DRIFT1 = 0!: DRIFT2 = 0!: REM DRIFT1 = 1-2, 3-4, 5-6;
15 REM DRIFT2 = STAT2-CCP5, STAT4-CCP10; 2-LLP5, 4-LLP10;
17 K = 800: REM NUMBER OF POINTS
18 DT = .008: REM TIME BETWEEN POINTS
19 IT = 203.48: REM INITIAL TENSION
20 CSA = .83: REM CROSSEC AREA
21 IP = 771142.5: REM INITIAL POSITION
23 IL = 3560: REM INITIAL LENGTH
25 WO = .001: REM LOWEST FREQUENCY
28 WM = 100: REM HIGHEST FREQUENCY
30 SS = .02: REM STEP SIZE IN W
31 NAV = 20: REM NUMBER OF POINTS FOR OFFSET CALCULATION
33 SMOOTH = 5
35 NW = 0: REM CALCULATE NUMBER OF DIFFERENT W VALUES
36 DO
37 NW = NW + 1: W = WO + NW * SS * W
38 LOOP WHILE W < WM
40 W = W0
45
46 REM ******************************************************************************
47 55 CNST = 180 / 3.1415926536#
50 OPEN INPFILES FOR INPUT AS #1
70 I = 1
75 WHILE NOT EOF(1)
80 INPUT #1, QQ, T(I), Q1, Q2, V(I), U(I)
85 U(I) = (U(I) + DRIFT1 + DRIFT2 - IT) / CSA
90 V(I) = (V(I) - IP) / IL
95 I = I + 1
100 WEND
110 CLOSE #1

295
120 FOR I = 1 TO K
130  DETECT = T(I + 1) - T(I)
140  IF DETECT > .5 THEN PRINT "DETECT=", DETECT: EXIT FOR
150  NEXT I
160 START = I + 2: PRINT "START AT ": START
170 FOR I = START TO (K - SMOOTH + 1)
180  SU = 0: SV = 0
190  FOR J = 0 TO SMOOTH - 1
200    SU = SU + U(I + J)
210    SV = SV + V(I + J)
220  NEXT J
230  U(I) = SU / SMOOTH
240  V(I) = SV / SMOOTH
250  NEXT I

320 REM OPEN OUTFILE$ FOR OUTPUT AS #1
330 PRINT "COMPUTING ..."
335 LOCATE CSRLIN + 1, 1, 0
340 PRINT "DONE: ";
341 W(0) = 0

345 Z = 1
350 DO
355 W(Z) = WO + Z * SS * W(Z - 1)
360 LOCATE CSRLIN, 7, 0
365 PRINT USING "###.# %"; 100 * Z / NW;
370 C = 0: DJ = 0: F = 0: GJ = 0
380 FOR I = START TO (K - SMOOTH + 1)
382  AN = W(Z) * I * DT
384  COSAN = COS(AN)
385  SINAN = SIN(AN)
386  VI = V(I)
388  UI = U(I)
400  C = C + VI * COSAN
410  DJ = DJ - VI * SINAN
420  F = F + UI * COSAN
430  GJ = GJ - UI * SINAN
440  NEXT
450 DN = F * F + GJ * GJ
460 N = C * F + DJ * GJ
470 NJ = F * DJ - C * GJ
480 A = N / DN: B = NJ / DN
490 GN(Z) = SQR(A * A + B * B)
500 FI(Z) = ATN(B / A) * CNST
510 IF A > 0 AND B < 0 THEN GOTO 600
515 IF A < 0 AND B < 0 THEN FI(Z) = FI(Z) - 180
520 IF A < 0 AND B > 0 THEN FI(Z) = FI(Z) - 180
525 REM IF A > 0 AND B > 0 THEN FI(Z) = FI(Z) - 360
600 PRINT USING "###.###,###.####,###.###,###.###"; W(Z); GN(Z); FI(Z); A; B

296
610 REM WRITE #1, W(Z), GN(Z), FI(Z), A, B
620 Z = Z + 1
630 LOOP WHILE W(Z - 1) < WM

640 REM ***********************************************
641 REM ******* THE SECOND PART OF THE PROGRAM *******
642 REM ***********************************************
650 REM ***************************************************************
660 REM REGRESSION ANALYSIS ON ER, TAUG AND TAUE
670 REM ***** GAIN(S)=(1+(TAUE)S)((1/ER)/(1+(TAUG)S)) **************
675 REM ***** **********
680 REM ***** TAUE=(ETA/K), TAUG=(ETA/K')(1+(K'/K)), ER=K'************
685 REM *** K: spring constant of the spring in series with the dashpot;**********
690 REM *** K': spring constant of the spring in parallel;**********
695 REM ***** ETA: VISCOSITY OF THE DASHPOT. **************
700 REM *** ER: RELAXED ELASTIC MODULUS; **************
705 REM ***** TAUE: RELAXATION TIME FOR CONSTANT STRAIN; **********
710 REM ***** TAUG: RELAXATION TIME FOR CONSTANT STRESS. **********
715 REM ***************************************************************
720 REM USING BOTH GAIN(WEIGHTED) AND PHI

730 REM INPUT "NUMBER OF POINTS IN FILE", Z
735 REM K = 143
740 REM INPUT "LOWEST TAU?", TL
745 TL = .1
750 REM INPUT "HIGHEST TAU?", TM
755 TM = 10
760 REM INPUT "NUMBER OF TAU POINTS?", N
765 N = 40
768 REM ************************************************************

770 REM ************************************************************
775 REM OPEN "C:\TEMP\AMAR18\CP15D.TXT" FOR INPUT AS #1
780 REM FOR I = 1 TO Z
785 REM INPUT #1, W(I), G(I), FI(I), QQ, QR
790 REM NEXT
795 REM CLOSE #1
800 REM ************************************************************
805 REM ************************************************************
810 REM OPEN OUTFILES FOR APPEND AS #1
815 REM LINE #836 AFTER THE FIRST FILE
820 REM PRINT #1, " " " TAUE", " TAUG", " ER", " SPRINGII", " VISCOSITY", " S2"
825 REM OPEN CHECKS FOR APPEND AS #2
830 REM LINE #842 AFTER THE FIRST FILE

835 REM **************************************************
842 PRINT #2, "", "IP", "IT", "IL", "CSA", "DRIFT1", "DRIFT2"

850 FOR J = 2 TO Z
855 SLOPE = (GN(J) - GN(J - 1)) / (W(J) - W(J - 1))
860 IF SLOPE > 0 THEN PRINT "NOISE STARTS AT #"; J: PRINT "w(rads/sec)=", W(J): PRINT "PHASE(DEG)=", FI(J - 1): EXIT FOR
865 NEXT

870 NP = 10: REM CHOOSE # OF PTS WILL BE USED TO CALCULATE GSS
875 GSUM = 0
880 FOR I = 1 TO NP
885 GSUM = GSUM + GN(I)
890 NEXT I
900 GSS = GSUM / NP

910 REM FROM HERE, THE PROGRAM DIFFERS FROM THE PROGRAM: GA96JY4.BAS
911 REM ************************************************************

915 K = J - 2
920 TAU = .5: TAU = 5: TAU = .01: TAU = 1
925 STEP = (TAU MAX - TAU MIN) / N: STEP = (TAU MAX - TAU MIN) / N
926 TAU = TAU MIN
930 SMIN = 100000

935 DO
940 TAU = TAU MIN
945 DO
947 LOCATE CSRLIN, 7, 0
948 PRINT USING "TAUG = ####.###, TAUE = ####.###"; TAU; TAU;

952 S = 0
955 FOR Z = 1 TO K
960 A = FI(Z) * .01745: REM CHANGE IN TO RADIUS
965 B = ATN(-W(Z) * TAU)
970 C = ATN(W(Z) * TAU)
975 PHASE1 = C + B
980 X = SQR(W(Z) * W(Z) * TAU * TAU + 1)
985 Y = SQR(W(Z) * W(Z) * TAU * TAU + 1)
990 GAIN1 = (GSS * Y) / X
995 WEIGHT = 100: REM WEIGHT FACTOR FOR GSS
1000 WTG = WEIGHT * (GN(Z) - GAIN1) / GSS
1005 ANG = A - PHASE1: REM PHASE ANGLE NOT WEIGHTED
1010 S = S + (WTG * WTG + ANG * ANG)
1015 NEXT Z
1020 IF S < SMIN THEN
1022 SMIN = S
1024 TAU OPT = TAU: TAU OPT = TAU
1025 END IF
1028 TAU = TAU + STEP
LOOP WHILE TAUG < TAU constexpr
1078 TAUE = TAUE + STEPE
1079 LOOP WHILE TAUE < TAU constexpr

1300 ER = 1 / GSS
1301 ETA = (TAU constexpr - TAU constexpr) / GSS
1310 SPRTNGII = ETA / TAU constexpr
1320 PRINT , "S2="; S; "RELAXED ELASTIC MODULUS IS"; ER; "mg/m²"
1330 PRINT , "VISCOSITY OF THE DASHPOT IS"; ETA
1340 PRINT , "RELAXATION TIME FOR CONSTANT STRAIN IS"; TAU constexpr; "SEC"
1350 PRINT , "RELAXATION TIME FOR CONSTANT STRESS IS"; TAU constexpr; "SEC"

1500 PRINT "TAUE="; TAU constexpr; "TAU constexpr; "TAU constexpr; "ER="; ER; "SPRINGII=";
SPRINGII; "VISCOSITY="; ETA
1510 PRINT #1, INPFIELD,
1520 PRINT #1, USING "##.####,##.####,####.####,####.####,##.###,#####.##";
TAU constexpr; TAU constexpr; ETA; S
1530 CLOSE #1
1540 PRINT #2, INPFIELD,
1550 PRINT #2, USING "######.#,####.##,#####.#,##.###,####.##,####.##"; IP; IT; IL; CSA;
DRIFT1; DRIFT2
1560 CLOSE #2

1570 TIMER2 = TIMER
1580 EXTIME = TIMER2 - TIMER1
1590 IF EXTIME < 0 THEN EXTIME = EXTIME + 86400
1600 PRINT USING "EXECUTION TIME: ###### seconds"; EXTIME
1610 BEEP
1620 END
3. program: Testken.bas  fitting Kelvin model to Gain and phase angle to estimate model parameters.

1 REM TESTKEN.BAS
2 REM TO TEST THE PROGRAM FITTING DATA TO KELVIN MODEL, OCTOBER 18, 1996.
3 REM INPUT IS A GENERATED BODE DIAGRAM OF KELVIN (KNOWN PARAMETERS)
4 REM OUTPUT SHOULD BE THE SAME PARAMETERS AS KNOWN
7 CLS
8 TIMER1 = TIMER
9 INPFILES = "b:\kelvin1.TXT"
55 CNST = 180 / 3.1415926536#
60 OPEN INPFILES FOR INPUT AS #1
70 I = 1
75 WHILE NOT EOF(1)
80 INPUT #1, W(I), GN(I), FI(I)
95 I = I + 1
100 WEND
110 CLOSE #1
320 REM OPEN OUTFILES FOR OUTPUT AS #1
330 PRINT "COMPUTING ..."
335 LOCATE CSRLIN + 1, 1, 0
340 PRINT "DONE: ";
341 W(0) = 0
660 REM REGRESSION ANALYSIS ON ER, TAUG AND TAUE
665 REM *************************************************
670 REM *** GAIN=(1+(TAUE)S)((1/ER)/(1+(TAUG)S))
680 REM *** TAUE=(ETA/K), TAUG=(ETA/K')(1+(K'/K)), ER=K'
685 REM *** K: SPRING CONSTANT OF THE SPRING IN SERIES WITH THE DASHPOAT;
690 REM *** K': SPRING CONSTANT OF THE SPRING IN PARALLEL;
695 REM *** ETA: VISCOSITY OF THE DASHPOAT.
700 REM *** ER: RELAXED ELASTIC MODULUS;
705 REM *** TAUE: RELAXATION TIME FOR CONSTANT STRAIN;
710 REM *** TAUG: RELAXATION TIME FOR CONSTANT STRESS.
715 REM *************************************************
720 REM USING BOTH GAIN(WEIGHTED) AND PHI
730 REM INPUT "NUMBER OF POINTS IN FILE", K
735 K = 143
760 REM INPUT "NUMBER OF TAU POINTS?", N
765 N = 40
870 NP = 10: REM CHOOSE # OF PTS WILL BE USED TO CALCULATE GSS
875 GSUM = 0
880 FOR I = 1 TO NP
885 GSUM = GSUM + GN(I)
890 NEXT I
900 GSS = GSUM / NP

920 TAUGMIN = .5: TAUGMAX = 5: TAUemin = .01: TAUemax = 1
925 STEPE = (TAUemax - TAUemin) / N: STEPG = (TAUGmax - TAUGmin) / N
926 TAUE = TAUemin
930 SMIN = 1000000

935 DO
940 TAUG = TAUGMIN
945 DO
947 LOCATE CSRLIN, 7, 0
948 PRINT USING "TAUG = ####.###, TAUE = ####.###"; TAUG; TAUE;

952 S = 0
955 FOR Z = 1 TO K
960 A = FI(Z) * .01745: REM CHANGE IN TO RADIUS
965 B = ATN(-W(Z) * TAUG)
970 C = ATN(W(Z) * TAUE)
975 PHASE1 = C + B
980 X = SQR(W(Z) * W(Z) * TAUG * TAUG + 1)
985 Y = SQR(W(Z) * W(Z) * TAUE * TAUE + 1)
990 GAIN1 = (GSS * Y) / X
995 WEIGHT = 100: REM WEIGHT FACTOR FOR GSS
1000 WTG = WEIGHT * (GN(Z) - GAIN1) / GSS
1005 ANG = A - PHASE1: REM PHASE ANGLE NOT WEIGHTED
1010 S = S + (WTG * WTG + ANG * ANG)
1015 NEXT Z
1020 IF S < SMIN THEN
1022 SMIN = S
1024 TAUGOPT = TAUG: TAUeOPT = TAUE
1026 END IF
1060 TAUG = TAUG + STEPG
1065 LOOP WHILE TAUG < TAUGMAX
1068 TAUE = TAUE + STEPE
1075 LOOP WHILE TAUE < TAUeMAX

1320 LPRINT, INPFILES
1330 LPRINT, "GSS="; GSS
1340 LPRINT, "TAUE="; TAUeOPT, "SEC"
1350 LPRINT, "TAUe="; TAUGOPT, "SEC"

1570 TIMER2 = TIMER
1580 EXTIME = TIMER2 - TIMER1
1590 IF EXTIME < 0 THEN EXTIME = EXTIME + 86400
1600 PRINT USING "EXECUTION TIME: ###### seconds"; EXTIME
1610 BEEP
1620 END
4. program: Boder.bas: adding random noise to the Bode diagram of perfect Kelvin model.

3 REM BODER.BAS
5 REM Generates Bode Data (OUTPUT 1) AND NOISY Bode DATA (OUTPUT2)
10 REM frequency analysis of control system
14 PY = 0
15 OPEN "C:\LU\KPP5.TXT" FOR OUTPUT AS #1
16 OPEN "C:\LU\KP5.TXT" FOR OUTPUT AS #2
20 INPUT "Transport Lag?", TL
25 RANDOMIZE (1)
30 INPUT "Gain Kc?", K
40 INPUT "Starting Frequency (rad/sec)?", SF
50 INPUT "Maximum Frequency (rad/sec)?", WF
60 INPUT "Step Factor between 0.1 and 1 ?", U
70 PRINT "************NUMERATOR************
80 INPUT "Order ?", R
90 K = .002: SF = .001: WF = 100: U = .01
95 II = 0
100 PRINT "EXPONENT", "COEFFICIENT"
110 FOR J = 0 TO R
120 PRINT
130 PRINT (R - J)
140 INPUT " ", C(R - J)
150 NEXT J
160 PRINT
170 PRINT "************DENOMINATOR************
180 INPUT "Order ?", N
190 FOR J = 0 TO N
200 PRINT
210 PRINT (N - J)
220 INPUT " ", D(N - J)
230 NEXT J
240 PRINT
250 PRINT
260 PRINT "Frequency", "Phase Angle", "Gain", "Gain"
270 'PRINT "Frequency", "Phase Angle", "Gain", "Gain"
280 PRINT "rad/sec", "degrees", "ratio", "decibels"
290 'PRINT "rad/sec", "degrees", "ratio", "decibels"
300 PRINT
310 PRINT
320 'PRINT
330 'PRINT
340 W = SF: TL = 0
350 GOTO 410
360 W0 = SF: II = II + 1: W = W0 + U * II * W
370 A = 0

302
380 B = 0
390 X = 0
400 Y = 0
410 FOR I = 2 TO R STEP 4
420 A = A - C(I) * W ^ I
430 NEXT I
440 FOR I = 0 TO R STEP 4
450 A = A + C(I) * W ^ I
460 NEXT I
470 FOR I = 3 TO R STEP 4
480 B = B - C(I) * W ^ I
490 NEXT I
500 FOR I = 1 TO R STEP 4
510 B = B + C(I) * W ^ I
520 NEXT I
530 FOR I = 0 TO N STEP 4
540 X = X + D(I) * W ^ I
550 NEXT I
560 FOR I = 1 TO N STEP 4
570 Y = Y + D(I) * W ^ I
580 NEXT I
590 FOR I = 3 TO N STEP 4
600 Y = Y - D(I) * W ^ I
610 NEXT I
620 FOR I = 2 TO N STEP 4
630 X = X - D(I) * W ^ I
640 NEXT I
650 IF R < 0 THEN 1020
660 IF R > 0 THEN 730
670 G = K / SQR(X * X + Y * Y)
680 P = Y
690 Q = X
700 GOSUB 890
710 PH = -PA - W * TL
720 GOTO 820
730 G = K * SQR(A * A + B * B) / SQR(X * X + Y * Y)
740 P = Y
750 Q = X
760 GOSUB 890
770 PY = PA
780 P = B
790 Q = A
800 GOSUB 890
810 PH = PA - PY - W * TL
820 PP = PH * 57.29578
830 DB = 8.68433 * LOG(G)
840 PRINT
850 PRINT W, PP, G, DB
860 WRITE #1, W, G, PP
861 RANDOMIZE TIMER
862 SI = (0.5 - RND)
863 IF W < 1! THEN 864 ELSE 869
864 WRITE #2, W, G, PP: GOTO 870

869 WRITE #2, W, G + .001 * SI, PP + 50 * SI
870 IF (W - WF) > 0 THEN 1020
880 GOTO 360
890 IF P > 0 THEN 930
900 IF Q = 0 THEN 1020
910 IF Q < 0 THEN 960
920 IF Q > 0 THEN 980
930 IF Q = 0 THEN 1020
940 IF Q < 0 THEN 960
950 IF Q > 0 THEN 1000
960 PA = 3.1417 + ATN(P / Q)
970 RETURN
980 PA = 6.2834 + ATN(P / Q)
990 RETURN
1000 PA = ATN(P / Q)
1010 RETURN
1020 END
5. program: Autocord.bas: auto correlation of the gain and the phase angle to find bandwidth of the noise.

10 REM FILE: AUTOCORD.BAS
20 REM AUTOCORRELATION OF BODE DATA TO FIND RMS ERROR, APRIL, 97
30 INPUT "RUN NUMBER = ", NO$
40 INPUT "NUMBER OF POINTS = ", K
50 ' INPUT "FREQUENCY RANGE =", DELOHM
60 DIM U(1000), V(1000), RU(1000), RV(1000), W(1000)
70 OPEN "C:\LU\KP3.TXT" FOR INPUT AS #1
80 OPEN "C:\LU\KP3A.TXT" FOR OUTPUT AS #2
90 I=1
100 WHILE NOT EOF(1)
110 INPUT #1, W(I), U(I), V(I)
120 I=I+1: WEND
130 KK=K/2: J=1
140 RU(J)=0 : RV(J)=0
150 FOR I=1 TO K
160 RU(J)=RU(J)+U(I)*U(I+J): RV(J)=RV(J)+V(I+J)*V(I):NEXT
170 RU(J)=RU(J)/(KK): RV(J)=RV(J)/(KK)
180 IF J<(KK) THEN GOTO 190 ELSE 200
190 J=J+1: GOTO 140
200 FOR I=1 TO KK
210 IF SGN(RV(I)) >0 THEN RV(I) = SQR (RV(I)) ELSE RV(I)= -SQR(-RV(I))
220 IF SGN(RU(I)) >0 THEN RU(I) = SQR (RU(I)) ELSE RU(I)= -SQR(-RU(I))
230 WRITE #2, W(I), RU(I), -RV(I)
235 NEXT
240 PRINT "RUN NUMBER = ", NO$, "FILE NAME = C:\LU\KP3A.TXT"
260 END
6. program: Errordta.bas: calculate the sum of square of difference for (1) fitting the Kelvin to actual data; (2) fitting Kelvin to simulated data.

5 REM ERRORDTA.BAS
10 REM PROGRAM TO COMPARE ERRORS IN BODE DIAGRAMS GENERATED BY SOLUTION WITH KELVIN AND VOIGT MODELS
25 INPUT "RUN NUMBER = ", RN$
35 N=144
40 OPEN "C:\LU\MAR1L.TXT" FOR INPUT AS #1: REM DATA
45 DIM F(1000), KERF(1000), KERG(1000), VERG(1000), VERF(1000), G(1000)
50 OPEN "C:\LU\KRMAR1.TXT" FOR INPUT AS #2: REM KELVIN FIT
55 DIM WV(1000), GV(1000), FV(1000), WK(1000), GK(1000), FK(1000), W(1000)
60 I=1
65 REM INPUT DATA
70 WHILE NOT EOF(1)
80 INPUT # 1, WV(I), GV(I), FV(I)
90 I=I+1
100 WEND
105 REM INPUT KELVIN FITTED DATA
110 I=1
120 WHILE NOT EOF(2)
130 INPUT #2, WK(I), GK(I), FK(I)
140 I=I+1
150 WEND
155 CLOSE #1 : CLOSE #2
160 OPEN "C:\LU\KMAR1.TXT" FOR INPUT AS #3
165 REM INPUT INITIAL MODEL BASE CONDITION
170 I=1
175 AVERGT=0: AVERFT=0: AKERGT=0: AKERFT=0
180 WHILE NOT EOF (3)
190 INPUT #3, W(I), G(I), F(I)
195 I=I+1: WEND
197 CLOSE #3
198 OPEN "C:\LU\ERROR1.TXT" FOR OUTPUT AS #1
200 FOR I=1 TO N
210 VERG(I)=(G(I)-GV(I)): KERG(I)=(G(I)-GK(I))
220 SQVG=SQVG+VERG(I)*VERG(I) : SQKG=SQKG+KERG(I)*KERG(I)
230 VERG=VERG+ABS(VERG(I)): KERG=KERG +ABS (KERG(I))
250 VERF(I)=(F(I)-FV(I)): KERF(I)=(F(I)-FK(I))
260 SQVF=SQVF+VERF(I)*VERF(I) : SQKF=SQKF + KERF(I)*KERF(I)
270 VERF=VERF+ABS(VERF(I)) : KERF=KERF +ABS (KERF(I))
275 WRITE # 1, WV(I), WK(I), W(I), VERG(I),VERF(I), KERG(I), KERF(I)
280 NEXT
290 SDVG=SQR(SQVG)/N : SDKG=(SQR(SQKG))/N
295 SDVF=SQR(SQVF)/N : SDKF=(SQR(SQKF))/N
300 AVERGT=VERG/N : AVERFT=VERF/N
320 AKERGT=KERT/N: AKERFT=KERFT/N
350 PRINT " ERRORS IN DATA: GAIN ="; AVERGT; " ANGLE="; AVERFT
355 PRINT "STANDARD DEVIATIONS: "; SDVG, SDVF
360 PRINT " ERRORS IN KELVIN: GAIN ="; AKERGT; " ANGLE="; AKERFT
365 PRINT "STANDARD DEVIATIONS: "; SDKG, SDKF
380 END
Appendix 3C: Other Programs Used in Data Analysis

1. program: Ga96jy4.bas: final program to fit data to Voigt model.

1 REM FOURIER TRANSFORM OF A PULSE RESPONSE. GA96JY4.BAS JULY 20, 1996.
2 REM OMEGA RANGE SCANNED
3 REM DATA SMOOTHED BY AVERAGING, TAU ESTIMATED BY "LEAST SQUARES" METHOD
6 DIM TAU(1000), S(500), G(1000)
7 REM *************************************************************************************************************
8 CLS
9 TIMER 1 = TIMER
10 INPFILES = "b:\june26B\CCP15.TXT"
11 OUTFILES = "c:\temp\june26\CPB.TXT"
12 DRIFT 1 = 13: DRIFT2 = 20: REM DRIFT1 RE STAT2,4,6; DRIFT2 RE AFTRLAST PLS
13 REM DRIFT1 RELATIVE TO STAT1,3,5; DRIFT2 RELATIVE TO STAT2,4,6.
14 K = 800: REM NUMBER OF POINTS
15 DT = .008: REM TIME BETWEEN POINTS
16 IT = 210: REM INITIAL TENSION
17 CSA = 511: REM CROSSEC AREA
18 IP = 771920: REM INITIAL POSITION
19 IL = 3700: REM INITIAL LENGTH
20 WO = .001: REM LOWEST FREQUENCY
21 WM = 100: REM HIGHEST FREQUENCY
22 SS = .05: REM STEP SIZE IN W
23 NAV = 20: REM NUMBER OF POINTS FOR OFFSET CALCULATION
24 SMOOTH = 5
25 NW = 0: REM CALCULATE NUMBER OF DIFFERENT W VALUES
26 DO
27 NW = NW + 1: W = WO + NW * SS * W
28 LOOP WHILE W < WM
29 W = W0
30 REM *************************************************************************************************************
31 CNST = 180 / 3.1415926536#
32 OPEN INPFILES FOR INPUT AS #1
33 I = 1
34 WHILE NOT EOF(1)
35 INPUT #1, QQ, T(I), Q1, Q2, V(I), U(I)
36 U(I) = (U(I) + DRIFT1 + DRIFT2 - IT) / CSA
37 V(I) = (V(I) - IP) / IL
38 I = I + 1

308
100 WEND
110 CLOSE #1

120 FOR I = 1 TO K
130   DETECT = T(I + 1) - T(I)
140   IF DETECT > .5 THEN PRINT "DETECT=", DETECT: EXIT FOR
150 NEXT I
160 START = I + 2: PRINT "START AT ": START
170 FOR I = START TO (K - SMOOTH + 1)
180   SU = 0: SV = 0
190   FOR J = 0 TO SMOOTH - 1
200     SU = SU + U(I + J)
210     SV = SV + V(I + J)
220   NEXT J
230   U(I) = SU/ SMOOTH
240   V(I) = SV/ SMOOTH
250 NEXT I

320 REM OPEN OUTFILES FOR OUTPUT AS #1
330 PRINT "COMPUTING ..."
335 LOCATE CSRLIN + 1, 1, 0
340 PRINT "DONE: ";
341 W(0) = 0

345 Z = 1
350 DO
355 W(Z) = WO + Z * SS * W(Z - 1)
360 LOCATE CSRLIN, 7, 0
365 PRINT USING "###.# %"; 100 * Z / NW;
370 C = 0: DJ = 0: F = 0: GJ = 0
380 FOR I = START TO (K - SMOOTH + 1)
385   AN = W(Z) * I * DT
388   COSAN = COS(AN)
390   SINAN = SIN(AN)
395   VI = V(I)
400   UI = U(I)
405   C = C + VI * COSAN
410   DJ = DJ - VI * SINAN
420   F = F + UI * COSAN
425   GJ = GJ - UI * SINAN
430 NEXT
450 DN = F * F + GJ * GJ
460 N = C * F + DJ * GJ
470 NJ = F * DJ - C * GJ
480 A = N / DN: B = NJ / DN
490 GN(Z) = SQR(A * A + B * B)
500 FI(Z) = ATN(B / A) * CNST
510 IF A > 0 AND B < 0 THEN GOTO 600
515 IF A < 0 AND B < 0 THEN FI(Z) = FI(Z) - 180
520 IF A < 0 AND B > 0 THEN FI(Z) = FI(Z) - 180
525 REM IF A > 0 AND B > 0 THEN FI(Z) = FI(Z) - 360
600 PRINT USING "###.###,##.###,###.####,###.###,###.###"; W(Z); GN(Z); FI(Z); A; B
610 REM WRITE #1, W(Z), GN(Z), FI(Z), A, B
620 Z = Z + 1
630 LOOP WHILE W(Z - 1) < WM
640 REM *******************************************************
641 REM ***** ********
642 REM ***** THE SECOND PART OF THE PROGRAM ********
643 REM *****
650 REM *******************************************************
710 REM REGRESSION ANALYSIS ON TAU USING BOTH GAIN(WEIGHTED) AND PHI
730 REM INPUT "NUMBER OF POINTS IN FILE", Z
735 REM K = 143
740 REM INPUT "LOWEST TAU?", TL
745 TL = .1
750 REM INPUT "HIGHEST TAU?", TM
755 TM = 10
760 REM INPUT "NUMBER OF TAU POINTS?", N
765 N = 500
768 REM *******************************************************
780 REM *******************************************************
790 REM OPEN "C:\TEMP\AMAR18\CP15D.TXT" FOR INPUT AS #1
800 REM FOR I = 1 TO Z
810 REM INPUT #1, W(I), G(I), FI(I), QQ, QR
820 REM NEXT
830 REM CLOSE #1
835 REM *******************************************************
840 OPEN OUTFILES FOR APPEND AS #1
842 REM LINE #845 AFTER THE FIRST FILE
845 REM PRINT #1, "", "MINUM TAU", "S^2", "GSS"
850 DTAU = (TM - TL) / N
860 TAU(I) = TL
870 GSUM = 0
880 FOR J = 2 TO Z
882 SLOPE = (GN(J) - GN(J - 1)) / (W(J) - W(J - 1))
883 IF SLOPE > 0 THEN PRINT "NOISE STARTS AT #"; J: PRINT "w(rads/sec)="; W(J):
PRINT "PHASE(DEG)="; FI(J - 1): EXIT FOR
885 NEXT
886 NP = 10: REM CHOOSE # OF PTS WILL BE USED TO CALCULATE G SS
888 FOR I = 1 TO NP
890 GSUM = GSUM + GN(I)
900 NEXT I
910 GSS = GSUM / NP

950 K = J - 2
955 L = 1
956 DO
960 S(L) = 0
990 FOR Z = 1 TO K
1000 A = F1(Z) * .01745
1010 B = ATN(-W(Z) * TAU(L))
1015 Y = 1 + W(Z) * W(Z) * TAU(L) * TAU(L)
1018 GG = GSS / SQR(Y)
1020 WEIGHT = 100: REM WEIGHT FACTOR FOR GSS
1025 CC = WEIGHT * (GN(Z) - GG) / GSS
1030 S(L) = S(L) + ((A - B) * (A - B) + CC * CC)
1040 NEXT Z
1045 REM WRITE #1, TAU(L), S(L)
1050 REM PRINT K, TL, TM, N
1054 LOCATE CSRLIN, 7, 0
1055 PRINT L; " : TAU = "; TAU(L);
1060 L = L + 1
1070 TAU(L) = TAU(L - 1) + DTAU
1080 LOOP WHILE TAU(L) < TM
1085 PRINT " "
1100 FOR L = 2 TO N
1110 C = (S(L) - S(L - 1)) / (TAU(L) - TAU(L - 1))
1120 IF C >= 0 THEN EXIT FOR
1150 NEXT
1160 PRINT "MINIMUM TAU="; TAU(L - 1); " S^2="; S(L - 1); " GSS="; GSS
1161 PRINT #1, INPFILES,
1162 PRINT #1, USING "##.####,####.####,###.#########"; TAU(L - 1); S(L - 1); GSS
1167 CLOSE #1

1170 TIMER2 = TIMER
1172 EXTIME = TIMER2 - TIMER1
1173 IF EXTIME < 0 THEN EXTIME = EXTIME + 86400
1174 PRINT USING "EXECUTION TIME: ###### seconds"; EXTIME
1179 BEEP
1180 END
2. program: Vkcomp.bas: compare the fitting of Voigt and Kelvin to the same data by the sum of square of the difference.

3 REM FILE: VOIKELCOMPARSES (VKCOMP.BAS)
5 REM FOURIER TRANSFORM OF A PULSE RESPONSE. JUNE.1996. KLP & LW
6 REM REVISED SEPT/96 TO ADD FILTER,TESTS AND TO FIT TO FIRST ORDER LAG
7 REM REVISED OCT/96 TO FIT THE KELVIN MODEL AND COMPARE WITH VOIGT.
9 'INPUT "RUN NUMBER? "; NOS
15 INPUT "VOIGT TAU = "; TAUVO
20 INPUT "VOIGT GAIN = "; GAINVO
25 INPUT "TAUHAT ="; TAUHAT
30 INPUT "TEHAT ="; TEHAT
35 INPUT "GSS = "; GSS
40 DIM U(1000), V(1000), T(1000), TE(1000):
41 DIM W(1000), GN(1000), FI(1000), SR(1000)
42 CSA = .624: IL = 3010: WO = .001: WM = 100: SS = .005: X = 5
45 IP = 771112: IT = 154.07: K = 800: DT = .008
46 TAUMIN = .5: TAUMAX = 5: TEMIN = .005: TEMAX = 1: TAUE = .01
47 REM THE INPUT COLUMN AND THE OUTPUT COLUMN SHOULD BE IN TWO DIFFERENT FILES
48 REM ***********************************************
49 OPEN "C:\MAY15\LLP12.TXT " FOR INPUT AS #1
50 REM THAT IS THE INPUT COLUMN
52 I = 1
54 WHILE NOT EOF(1)
56 INPUT #1, QQ, T(I), Q1, Q2, V(I), U(I)
58 U(I) = (U(I) - IT) / CSA: V(I) = (V(I) - IP) / IL
60 I = I + 1
62 WEND
64 CLOSE #1: REM ::DETECT BREAK IN TIME RECORD
65 'LPRINT " RUN NUMBER"," NOS
66 FOR I = 1 TO K
68 DET = T(I+1) - T(I)
70 IF DET > .5 THEN PRINT "SPACE AT ", DET; "(SEC)": GOTO 74
72 NEXT I
74 START = I + 2
76 PRINT "START AT POINT ":, START
86 REM SMOOTH DATA USING 5 POINT AVERAGING
88 FOR I = (START + 2) TO (K - (X + 1))
90 U(I) = U(I) / (X + 1): V(I) = V(I) / (X + 1)
92 NEXT I
97 REM CHANGE IN BASE-LINE OCCURS AT PEAK OF PULSE (MAX)
100 FOR I = (START + 2) TO (K - (X + 1))
102 SLOPE = (V(I) - V(I + 1)) / (T(I) - T(I + 1))
104 IF SLOPE < 0 THEN MAX = (I + 1): GOTO 118
116 NEXT I
118 PRINT "MAX ", MAX
130 OPEN "C:\MY15LLPX.TXT" FOR OUTPUT AS #1
132 REM**********************************************************************
140 NP = (K - (X + 1)) - (START + (X + 1))
150 Z = 1: W(0) = 0
160 W(Z) = W0 + Z * SS * W(Z - 1)
170 C = 0: DJ = 0: F = 0: GJ = 0
180 FOR I = (START + 2) TO (K - (X + 1))
190 AN = W(Z) * I * DT
200 C = C + V(I) * COS(AN)
210 DI = DJ - V(I) * SIN(AN)
220 F = F + U(I) * COS(AN)
230 GJ = GJ - U(I) * SIN(AN)
240 NEXT
250 DN = F * F + GJ * GJ
260 N = C * F + DJ * GJ
270 NJ = F * DJ - C * GJ
280 A = N / DN: B = NJ / DN
290 GN(Z) = SQR(A * A + B * B)
300 FI(Z) = (ATN(B / A)) * (180 / 3.1416)
310 IF A > 0 AND B < 0 THEN 400
320 IF A < 0 AND B < 0 THEN FI(Z) = -180 + FI(Z): GOTO 400
330 IF A < 0 AND B > 0 THEN FI(Z) = -180 + FI(Z): GOTO 400
340 IF A > 0 AND B > 0 THEN 400
400 'PRINT USING "###.###,##.#####,###.#,#.###,#.######"; W(Z); GN(Z); FI(Z); A; B
410 WRITE #1, W(Z), GN(Z), FI(Z)
420 IF W(Z) > WM THEN GOTO 650
430 Z = Z + 1: GOTO 160

510 REM REGRESSION ANALYSIS ON TAU USING PHI AND GAIN (TOTFIT)
520 REM JUNE 1996; KLP & LW
522 REM TAUFITF2.BAS
530 REM *********************************************
540 TB = .1: TM = 10: N = 200
550 'CLOSE #1
560 I = 1
570 DIM TAU(2500), S(2500)
590 'OPEN "C:\LUCP7MT.TXT" FOR INPUT AS #1
595 REM**********************************************************************
600 OPEN "B:\AVGC3OUT.TXT" FOR OUTPUT AS #1
630 DTAU = (TM - TL) / N: GSUM = 0
650 TAU(I) = TL
660 'LPRINT
670 'LPRINT , "RUN NUMBER ", RN$
1820 SLOPE = (FI(J) - FI(J - 1)) / (W(J) - W(J - 1))
1830 IF SLOPE > 0 THEN PRINT "NOISE STARTS AT ", J: GOTO 1850
1840 NEXT
1850 FOR I = 1 TO 10: GSUM = GSUM + GN(I): NEXT
1852 GSS = GSUM / 10
1854 FOR I = 1 TO 10: GGSUM = ((GN(I) - GSS) * (GN(I) - GSS)) / 10: NEXT
1856 STDEV = SQR(GGSUM)
2295
2362
3430 PRINT "#########################################################"
3490 SKPHI = 0: SKGN = 0: SVPHI = 0: SVGN = 0
3500 FOR Z = 1 TO K
3510 REM KELVIN MODEL ERRORS: FIT TO BODE CURVES
3520 A = FI(Z) * 0.01745: B = ATN(-W(Z) * TAUHAT): C = ATN(W(Z) * TEHAT)
3530 D = B + C: Y = 1 + W(Z) * W(Z) + TAUHAT * TAUHAT: GG = GSS / SQR(Y)
3540 GH = SQR(TEHAT * TEHAT * W(Z) * W(Z) + 1): BB = ATN(-W(Z) * TAUVO)
3542 GVO = GAINVO / (SQR(W(Z) * W(Z) * TAUVO * TAUVO + 1))
3550 SKPHI = SKPHI + (A - D) * (A - D)
3560 SKGN = SKGN + (GN(Z) - (GG * GH))^2
3570 SVPHI = SVPHI + (A - BB) * (A - BB)
3580 SVGN = SVGN + (GN(Z) - GVO)^2
3585 NEXT
3590 SKPHI = SKPHI / K: SKGN = SKGN / K: SVPHI = SVPHI / K: SVGN = SVGN / K
3600 PRINT "MODEL COMPARISON"
3610 PRINT "ERRORS IN KELVIN MODEL"
3620 PRINT "ERROR IN PHI = "; SKPHI; "ERROR IN GAIN = "; SKGN
3630 PRINT "ERRORS IN VOIGT MODEL"
3640 PRINT "ERROR IN PHI = "; SVPHI; "ERROR IN GAIN = "; SVGN

314
3. program: FFTLU557.BAS: calculate the absolute gain and phase angle at each frequency for sinusoidal oscillation data.

3 REM FILE: FFTLU557.BAS
5 REM FOURIER TRANSFORM OF A sine wave RESPONSE. JUNE.1996.
6 REM REVISED JUNE/96 TO ALLOW FREQUENCY CALCULATION AND MODEL FILTER.
7 INPUT "RUN NUMBER? "; NO$
10 INPUT "NUMBER OF POINTS?"; K
25 INPUT "TIME BETWEEN POINTS?"; DT
40 DIM U(1000), V(1000), T(1000), S(1000), A(1000), PHI(1000), SS(1000)
45 IP = 771648: IT = 293
47 REM THE INPUT COLUMN AND THE OUTPUT COLUMN SHOULD BE IN TWO DIFFERENT FILES
48 OPEN "B:\JUN28DYN\CIRC\DYN5.TXT" FOR INPUT AS #1
49 REM **********************************************
50 REM THAT IS THE INPUT COLUMN
52 I = 1
54 WHILE NOT EOF(1)
56 INPUT #1, Q, T(I), QQ, QR, V(I), U(I)
58 U(I) = (U(I) - IT) / CSA: V(I) = (V(I) - IP) / IL
60 I = I + 1
62 WEND
65 IF V(4) - V(1) > 0 THEN GOTO 67 ELSE GOTO 90
67 HI = V(1)
69 FOR I = 4 TO K
70 IF V(I) > HI THEN HI = V(I) ELSE GOTO 74
72 NEXT
74 Z = I: T = T(I): LOW = V(I)
76 FOR I = (Z + 3) TO K
78 IF V(I) < LOW THEN LOW = V(I) ELSE GOTO 82
80 NEXT
82 TB = T(I)
84 F = .5 / (TB - T): GOTO 110
90 LOW = V(I)
92 FOR I = 4 TO K
94 IF V(I) < LOW THEN LOW = V(I) ELSE GOTO 98
96 NEXT
98 ZZ = I + 1: TT = T(I): HII = V(I)
100 FOR I = (ZZ + 2) TO K
102 IF V(I) > HII THEN HII = V(I) ELSE GOTO 106
104 NEXT
106 TTB = T(I)
108 F = .5 / (TTB - TT)
110 W = 6.283 * F
115 REM SMOOTH DATA USING 5 POINT AVERAGING
FOR I = 3 TO (K - 2)
U(I) = (U(I) + U(I + 1) + U(I + 2) + U(I - 1) + U(I - 2)) / 5
V(I) = (V(I) + V(I + 1) + V(I + 2) + V(I - 1) + V(I - 2)) / 5: NEXT
LPRINT: LPRINT "********************************************
LPRINT: LPRINT "RUN NUMBER=", J28, "CIRC\DY", NO$
LPRINT: LPRINT "SAMPLE TIME =", DT, "No. OF POINTS =", K
N = 0
FOR I = 3 TO (K - 2)
SUMU = SUMU + U(I): SUMV = SUMV + V(I): NEXT
UAV = SUMU / (K - 4): VAV = SUMV / (K - 4)
LPRINT "AVERAGE TENSION=", UAV; "AVERAGE POSITION=", VAV
FOR I = 3 TO (K - 2): U(I) = U(I) - UAV: NEXT
J = 1: PHI(J) = 0: DPHI = .06283: LOW = 1000000
SS(J) = 0
FOR I = 3 TO (K - 2)
SS(J) = SS(J) + (AA * (SIN(W * I * DT + PHI(J))) - U(I)) ^ 2
NEXT
IF SS(J) < LOW THEN LOW = SS(J): LPHI = PHI(J)
J = J + 1
PHI(J) = PHI(J - 1) + DPHI
IF PHI(J) < 6.283 THEN GOTO 141
PHI = LPHI: J = 1: A(J) = LA: DA = (MA - LA) / CP: LLOW = 2000000
S(J) = 0
FOR I = 3 TO (K - 2)
S(J) = S(J) + (A(J) * (SIN(W * I * DT + PHI)) - U(I)) ^ 2
NEXT
IF S(J) < LLOW THEN LLOW = S(J): A = A(J)
J = J + 1: A(J) = A(J - 1) + DA
IF A(J) < MA THEN GOTO 155
N = N + 1
LPRINT, "FITTED A =", A; "PHI=", PHI
IF N > 1 THEN GOTO 300
PHI1 = PHI: A1 = A
AA = AA / 200: LA = .005: MA = .5
FOR I = 2 TO (K - 2): U(I) = V(I): NEXT
GOTO 140
ANGLE = PHI1 - PHI: DANGLE = ANGLE * 57.2958
GAIN = A / A1
GOTO 140
LPRINT, "ANGLE = ", ANGLE, "RADIANS OR ", DANGLE, " DEGREES"
GOTO 140
LPRINT, "GAIN = ", GAIN: LPRINT, "FREQUENCY = ", W: LPRINT
END: STOP
Appendix 3D: Results of the Physiological Experiments

1. Table Appendix 3D.1 The result of the monitored pH of the solution in the tissue bath.

<table>
<thead>
<tr>
<th>Description</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>De-ironized H₂O</td>
<td>7.17</td>
</tr>
<tr>
<td>Freshly made Kreb’s solution</td>
<td>7.48</td>
</tr>
<tr>
<td>Kreb’s at 37°C</td>
<td>7.49</td>
</tr>
<tr>
<td>Oxygenated Kreb’s at 37°C</td>
<td>7.45</td>
</tr>
<tr>
<td>Oxygenated Kreb’s at 37°C (with Aluminum clips)</td>
<td>7.47</td>
</tr>
<tr>
<td>Oxygenated Kreb’s at 37°C (with tissue sample for 1 hour)</td>
<td>7.63</td>
</tr>
<tr>
<td>Oxygenated Kreb’s at 37°C (with tissue sample for 1.5 hours)</td>
<td>7.52</td>
</tr>
<tr>
<td>Oxygenated Kreb’s at 37°C (with tissue sample for 2.5 hours)</td>
<td>7.59</td>
</tr>
<tr>
<td>Oxygenated Kreb’s at 37°C (with tissue sample for 4 hours)</td>
<td>7.56</td>
</tr>
</tbody>
</table>
2. Table Appendix 3D.2 The steady-state stiffness (Static pre-loading experiments)

<table>
<thead>
<tr>
<th>Age (month)</th>
<th>Circumferential (kPa)</th>
<th>Longitudinal (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>2.42591</td>
<td>5.36538</td>
</tr>
<tr>
<td>23.5</td>
<td>7.90146</td>
<td>13.64993</td>
</tr>
<tr>
<td>1.75</td>
<td>16.60804</td>
<td>24.44269</td>
</tr>
<tr>
<td>20</td>
<td>1.43775</td>
<td>11.23775</td>
</tr>
<tr>
<td>8.5</td>
<td>9.81275</td>
<td>14.49526</td>
</tr>
<tr>
<td>2.25</td>
<td>11.96702</td>
<td>17.17976</td>
</tr>
<tr>
<td>2.25</td>
<td>5.88345</td>
<td>8.88874</td>
</tr>
<tr>
<td>19</td>
<td>10.10744</td>
<td>7.02936</td>
</tr>
<tr>
<td>11</td>
<td>13.67534</td>
<td>14.81379</td>
</tr>
<tr>
<td>2.75</td>
<td>5.90366</td>
<td>27.92779</td>
</tr>
<tr>
<td>14</td>
<td>6.88505</td>
<td>23.65387</td>
</tr>
<tr>
<td>2.5</td>
<td>7.85124</td>
<td>19.45176</td>
</tr>
<tr>
<td>2.5</td>
<td>10.03435</td>
<td>25.95667</td>
</tr>
<tr>
<td>2.5</td>
<td>5.68882</td>
<td>6.66482</td>
</tr>
<tr>
<td>2</td>
<td>5.86177</td>
<td>13.29549</td>
</tr>
<tr>
<td>2.63</td>
<td>15.88867</td>
<td>24.67735</td>
</tr>
<tr>
<td>2.63</td>
<td>8.03321</td>
<td>15.32822</td>
</tr>
<tr>
<td>2.63</td>
<td>15.45752</td>
<td>17.06214</td>
</tr>
<tr>
<td>2.63</td>
<td>3.69611</td>
<td>13.82651</td>
</tr>
<tr>
<td>3.75</td>
<td>6.96873</td>
<td>8.638</td>
</tr>
<tr>
<td>2.75</td>
<td>5.94182</td>
<td>9.07817</td>
</tr>
<tr>
<td>14</td>
<td>6.82668</td>
<td>18.77546</td>
</tr>
<tr>
<td>12</td>
<td>4.86076</td>
<td>15.74142</td>
</tr>
<tr>
<td>19.5</td>
<td>10.40939</td>
<td>20.03516</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>19</td>
<td>5.70177</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>3.97472</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>3.64912</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>7.92648</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>1.50623</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>3.29165</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>3.14587</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2.52794</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>4.93973</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2.61682</td>
<td></td>
</tr>
<tr>
<td>8.5</td>
<td>3.1858</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>3.4544</td>
<td></td>
</tr>
<tr>
<td>8.5</td>
<td>1.97152</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>4.95817</td>
<td></td>
</tr>
<tr>
<td>1.75</td>
<td>4.66505</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>2.3748</td>
<td></td>
</tr>
</tbody>
</table>
3. **Table Appendix 3D.3 The steady-state stiffness \( (\mu_2) \) (Pulse tests)**

<table>
<thead>
<tr>
<th>Age</th>
<th>C (10% )</th>
<th>C (20% )</th>
<th>C (30% )</th>
<th>L (10% )</th>
<th>L (20% )</th>
<th>L (30% )</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>3.40862</td>
<td>5.33442</td>
<td>6.91332</td>
<td>4.75521</td>
<td>7.39819</td>
<td>8.36317</td>
</tr>
<tr>
<td>23.5</td>
<td>4.64269</td>
<td>7.26667</td>
<td>6.24046</td>
<td>10.61688</td>
<td>13.60611</td>
<td>15.47319</td>
</tr>
<tr>
<td>1.75</td>
<td>11.5684</td>
<td>17.09059</td>
<td>17.96703</td>
<td>21.09677</td>
<td>23.19149</td>
<td>26.80328</td>
</tr>
<tr>
<td>20</td>
<td>0.84409</td>
<td>1.8869</td>
<td>2.16748</td>
<td>6.62838</td>
<td>11.83354</td>
<td>14.25872</td>
</tr>
<tr>
<td>8.5</td>
<td>5.06977</td>
<td>9.15966</td>
<td>14.4904</td>
<td>7.91129</td>
<td>15.37617</td>
<td>17.90146</td>
</tr>
<tr>
<td>11</td>
<td>0.05737</td>
<td>16.43216</td>
<td>13.87553</td>
<td>11.26292</td>
<td>15.23292</td>
<td>16.35</td>
</tr>
<tr>
<td>2.75</td>
<td>4.85403</td>
<td>5.96715</td>
<td>8.82194</td>
<td>21.1879</td>
<td>25.48052</td>
<td>29.72727</td>
</tr>
<tr>
<td>2.5</td>
<td>4.19231</td>
<td>7.05755</td>
<td>9.08333</td>
<td>1.43799</td>
<td>11.52761</td>
<td>15.40031</td>
</tr>
<tr>
<td>2.5</td>
<td>8.15461</td>
<td>10.32632</td>
<td>8.81402</td>
<td>20.39501</td>
<td>21.56044</td>
<td>26.87671</td>
</tr>
<tr>
<td>2.5</td>
<td>1.35535</td>
<td>5.51124</td>
<td>7.60465</td>
<td>7.50574</td>
<td>6.69168</td>
<td>8.30652</td>
</tr>
<tr>
<td>2.63</td>
<td>4.76908</td>
<td>5.68366</td>
<td>2.75717</td>
<td>8.25758</td>
<td>11.80505</td>
<td>13.68201</td>
</tr>
<tr>
<td>3.75</td>
<td>3.21745</td>
<td>4.42889</td>
<td>6.48381</td>
<td>5.0179</td>
<td>7.62238</td>
<td>9.65551</td>
</tr>
<tr>
<td>2.75</td>
<td>1.19663</td>
<td>3.31419</td>
<td>5.1014</td>
<td>5.27987</td>
<td>8.30652</td>
<td>9.15112</td>
</tr>
<tr>
<td>14</td>
<td>1.96514</td>
<td>6.78423</td>
<td>7.63424</td>
<td>14.07461</td>
<td>18</td>
<td>20.2268</td>
</tr>
<tr>
<td>12</td>
<td>2.59455</td>
<td>5.21254</td>
<td>5.46214</td>
<td>14.42647</td>
<td>15.30421</td>
<td>16.18812</td>
</tr>
<tr>
<td></td>
<td>C: Circumferential samples, L: Longitudinal samples. %: the strain value. The unit of age is month. All other values in kPa.</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>----</td>
<td>----------------------------------------------------------------------------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>6.04809 5.43189 6.59718 8.55275 11.81927 11.93431</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>2.34465 4.58197 5.86722 15.47319 15.62102 13.89518</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>0.61784 3.26456 4.44696 13.64395 17.96703 19.19765</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>1.73505 2.05963 2.30552 10.76839 11.12245 11.14773</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>2.41507 3.61993 4.4269 10 12.82353 12.40202</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>2.26454 3.60529 4.54167 5.75704 6.04809 6.65536</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1.05552 2.82546 3.59868 3.89905 6.19709 6.49669</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.981 1.4418 1.26826 6.90359 7.86688 6.0295</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1.87715 2.16032 3.12222 4.2951 4.43691 5.03852</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.5</td>
<td>2.37473 2.63426 2.63426 4.46315 5.85672 5.85672</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>2.54277 2.8534 3.93186 7.65808 7.41497 6.32903</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.5</td>
<td>1.77268 2.35365 1.99918 1.68934 2.68033 2.73107</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>3.13318 4.13053 4.84684 4.15854 5.93108 7.47144</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.75</td>
<td>5.63793 5.63793 5.09875 3.33447 5.70349 9.14259</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>1.22426 1.94181 2.3921 8.12759 8.94257 12.60925</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
4. Table Appendix 3D. 4 The time constants (fitting pulse data to Kelvin model)
Circumferential samples, at 10% strain.

<table>
<thead>
<tr>
<th>Age (month)</th>
<th>$\tau_e$ (Mean)</th>
<th>$\tau_e$ (SD)</th>
<th>$\tau_\sigma$ (Mean)</th>
<th>$\tau_\sigma$ (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>0.0694</td>
<td>0.0675</td>
<td>1.4</td>
<td>0.1232</td>
</tr>
<tr>
<td>23.5</td>
<td>0.208</td>
<td>0.1272</td>
<td>0.8825</td>
<td>0.09</td>
</tr>
<tr>
<td>1.75</td>
<td>0.10405</td>
<td>0.0804</td>
<td>1.1525</td>
<td>0.045</td>
</tr>
<tr>
<td>20</td>
<td>0.0793</td>
<td>0.08488</td>
<td>2.2775</td>
<td>0.2181</td>
</tr>
<tr>
<td>8.5</td>
<td>0.0892</td>
<td>0.08342</td>
<td>1.4225</td>
<td>0.14925</td>
</tr>
<tr>
<td>2.25</td>
<td>0.39115</td>
<td>0.04021</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2.25</td>
<td>0.2285</td>
<td>0.11776</td>
<td>1.2425</td>
<td>0.05511</td>
</tr>
<tr>
<td>19</td>
<td>0.1882</td>
<td>0.08771</td>
<td>0.7475</td>
<td>0.045</td>
</tr>
<tr>
<td>11</td>
<td>0.01</td>
<td>0</td>
<td>4.01</td>
<td>1.755</td>
</tr>
<tr>
<td>2.75</td>
<td>0.0991</td>
<td>0.10217</td>
<td>1.2875</td>
<td>0.07115</td>
</tr>
<tr>
<td>14</td>
<td>0.27235</td>
<td>0.07442</td>
<td>1.04</td>
<td>0.045</td>
</tr>
<tr>
<td>2.5</td>
<td>0.27235</td>
<td>0.07275</td>
<td>1.1525</td>
<td>0.045</td>
</tr>
<tr>
<td>2.5</td>
<td>0.1684</td>
<td>0.14053</td>
<td>0.7475</td>
<td>0.11023</td>
</tr>
<tr>
<td>2.5</td>
<td>0.01</td>
<td>0</td>
<td>4.2125</td>
<td>0.78266</td>
</tr>
<tr>
<td>2</td>
<td>0.24265</td>
<td>0.08371</td>
<td>0.77</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.63</td>
<td>0.2872</td>
<td>0.0396</td>
<td>0.6125</td>
<td>0</td>
</tr>
<tr>
<td>2.63</td>
<td>0.01</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2.63</td>
<td>0.307</td>
<td>0.06823</td>
<td>0.86</td>
<td>0.045</td>
</tr>
<tr>
<td>2.63</td>
<td>0.1585</td>
<td>0.12325</td>
<td>0.7475</td>
<td>0.11023</td>
</tr>
<tr>
<td>3.75</td>
<td>0.16345</td>
<td>0.12946</td>
<td>0.8825</td>
<td>0.09</td>
</tr>
<tr>
<td>2.75</td>
<td>0.02485</td>
<td>0.0297</td>
<td>3.83</td>
<td>0.41852</td>
</tr>
<tr>
<td>14</td>
<td>0.0298</td>
<td>0.0396</td>
<td>3.2</td>
<td>0.34857</td>
</tr>
<tr>
<td>12</td>
<td>0.20305</td>
<td>0.13948</td>
<td>1.445</td>
<td>0.18279</td>
</tr>
<tr>
<td>19.5</td>
<td>0.30205</td>
<td>0.097</td>
<td>0.7025</td>
<td>0.045</td>
</tr>
<tr>
<td>19</td>
<td>0.32185</td>
<td>0.03357</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>------</td>
<td>---------</td>
<td>---------</td>
<td>------</td>
<td>-----</td>
</tr>
<tr>
<td>19</td>
<td>0.17335</td>
<td>0.1414</td>
<td>1.49</td>
<td>0.11023</td>
</tr>
<tr>
<td>29</td>
<td>0.05455</td>
<td>0.0891</td>
<td>3.74</td>
<td>1.04667</td>
</tr>
<tr>
<td>30</td>
<td>0.16345</td>
<td>0.11222</td>
<td>1.04</td>
<td>0.1312</td>
</tr>
<tr>
<td>30</td>
<td>0.0397</td>
<td>0.03638</td>
<td>1.355</td>
<td>0.39997</td>
</tr>
<tr>
<td>2.5</td>
<td>0.1684</td>
<td>0.14053</td>
<td>1.085</td>
<td>0.11023</td>
</tr>
<tr>
<td>2.5</td>
<td>0.05455</td>
<td>0.07732</td>
<td>1.355</td>
<td>0.11473</td>
</tr>
<tr>
<td>9</td>
<td>0.10405</td>
<td>0.11545</td>
<td>2.0525</td>
<td>0.16534</td>
</tr>
<tr>
<td>9</td>
<td>0.01</td>
<td>0</td>
<td>4.01</td>
<td>1.755</td>
</tr>
<tr>
<td>9</td>
<td>0.1387</td>
<td>0.1584</td>
<td>1.6025</td>
<td>0.14925</td>
</tr>
<tr>
<td>8.5</td>
<td>0.3367</td>
<td>0.31205</td>
<td>0.8375</td>
<td>0.25654</td>
</tr>
<tr>
<td>2.5</td>
<td>0.54955</td>
<td>0.31047</td>
<td>0.6575</td>
<td>0.135</td>
</tr>
<tr>
<td>8.5</td>
<td>0.1981</td>
<td>0.13789</td>
<td>0.77</td>
<td>0.24233</td>
</tr>
<tr>
<td>12</td>
<td>0.1684</td>
<td>0.1361</td>
<td>0.9275</td>
<td>0.08419</td>
</tr>
<tr>
<td>1.75</td>
<td>0.47035</td>
<td>0.37718</td>
<td>0.6575</td>
<td>0.22045</td>
</tr>
<tr>
<td>4.5</td>
<td>0.59905</td>
<td>0.48104</td>
<td>0.68</td>
<td>0.26239</td>
</tr>
</tbody>
</table>

*Time constants in seconds*
5. *Table Appendix 3D. 5 The time constants (fitting pulse data to Kelvin model)*
*Circumferential samples, at 20% strain.*

<table>
<thead>
<tr>
<th>Age (month)</th>
<th>$\tau_e$ (Mean)</th>
<th>$\tau_e$ (SD)</th>
<th>$\tau_\sigma$ (Mean)</th>
<th>$\tau_\sigma$ (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>0.10405</td>
<td>0.09573</td>
<td>0.9275</td>
<td>0.045</td>
</tr>
<tr>
<td>23.5</td>
<td>0.1288</td>
<td>0.07889</td>
<td>0.6125</td>
<td>0</td>
</tr>
<tr>
<td>1.75</td>
<td>0.1486</td>
<td>0.10096</td>
<td>0.815</td>
<td>0.08419</td>
</tr>
<tr>
<td>20</td>
<td>0.12385</td>
<td>0.11353</td>
<td>1.085</td>
<td>0.08419</td>
</tr>
<tr>
<td>8.5</td>
<td>0.2971</td>
<td>0.05096</td>
<td>1.13</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.25</td>
<td>0.17335</td>
<td>0.0459</td>
<td>0.5225</td>
<td>0.045</td>
</tr>
<tr>
<td>2.25</td>
<td>0.1288</td>
<td>0.09313</td>
<td>0.59</td>
<td>0.08419</td>
</tr>
<tr>
<td>19</td>
<td>0.1882</td>
<td>0.09181</td>
<td>0.7475</td>
<td>0.1312</td>
</tr>
<tr>
<td>11</td>
<td>0.2377</td>
<td>0.08194</td>
<td>0.86</td>
<td>0.045</td>
</tr>
<tr>
<td>2.75</td>
<td>0.23275</td>
<td>0.11818</td>
<td>1.3325</td>
<td>0.05511</td>
</tr>
<tr>
<td>14</td>
<td>0.1585</td>
<td>0.15257</td>
<td>1.1975</td>
<td>0.045</td>
</tr>
<tr>
<td>2.5</td>
<td>0.1882</td>
<td>0.10547</td>
<td>0.7475</td>
<td>0.045</td>
</tr>
<tr>
<td>2.5</td>
<td>0.22285</td>
<td>0.05556</td>
<td>0.5225</td>
<td>0.045</td>
</tr>
<tr>
<td>2.5</td>
<td>0.1387</td>
<td>0.12064</td>
<td>1.0175</td>
<td>0.05511</td>
</tr>
<tr>
<td>2</td>
<td>0.2476</td>
<td>0.0985</td>
<td>0.995</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.63</td>
<td>0.2377</td>
<td>0.11651</td>
<td>0.7475</td>
<td>0.045</td>
</tr>
<tr>
<td>2.63</td>
<td>0.1486</td>
<td>0.16009</td>
<td>1.2875</td>
<td>0</td>
</tr>
<tr>
<td>2.63</td>
<td>0.1981</td>
<td>0.08659</td>
<td>0.6575</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.63</td>
<td>0.1387</td>
<td>0.05512</td>
<td>0.545</td>
<td>0.05511</td>
</tr>
<tr>
<td>3.75</td>
<td>0.21295</td>
<td>0.10663</td>
<td>0.59</td>
<td>0.045</td>
</tr>
<tr>
<td>2.75</td>
<td>0.1189</td>
<td>0.1352</td>
<td>0.995</td>
<td>0.05511</td>
</tr>
<tr>
<td>14</td>
<td>0.109</td>
<td>0.07827</td>
<td>1.13</td>
<td>0.05511</td>
</tr>
<tr>
<td>12</td>
<td>0.14365</td>
<td>0.08516</td>
<td>0.6575</td>
<td>0.05511</td>
</tr>
<tr>
<td>19.5</td>
<td>0.2179</td>
<td>0.0459</td>
<td>0.68</td>
<td>0.05511</td>
</tr>
<tr>
<td>Time (s)</td>
<td>x1</td>
<td>x2</td>
<td>x3</td>
<td>x4</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
<td>-------</td>
<td>-------</td>
<td>------</td>
</tr>
<tr>
<td>19</td>
<td>0.19315</td>
<td>0.07604</td>
<td>0.6125</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>0.1981</td>
<td>0.09974</td>
<td>0.86</td>
<td>0.08419</td>
</tr>
<tr>
<td>29</td>
<td>0.1684</td>
<td>0.13789</td>
<td>1.04</td>
<td>0.045</td>
</tr>
<tr>
<td>30</td>
<td>0.2377</td>
<td>0.12266</td>
<td>0.9275</td>
<td>0.08419</td>
</tr>
<tr>
<td>30</td>
<td>0.1684</td>
<td>0.14483</td>
<td>0.95</td>
<td>0</td>
</tr>
<tr>
<td>2.5</td>
<td>0.23275</td>
<td>0.03131</td>
<td>0.68</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.5</td>
<td>0.2872</td>
<td>0.03638</td>
<td>0.8375</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>0.1585</td>
<td>0.10845</td>
<td>0.725</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>0.22285</td>
<td>0.11672</td>
<td>0.9725</td>
<td>0.045</td>
</tr>
<tr>
<td>9</td>
<td>0.0892</td>
<td>0.097</td>
<td>1.175</td>
<td>0</td>
</tr>
<tr>
<td>8.5</td>
<td>0.01</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2.5</td>
<td>0.2575</td>
<td>0.13646</td>
<td>0.5225</td>
<td>0.045</td>
</tr>
<tr>
<td>8.5</td>
<td>0.32185</td>
<td>0.17403</td>
<td>0.545</td>
<td>0.05511</td>
</tr>
<tr>
<td>12</td>
<td>0.1684</td>
<td>0.0485</td>
<td>0.725</td>
<td>0.1423</td>
</tr>
<tr>
<td>1.75</td>
<td>0.56935</td>
<td>0.10454</td>
<td>0.5225</td>
<td>0.045</td>
</tr>
<tr>
<td>4.5</td>
<td>0.802</td>
<td>0.396</td>
<td>0.5225</td>
<td>0.045</td>
</tr>
</tbody>
</table>

*Time constants in seconds.*
6. Table Appendix 3D. The time constants (fitting pulse data to Kelvin model)
Circumferential samples, at 30% strain.

<table>
<thead>
<tr>
<th>Age (month)</th>
<th>$\tau_\varepsilon$ (Mean)</th>
<th>$\tau_\varepsilon$ (SD)</th>
<th>$\tau_\sigma$ (Mean)</th>
<th>$\tau_\sigma$ (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>0.1486</td>
<td>0.10454</td>
<td>1.085</td>
<td>0.045</td>
</tr>
<tr>
<td>23.5</td>
<td>0.23275</td>
<td>0.13374</td>
<td>0.8825</td>
<td>0.055</td>
</tr>
<tr>
<td>1.75</td>
<td>0.1684</td>
<td>0.12085</td>
<td>0.77</td>
<td>0.11473</td>
</tr>
<tr>
<td>20</td>
<td>0.20305</td>
<td>0.07889</td>
<td>1.1525</td>
<td>0.045</td>
</tr>
<tr>
<td>8.5</td>
<td>0.208</td>
<td>0.146</td>
<td>1.1075</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.25</td>
<td>0.109</td>
<td>0.11818</td>
<td>0.68</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.25</td>
<td>0.1981</td>
<td>0.11135</td>
<td>0.7925</td>
<td>0.09</td>
</tr>
<tr>
<td>19</td>
<td>0.1585</td>
<td>0.12125</td>
<td>0.77</td>
<td>0.05511</td>
</tr>
<tr>
<td>11</td>
<td>0.18325</td>
<td>0.15014</td>
<td>0.905</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.75</td>
<td>0.2179</td>
<td>0.14226</td>
<td>1.1975</td>
<td>0.08419</td>
</tr>
<tr>
<td>14</td>
<td>0.24265</td>
<td>0.13153</td>
<td>1.1075</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.5</td>
<td>0.1387</td>
<td>0.12165</td>
<td>0.7025</td>
<td>0.08419</td>
</tr>
<tr>
<td>2.5</td>
<td>0.17335</td>
<td>0.0459</td>
<td>0.7025</td>
<td>0.045</td>
</tr>
<tr>
<td>2.5</td>
<td>0.1486</td>
<td>0.11135</td>
<td>0.77</td>
<td>0.05511</td>
</tr>
<tr>
<td>2</td>
<td>0.16345</td>
<td>0.12755</td>
<td>1.0175</td>
<td>0.09</td>
</tr>
<tr>
<td>2.63</td>
<td>0.0793</td>
<td>0.08488</td>
<td>0.9275</td>
<td>0.045</td>
</tr>
<tr>
<td>2.63</td>
<td>0.22285</td>
<td>0.07275</td>
<td>1.0175</td>
<td>0.09</td>
</tr>
<tr>
<td>2.63</td>
<td>0.1882</td>
<td>0.08631</td>
<td>0.6575</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.63</td>
<td>0.109</td>
<td>0.08134</td>
<td>1.04</td>
<td>0.08419</td>
</tr>
<tr>
<td>3.75</td>
<td>0.5446</td>
<td>0.13059</td>
<td>0.59</td>
<td>0.11023</td>
</tr>
<tr>
<td>2.75</td>
<td>0.16345</td>
<td>0.13411</td>
<td>0.77</td>
<td>0.05511</td>
</tr>
<tr>
<td>14</td>
<td>0.1981</td>
<td>0.07764</td>
<td>1.3325</td>
<td>0.05511</td>
</tr>
<tr>
<td>12</td>
<td>0.17335</td>
<td>0.12483</td>
<td>0.7025</td>
<td>0.08419</td>
</tr>
<tr>
<td>19.5</td>
<td>0.17335</td>
<td>0.1414</td>
<td>0.8825</td>
<td>0.05511</td>
</tr>
<tr>
<td>Time (s)</td>
<td>D1</td>
<td>D2</td>
<td>D3</td>
<td>D4</td>
</tr>
<tr>
<td>---------</td>
<td>----</td>
<td>----</td>
<td>----</td>
<td>----</td>
</tr>
<tr>
<td>19</td>
<td>0.20305</td>
<td>0.03284</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>0.19315</td>
<td>0.14567</td>
<td>0.905</td>
<td>0.05511</td>
</tr>
<tr>
<td>29</td>
<td>0.15355</td>
<td>0.0891</td>
<td>0.9725</td>
<td>0.11023</td>
</tr>
<tr>
<td>30</td>
<td>0.18325</td>
<td>0.15014</td>
<td>0.8825</td>
<td>0.09</td>
</tr>
<tr>
<td>30</td>
<td>0.208</td>
<td>0.12813</td>
<td>0.995</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.5</td>
<td>0.1585</td>
<td>0.07669</td>
<td>0.7925</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.5</td>
<td>0.17335</td>
<td>0.1414</td>
<td>0.905</td>
<td>0.05511</td>
</tr>
<tr>
<td>9</td>
<td>0.0793</td>
<td>0.07241</td>
<td>0.8375</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>0.26245</td>
<td>0.13592</td>
<td>1.1525</td>
<td>0.045</td>
</tr>
<tr>
<td>9</td>
<td>0.16345</td>
<td>0.1455</td>
<td>1.1075</td>
<td>0.05511</td>
</tr>
<tr>
<td>8.5</td>
<td>0.81685</td>
<td>0.21152</td>
<td>0.725</td>
<td>0.18825</td>
</tr>
<tr>
<td>2.5</td>
<td>0.49015</td>
<td>0.10686</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>8.5</td>
<td>0.2872</td>
<td>0.16521</td>
<td>0.59</td>
<td>0.1312</td>
</tr>
<tr>
<td>12</td>
<td>0.1981</td>
<td>0.08073</td>
<td>0.6125</td>
<td>0.17428</td>
</tr>
<tr>
<td>1.75</td>
<td>0.7723</td>
<td>0.0995</td>
<td>0.68</td>
<td>0.135</td>
</tr>
<tr>
<td>4.5</td>
<td>1</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
</tr>
</tbody>
</table>

*Time constants in seconds.*
Table Appendix 3D. The time constants (fitting pulse data to Kelvin model)
Longitudinal samples, at 10% strain.

<table>
<thead>
<tr>
<th>Age (month)</th>
<th>$\tau_\epsilon$ (Mean)</th>
<th>$\tau_\epsilon$ (SD)</th>
<th>$\tau_\sigma$ (Mean)</th>
<th>$\tau_\sigma$ (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>0.1585</td>
<td>0.0813</td>
<td>1.1525</td>
<td>0.2505</td>
</tr>
<tr>
<td>23.5</td>
<td>0.22285</td>
<td>0.0973</td>
<td>0.86</td>
<td>0.045</td>
</tr>
<tr>
<td>1.75</td>
<td>0.18325</td>
<td>0.0383</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>0.1585</td>
<td>0.11069</td>
<td>1.22</td>
<td>0.09</td>
</tr>
<tr>
<td>8.5</td>
<td>0.2179</td>
<td>0.1498</td>
<td>1.265</td>
<td>0.045</td>
</tr>
<tr>
<td>2.25</td>
<td>0.3466</td>
<td>0.05773</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2.25</td>
<td>0.0595</td>
<td>0.03834</td>
<td>1.0625</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>0.1288</td>
<td>0.12755</td>
<td>1.355</td>
<td>0.05511</td>
</tr>
<tr>
<td>11</td>
<td>0.23275</td>
<td>0.12325</td>
<td>0.68</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.75</td>
<td>0.208</td>
<td>0.11609</td>
<td>0.7475</td>
<td>0.08419</td>
</tr>
<tr>
<td>14</td>
<td>0.16345</td>
<td>0.08043</td>
<td>0.5675</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.5</td>
<td>0.0199</td>
<td>0.0198</td>
<td>4.8425</td>
<td>0.09</td>
</tr>
<tr>
<td>2.5</td>
<td>0.2179</td>
<td>0.09974</td>
<td>0.635</td>
<td>0.11023</td>
</tr>
<tr>
<td>3.75</td>
<td>0.1189</td>
<td>0.0947</td>
<td>1.2425</td>
<td>0.09</td>
</tr>
<tr>
<td>2.75</td>
<td>0.01495</td>
<td>0.0099</td>
<td>1.58</td>
<td>0.09</td>
</tr>
<tr>
<td>14</td>
<td>0.18325</td>
<td>0.11921</td>
<td>0.7475</td>
<td>0.045</td>
</tr>
<tr>
<td>12</td>
<td>0.2872</td>
<td>0.08771</td>
<td>0.5225</td>
<td>0.045</td>
</tr>
<tr>
<td>19.5</td>
<td>0.3169</td>
<td>0.03704</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Time (s)</td>
<td>Value 1</td>
<td>Value 2</td>
<td>Value 3</td>
<td>Value 4</td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td>19</td>
<td>0.1684</td>
<td>0.10913</td>
<td>0.9725</td>
<td>0.045</td>
</tr>
<tr>
<td>19</td>
<td>0.3367</td>
<td>0.05512</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>29</td>
<td>0.2674</td>
<td>0.07275</td>
<td>0.815</td>
<td>0.045</td>
</tr>
<tr>
<td>30</td>
<td>0.19315</td>
<td>0.10725</td>
<td>0.77</td>
<td>0.05511</td>
</tr>
<tr>
<td>30</td>
<td>0.25255</td>
<td>0.10573</td>
<td>0.5225</td>
<td>0.045</td>
</tr>
<tr>
<td>2.5</td>
<td>0.31195</td>
<td>0.0594</td>
<td>0.905</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.5</td>
<td>0.2476</td>
<td>0.07604</td>
<td>0.6125</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>0.1288</td>
<td>0.09313</td>
<td>0.995</td>
<td>0.05511</td>
</tr>
<tr>
<td>9</td>
<td>0.2476</td>
<td>0.11024</td>
<td>0.7475</td>
<td>0.045</td>
</tr>
<tr>
<td>9</td>
<td>0.1387</td>
<td>0.06895</td>
<td>0.7925</td>
<td>0.05511</td>
</tr>
<tr>
<td>8.5</td>
<td>0.28225</td>
<td>0.0495</td>
<td>0.5675</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.5</td>
<td>0.22285</td>
<td>0.11567</td>
<td>0.5225</td>
<td>0.045</td>
</tr>
<tr>
<td>8.5</td>
<td>0.08425</td>
<td>0.12523</td>
<td>1.5125</td>
<td>0.33373</td>
</tr>
<tr>
<td>12</td>
<td>0.2179</td>
<td>0.08938</td>
<td>0.77</td>
<td>0.16838</td>
</tr>
<tr>
<td>1.75</td>
<td>0.802</td>
<td>0.396</td>
<td>0.59</td>
<td>0.18</td>
</tr>
<tr>
<td>4.5</td>
<td>1</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
</tr>
</tbody>
</table>

*Time constants in seconds.*
Table Appendix 3D. The time constants (fitting pulse data to Kelvin model)
Longitudinal samples, at 20% strain.

<table>
<thead>
<tr>
<th>Age (month)</th>
<th>$\tau_\varepsilon$ (Mean)</th>
<th>$\tau_\varepsilon$ (SD)</th>
<th>$\tau_\sigma$ (Mean)</th>
<th>$\tau_\sigma$ (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>0.25255</td>
<td>0.04799</td>
<td>0.6125</td>
<td>0</td>
</tr>
<tr>
<td>23.5</td>
<td>0.13375</td>
<td>0.06454</td>
<td>0.5675</td>
<td>0.05511</td>
</tr>
<tr>
<td>1.75</td>
<td>0.29215</td>
<td>0.03357</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>0.19315</td>
<td>0.09598</td>
<td>0.77</td>
<td>0.05511</td>
</tr>
<tr>
<td>8.5</td>
<td>0.17335</td>
<td>0.03704</td>
<td>0.545</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.25</td>
<td>0.35155</td>
<td>0.01852</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2.25</td>
<td>0.208</td>
<td>0.08134</td>
<td>0.635</td>
<td>0.045</td>
</tr>
<tr>
<td>19</td>
<td>0.2377</td>
<td>0.08342</td>
<td>0.6575</td>
<td>0.05511</td>
</tr>
<tr>
<td>11</td>
<td>0.23275</td>
<td>0.01565</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2.75</td>
<td>0.21295</td>
<td>0.0594</td>
<td>0.5675</td>
<td>0.05511</td>
</tr>
<tr>
<td>14</td>
<td>0.2278</td>
<td>0.05285</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2.5</td>
<td>0.22285</td>
<td>0.05096</td>
<td>0.6575</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.5</td>
<td>0.1684</td>
<td>0.08073</td>
<td>0.5675</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.5</td>
<td>0.7327</td>
<td>0.07572</td>
<td>0.7025</td>
<td>0.08419</td>
</tr>
<tr>
<td>2</td>
<td>0.25255</td>
<td>0.0594</td>
<td>0.5675</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.63</td>
<td>0.23275</td>
<td>0.05192</td>
<td>0.5675</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.63</td>
<td>0.14365</td>
<td>0.05981</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2.63</td>
<td>0.20305</td>
<td>0.0653</td>
<td>0.59</td>
<td>0.045</td>
</tr>
<tr>
<td>2.63</td>
<td>0.15355</td>
<td>0.10072</td>
<td>0.6125</td>
<td>0</td>
</tr>
<tr>
<td>3.75</td>
<td>0.1585</td>
<td>0.12424</td>
<td>0.7475</td>
<td>0.045</td>
</tr>
<tr>
<td>2.75</td>
<td>0.19315</td>
<td>0.13966</td>
<td>0.77</td>
<td>0.05511</td>
</tr>
<tr>
<td>14</td>
<td>0.109</td>
<td>0.0495</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>0.30205</td>
<td>0.04258</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>19.5</td>
<td>0.31195</td>
<td>0.0099</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Value</td>
<td>Column 1</td>
<td>Column 2</td>
<td>Column 3</td>
<td>Column 4</td>
</tr>
<tr>
<td>-------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>19</td>
<td>0.2278</td>
<td>0.0594</td>
<td>0.6125</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>0.3466</td>
<td>0.01213</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>29</td>
<td>0.1981</td>
<td>0.1229</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>0.1486</td>
<td>0.07275</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>0.2971</td>
<td>0.04021</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2.5</td>
<td>0.1783</td>
<td>0.07241</td>
<td>0.5675</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.5</td>
<td>0.1486</td>
<td>0.10217</td>
<td>0.5225</td>
<td>0.045</td>
</tr>
<tr>
<td>9</td>
<td>0.13375</td>
<td>0.06823</td>
<td>0.545</td>
<td>0.05511</td>
</tr>
<tr>
<td>9</td>
<td>0.1882</td>
<td>0.0653</td>
<td>0.545</td>
<td>0.05511</td>
</tr>
<tr>
<td>9</td>
<td>0.18325</td>
<td>0.09392</td>
<td>0.6575</td>
<td>0.05511</td>
</tr>
<tr>
<td>8.5</td>
<td>0.01</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2.5</td>
<td>0.5545</td>
<td>0.25769</td>
<td>0.635</td>
<td>0.16534</td>
</tr>
<tr>
<td>8.5</td>
<td>0.34165</td>
<td>0.33265</td>
<td>0.7925</td>
<td>0.1526</td>
</tr>
<tr>
<td>12</td>
<td>0.19315</td>
<td>0.14312</td>
<td>0.5675</td>
<td>0.05511</td>
</tr>
<tr>
<td>1.75</td>
<td>0.01</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>4.5</td>
<td>0.7921</td>
<td>0.39152</td>
<td>0.7025</td>
<td>0.30521</td>
</tr>
</tbody>
</table>

*Time constants in seconds.*
Table Appendix 3D. The time constants (fitting pulse data to Kelvin model)
Longitudinal samples, at 30% strain.

<table>
<thead>
<tr>
<th>Age (month)</th>
<th>$\tau_\epsilon$ (Mean)</th>
<th>$\tau_\epsilon$ (SD)</th>
<th>$\tau_\sigma$ (Mean)</th>
<th>$\tau_\sigma$ (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>0.21295</td>
<td>0.05285</td>
<td>0.5675</td>
<td>0.09</td>
</tr>
<tr>
<td>23.5</td>
<td>0.11395</td>
<td>0.06714</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>1.75</td>
<td>0.3763</td>
<td>0.0099</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>0.23275</td>
<td>0.05423</td>
<td>0.635</td>
<td>0.045</td>
</tr>
<tr>
<td>8.5</td>
<td>0.2575</td>
<td>0.07173</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2.25</td>
<td>0.3961</td>
<td>0.01213</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2.25</td>
<td>0.1684</td>
<td>0.13338</td>
<td>0.6575</td>
<td>0.05511</td>
</tr>
<tr>
<td>19</td>
<td>0.34165</td>
<td>0.0485</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>11</td>
<td>0.31195</td>
<td>0.04258</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2.75</td>
<td>0.208</td>
<td>0.08429</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>0.3862</td>
<td>0.02425</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2.5</td>
<td>0.24265</td>
<td>0.03357</td>
<td>0.5225</td>
<td>0.045</td>
</tr>
<tr>
<td>2.5</td>
<td>0.2575</td>
<td>0.06454</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2.5</td>
<td>0.39115</td>
<td>0.31911</td>
<td>0.5675</td>
<td>0.09</td>
</tr>
<tr>
<td>2</td>
<td>0.2674</td>
<td>0.02524</td>
<td>0.5675</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.63</td>
<td>0.1288</td>
<td>0.09826</td>
<td>0.59</td>
<td>0.045</td>
</tr>
<tr>
<td>2.63</td>
<td>0.2773</td>
<td>0.03638</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2.63</td>
<td>0.42085</td>
<td>0.01213</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2.63</td>
<td>0.24265</td>
<td>0.0198</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>3.75</td>
<td>0.21295</td>
<td>0.10312</td>
<td>0.59</td>
<td>0.045</td>
</tr>
<tr>
<td>2.75</td>
<td>0.23275</td>
<td>0.05644</td>
<td>0.68</td>
<td>0.05511</td>
</tr>
<tr>
<td>14</td>
<td>0.21295</td>
<td>0.05048</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>12</td>
<td>0.3664</td>
<td>0.02524</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>19.5</td>
<td>0.3466</td>
<td>0.02524</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>Value</td>
<td>Time 1</td>
<td>Time 2</td>
<td>Time 3</td>
<td>Time 4</td>
</tr>
<tr>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
<td>--------</td>
</tr>
<tr>
<td>19</td>
<td>0.208</td>
<td>0.08283</td>
<td>0.5675</td>
<td>0.05511</td>
</tr>
<tr>
<td>19</td>
<td>0.26245</td>
<td>0.04537</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>29</td>
<td>0.30205</td>
<td>0.05048</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>0.21295</td>
<td>0.02425</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>30</td>
<td>0.35155</td>
<td>0.02425</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>2.5</td>
<td>0.15355</td>
<td>0.08631</td>
<td>0.545</td>
<td>0.05511</td>
</tr>
<tr>
<td>2.5</td>
<td>0.22285</td>
<td>0.05096</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>0.1585</td>
<td>0.10265</td>
<td>0.5225</td>
<td>0.045</td>
</tr>
<tr>
<td>9</td>
<td>0.01</td>
<td>0</td>
<td>0.635</td>
<td>0.08419</td>
</tr>
<tr>
<td>9</td>
<td>0.21295</td>
<td>0.05048</td>
<td>0.5225</td>
<td>0.045</td>
</tr>
<tr>
<td>8.5</td>
<td>0.66835</td>
<td>0.12678</td>
<td>0.68</td>
<td>0.11473</td>
</tr>
<tr>
<td>2.5</td>
<td>0.3961</td>
<td>0.25845</td>
<td>0.5675</td>
<td>0.135</td>
</tr>
<tr>
<td>8.5</td>
<td>0.26245</td>
<td>0.19387</td>
<td>0.59</td>
<td>0.045</td>
</tr>
<tr>
<td>12</td>
<td>0.4654</td>
<td>0.1719</td>
<td>0.545</td>
<td>0.09</td>
</tr>
<tr>
<td>1.75</td>
<td>0.77725</td>
<td>0.26286</td>
<td>0.68</td>
<td>0.22045</td>
</tr>
<tr>
<td>4.5</td>
<td>0.9505</td>
<td>0.05192</td>
<td>0.7475</td>
<td>0.08419</td>
</tr>
</tbody>
</table>

*Time constants in seconds.*
10. Table Appendix 3D. 10 The stiffness of spring 1 ($\mu_1$) (fitting pulse data to Kelvin model)

<table>
<thead>
<tr>
<th>Age</th>
<th>C (10%)</th>
<th>C (20%)</th>
<th>C (30%)</th>
<th>L (10%)</th>
<th>L (20%)</th>
<th>L (30%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>65.35317</td>
<td>42.21651</td>
<td>43.56415</td>
<td>29.82132</td>
<td>10.54436</td>
<td>13.92422</td>
</tr>
<tr>
<td>23.5</td>
<td>15.05526</td>
<td>27.28951</td>
<td>17.421</td>
<td>30.3547</td>
<td>44.12449</td>
<td>52.42146</td>
</tr>
<tr>
<td>1.75</td>
<td>116.56789</td>
<td>76.64313</td>
<td>64.18625</td>
<td>36.46604</td>
<td>16.49958</td>
<td>8.81096</td>
</tr>
<tr>
<td>20</td>
<td>23.39822</td>
<td>14.64347</td>
<td>10.13501</td>
<td>44.39133</td>
<td>35.34133</td>
<td>24.64262</td>
</tr>
<tr>
<td>8.5</td>
<td>75.77942</td>
<td>25.67849</td>
<td>17.421</td>
<td>38.01703</td>
<td>32.96541</td>
<td>16.85866</td>
</tr>
<tr>
<td>2.25</td>
<td>17.0518</td>
<td>27.50754</td>
<td>22.52101</td>
<td>114.99892</td>
<td>19.88036</td>
<td>29.86594</td>
</tr>
<tr>
<td>19</td>
<td>24.54019</td>
<td>30.30532</td>
<td>42.42984</td>
<td>75.31695</td>
<td>23.06971</td>
<td>10.24053</td>
</tr>
<tr>
<td>11</td>
<td>22.948</td>
<td>43.01949</td>
<td>54.65028</td>
<td>21.64271</td>
<td>17.49086</td>
<td>9.85612</td>
</tr>
<tr>
<td>2.75</td>
<td>58.20918</td>
<td>28.19494</td>
<td>39.66027</td>
<td>54.95612</td>
<td>42.42366</td>
<td>41.73251</td>
</tr>
<tr>
<td>14</td>
<td>20.21243</td>
<td>49.05155</td>
<td>46.80679</td>
<td>50.94624</td>
<td>25.76274</td>
<td>7.263</td>
</tr>
<tr>
<td>2.5</td>
<td>13.54823</td>
<td>20.9739</td>
<td>36.92272</td>
<td>348.48495</td>
<td>22.48362</td>
<td>17.76129</td>
</tr>
<tr>
<td>2.5</td>
<td>569.58584</td>
<td>34.91909</td>
<td>31.80033</td>
<td>367.78126</td>
<td>-0.27581</td>
<td>3.745</td>
</tr>
<tr>
<td>2</td>
<td>13.45111</td>
<td>15.96348</td>
<td>42.32754</td>
<td>36.30437</td>
<td>16.87427</td>
<td>16.9119</td>
</tr>
<tr>
<td>2.63</td>
<td>13.37111</td>
<td>30.36032</td>
<td>228.60274</td>
<td>32.75484</td>
<td>34.16252</td>
<td>92.92886</td>
</tr>
<tr>
<td>2.63</td>
<td>351.12469</td>
<td>24.04405</td>
<td>16.95637</td>
<td>46.86422</td>
<td>40.22396</td>
<td>13.30815</td>
</tr>
<tr>
<td>2.63</td>
<td>13.53047</td>
<td>32.54604</td>
<td>40.70292</td>
<td>4.60766</td>
<td>22.68787</td>
<td>3.44213</td>
</tr>
<tr>
<td>2.63</td>
<td>17.72232</td>
<td>16.64939</td>
<td>23.54977</td>
<td>32.2068</td>
<td>35.28445</td>
<td>14.51088</td>
</tr>
<tr>
<td>3.75</td>
<td>14.15422</td>
<td>7.84181</td>
<td>0.54052</td>
<td>47.41894</td>
<td>28.32544</td>
<td>17.09608</td>
</tr>
<tr>
<td>2.75</td>
<td>183.23367</td>
<td>24.4202</td>
<td>18.93089</td>
<td>552.72646</td>
<td>24.80775</td>
<td>17.5847</td>
</tr>
<tr>
<td>14</td>
<td>209.0566</td>
<td>63.5477</td>
<td>43.71672</td>
<td>43.33751</td>
<td>64.56881</td>
<td>27.2651</td>
</tr>
<tr>
<td>12</td>
<td>15.8695</td>
<td>18.64576</td>
<td>16.67315</td>
<td>11.81946</td>
<td>10.02969</td>
<td>5.90266</td>
</tr>
<tr>
<td>19.5</td>
<td>11.56075</td>
<td>22.27413</td>
<td>41.41518</td>
<td>11.42756</td>
<td>12.34588</td>
<td>9.29713</td>
</tr>
<tr>
<td>----</td>
<td>---------</td>
<td>----------</td>
<td>---------</td>
<td>----------</td>
<td>----------</td>
<td>----------</td>
</tr>
<tr>
<td>19</td>
<td>17.80838</td>
<td>15.30947</td>
<td>21.62351</td>
<td>7.50452</td>
<td>6.91363</td>
<td>12.57687</td>
</tr>
<tr>
<td>29</td>
<td>41.74186</td>
<td>16.89662</td>
<td>23.7176</td>
<td>27.94101</td>
<td>27.38135</td>
<td>12.58128</td>
</tr>
<tr>
<td>30</td>
<td>30.58669</td>
<td>19.27447</td>
<td>37.20998</td>
<td>22.48499</td>
<td>22.07239</td>
<td>12.52232</td>
</tr>
<tr>
<td>30</td>
<td>57.48391</td>
<td>9.55942</td>
<td>8.72329</td>
<td>11.5103</td>
<td>7.59591</td>
<td>4.70738</td>
</tr>
<tr>
<td>2.5</td>
<td>13.14521</td>
<td>6.95602</td>
<td>17.7076</td>
<td>19.01106</td>
<td>27.99169</td>
<td>31.61687</td>
</tr>
<tr>
<td>2.5</td>
<td>53.98572</td>
<td>6.90805</td>
<td>19.16881</td>
<td>8.48443</td>
<td>15.21791</td>
<td>8.27702</td>
</tr>
<tr>
<td>9</td>
<td>392.4</td>
<td>4.8501</td>
<td>4.30107</td>
<td>13.93823</td>
<td>14.91447</td>
<td>376.84375</td>
</tr>
<tr>
<td>8.5</td>
<td>3.53212</td>
<td>129.07874</td>
<td>-0.29621</td>
<td>4.51059</td>
<td>286.97928</td>
<td>0.10209</td>
</tr>
<tr>
<td>2.5</td>
<td>0.49949</td>
<td>2.93651</td>
<td>0.07901</td>
<td>10.29726</td>
<td>1.07647</td>
<td>2.73869</td>
</tr>
<tr>
<td>8.5</td>
<td>5.1176</td>
<td>1.63187</td>
<td>2.10777</td>
<td>28.63857</td>
<td>3.53703</td>
<td>3.4085</td>
</tr>
<tr>
<td>1.75</td>
<td>2.24331</td>
<td>-0.46393</td>
<td>-0.60937</td>
<td>-0.88143</td>
<td>279.47101</td>
<td>-1.14393</td>
</tr>
<tr>
<td>4.5</td>
<td>0.16544</td>
<td>-0.67673</td>
<td>-1.19605</td>
<td>-4.0638</td>
<td>-1.01156</td>
<td>-2.69298</td>
</tr>
</tbody>
</table>

C: Circumferential samples, L: Longitudinal samples. %: the strain value. The unit of age is month. All other values in kPa.
### The viscosity of dashpot ($\eta$) (fitting pulse data to Kelvin model)

<table>
<thead>
<tr>
<th>Age</th>
<th>C (10%)</th>
<th>C (20%)</th>
<th>C (30%)</th>
<th>L (10%)</th>
<th>L (20%)</th>
<th>L (30%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>4.53551</td>
<td>4.39263</td>
<td>6.47363</td>
<td>4.72668</td>
<td>2.66298</td>
<td>2.96516</td>
</tr>
<tr>
<td>23.5</td>
<td>3.13149</td>
<td>3.51489</td>
<td>4.05474</td>
<td>6.76455</td>
<td>5.90165</td>
<td>5.97342</td>
</tr>
<tr>
<td>1.75</td>
<td>12.12889</td>
<td>11.38917</td>
<td>10.80897</td>
<td>6.68245</td>
<td>4.82035</td>
<td>3.31557</td>
</tr>
<tr>
<td>20</td>
<td>1.85548</td>
<td>1.81359</td>
<td>2.05791</td>
<td>7.03603</td>
<td>6.82618</td>
<td>5.73557</td>
</tr>
<tr>
<td>8.5</td>
<td>6.75952</td>
<td>7.62908</td>
<td>13.03411</td>
<td>8.28391</td>
<td>5.71455</td>
<td>4.3411</td>
</tr>
<tr>
<td>2.25</td>
<td>1.17731</td>
<td>4.09708</td>
<td>7.15391</td>
<td>2.1225</td>
<td>2.16388</td>
<td>1.69312</td>
</tr>
<tr>
<td>2.25</td>
<td>3.89634</td>
<td>3.54297</td>
<td>4.46141</td>
<td>6.84244</td>
<td>4.13511</td>
<td>5.02943</td>
</tr>
<tr>
<td>19</td>
<td>4.61846</td>
<td>5.70346</td>
<td>6.72513</td>
<td>9.70082</td>
<td>5.48367</td>
<td>3.49868</td>
</tr>
<tr>
<td>11</td>
<td>0.22948</td>
<td>10.22573</td>
<td>10.01466</td>
<td>5.03734</td>
<td>4.071</td>
<td>3.07462</td>
</tr>
<tr>
<td>2.75</td>
<td>5.76853</td>
<td>6.56237</td>
<td>8.64197</td>
<td>11.43087</td>
<td>9.03412</td>
<td>8.68036</td>
</tr>
<tr>
<td>14</td>
<td>5.50486</td>
<td>7.77467</td>
<td>11.35767</td>
<td>8.32716</td>
<td>5.86875</td>
<td>2.80497</td>
</tr>
<tr>
<td>2.5</td>
<td>3.68986</td>
<td>3.94729</td>
<td>5.12118</td>
<td>6.93485</td>
<td>5.01048</td>
<td>4.30978</td>
</tr>
<tr>
<td>2.5</td>
<td>4.72233</td>
<td>3.09428</td>
<td>4.66394</td>
<td>8.50676</td>
<td>8.60477</td>
<td>6.5176</td>
</tr>
<tr>
<td>2.5</td>
<td>5.69586</td>
<td>4.84328</td>
<td>4.72553</td>
<td>3.67781</td>
<td>-0.20209</td>
<td>1.46485</td>
</tr>
<tr>
<td>2</td>
<td>3.26391</td>
<td>3.95256</td>
<td>6.91844</td>
<td>7.19189</td>
<td>4.2616</td>
<td>4.52224</td>
</tr>
<tr>
<td>2.63</td>
<td>3.51125</td>
<td>3.57295</td>
<td>3.77873</td>
<td>6.73205</td>
<td>5.77817</td>
<td>3.69035</td>
</tr>
<tr>
<td>2.63</td>
<td>4.15385</td>
<td>6.44737</td>
<td>7.66029</td>
<td>1.64263</td>
<td>4.60677</td>
<td>1.44862</td>
</tr>
<tr>
<td>2.63</td>
<td>2.80899</td>
<td>2.30927</td>
<td>2.56693</td>
<td>6.53959</td>
<td>5.41793</td>
<td>3.52107</td>
</tr>
<tr>
<td>3.75</td>
<td>2.31351</td>
<td>1.66991</td>
<td>0.29436</td>
<td>5.63811</td>
<td>4.48958</td>
<td>3.64061</td>
</tr>
<tr>
<td>2.75</td>
<td>4.55336</td>
<td>2.90356</td>
<td>3.09425</td>
<td>8.26326</td>
<td>4.79162</td>
<td>4.09284</td>
</tr>
<tr>
<td>14</td>
<td>6.22989</td>
<td>6.9267</td>
<td>8.66028</td>
<td>7.9416</td>
<td>7.038</td>
<td>5.8061</td>
</tr>
<tr>
<td>12</td>
<td>3.2223</td>
<td>2.67846</td>
<td>2.89029</td>
<td>3.39455</td>
<td>3.02947</td>
<td>2.16273</td>
</tr>
<tr>
<td>19.5</td>
<td>3.49192</td>
<td>4.85353</td>
<td>7.17932</td>
<td>3.62139</td>
<td>3.8513</td>
<td>3.22238</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>1.07747</td>
<td>2.27786</td>
<td>1.95903</td>
<td>6.87727</td>
<td>4.54687</td>
<td>4.29038</td>
</tr>
<tr>
<td>19</td>
<td>3.08708</td>
<td>3.03281</td>
<td>4.17658</td>
<td>2.52677</td>
<td>2.39626</td>
<td>3.3008</td>
</tr>
<tr>
<td>29</td>
<td>2.27702</td>
<td>2.84539</td>
<td>3.64184</td>
<td>7.47143</td>
<td>5.42425</td>
<td>3.80017</td>
</tr>
<tr>
<td>30</td>
<td>4.99939</td>
<td>4.58154</td>
<td>6.81873</td>
<td>4.34298</td>
<td>3.27996</td>
<td>2.66663</td>
</tr>
<tr>
<td>30</td>
<td>2.28211</td>
<td>1.60981</td>
<td>1.81444</td>
<td>2.90693</td>
<td>2.25675</td>
<td>1.65488</td>
</tr>
<tr>
<td>2.5</td>
<td>2.21365</td>
<td>1.61901</td>
<td>2.80665</td>
<td>5.9305</td>
<td>4.99092</td>
<td>4.85477</td>
</tr>
<tr>
<td>2.5</td>
<td>2.94492</td>
<td>1.98399</td>
<td>3.32291</td>
<td>2.10074</td>
<td>2.26138</td>
<td>1.84453</td>
</tr>
<tr>
<td>9</td>
<td>2.05663</td>
<td>1.60062</td>
<td>2.72852</td>
<td>3.37736</td>
<td>2.54855</td>
<td>2.3648</td>
</tr>
<tr>
<td>9</td>
<td>3.924</td>
<td>1.08085</td>
<td>1.12881</td>
<td>3.4511</td>
<td>2.8069</td>
<td>3.76844</td>
</tr>
<tr>
<td>9</td>
<td>2.74777</td>
<td>2.34568</td>
<td>2.94753</td>
<td>2.80814</td>
<td>2.1042</td>
<td>1.55967</td>
</tr>
<tr>
<td>8.5</td>
<td>1.18926</td>
<td>1.29079</td>
<td>-0.24196</td>
<td>1.27311</td>
<td>2.86979</td>
<td>0.06823</td>
</tr>
<tr>
<td>2.5</td>
<td>0.27449</td>
<td>0.75615</td>
<td>0.03873</td>
<td>2.29474</td>
<td>0.59691</td>
<td>1.0848</td>
</tr>
<tr>
<td>8.5</td>
<td>1.0138</td>
<td>0.52522</td>
<td>0.60535</td>
<td>2.4128</td>
<td>1.20843</td>
<td>0.89456</td>
</tr>
<tr>
<td>12</td>
<td>2.3784</td>
<td>2.29905</td>
<td>2.00853</td>
<td>2.29593</td>
<td>2.2203</td>
<td>0.59473</td>
</tr>
<tr>
<td>1.75</td>
<td>1.05514</td>
<td>-0.26414</td>
<td>-0.47061</td>
<td>-0.70691</td>
<td>2.79471</td>
<td>-0.88912</td>
</tr>
<tr>
<td>4.5</td>
<td>0.0991</td>
<td>-0.54274</td>
<td>-1.19605</td>
<td>-4.0638</td>
<td>-0.80125</td>
<td>-2.55968</td>
</tr>
</tbody>
</table>

*C: Circumferential samples, L: Longitudinal samples. %: the strain value.
The unit of age is month. All other values in kPa s.*
Appendix 3E: Examples of the Tracing of Experiments

1. Photograph of the tracing of the static-preloading (Figure 3a.1)

![Photograph of the tracing of the static-preloading](image)

Photograph taken by author
2. Photograph of the tracing of the sinusoidal oscillation (Figure 3a. 2)
Appendix 4A: Program for Elastic Fiber Orientation Analysis

Fortran program: Morpho1.FOR: program takes in the measurements on single fibers and calculates the statistical distribution of different types of fibers.

* * * * * *

** THIS PROGRAM PERFORMS A FIBRE CLASS DISTRIBUTION ANALYSIS BASED ON A SET OF LENGTH, THICKNESS, LONGITUDINAL-DIRECTION SPAN AND CIRCUMFERENTIAL-DIRECTION SPAN MEASUREMENTS. THE REQUIRED INPUT CONSISTS OF NC DATA POINTS DESCRIBING THE CIRCUMFERENTIAL SECTION AND OF NL DATA POINTS DESCRIBING THE LONGITUDINAL SECTION OF THE SAME SAMPLE. **

* * * * * *

**

C

CHARACTER*1 ANS1
CHARACTER*12 INPFILE,OUTFILE
REAL L_C,T_C,A_C,B_C,L_L,T_L,A_L,B_L
REAL LT_CRIT,AB_CRIT
INTEGER CIRC_D,CIRC_L,CIRC_T,CIRC_A
INTEGER LONG_D,LONG_C,LONG_T,LONG_A
INTEGER CIRC_LD,CIRC_TD,LONG_CD,LONG_TD
INTEGER COMB_L,COMB_T,COMB_C,COMB_A

C

PARAMETER (OUTFILE='MORPH01.OUT')
PARAMETER (LT_Crit=2,AB_CRIT=2)
*

* LT_CRIT: CRITICAL LENGTH-TO-THICKNESS RATIO TO IDENTIFY A DOT
* AB_CRIT: CRITICAL A-TO-B RATIO TO IDENTIFY CLASSES L,C,T,A
*

DIMENSION L_C(1:500) ! LENGTH, CIRCUMFERENTIAL SECTION
DIMENSION T_C(1:500) ! THICKNESS, CIRCUMFERENTIAL SECTION
DIMENSION A_C(1:500) ! A-SPAN, CIRCUMFERENTIAL SECTION
DIMENSION B_C(1:500) ! B-SPAN, CIRCUMFERENTIAL SECTION
DIMENSION L_L(1:500) ! LENGTH, LONGITUDINAL SECTION
DIMENSION T_L(1:500) ! THICKNESS, LONGITUDINAL SECTION
DIMENSION A_L(1:500) ! A-SPAN, LONGITUDINAL SECTION
DIMENSION B_L(1:500) ! B-SPAN, LONGITUDINAL SECTION

340
900 FORMAT(I4,4F14.8)
901 FORMAT(I7,' D',I7,' L',I7,' T',I7,' A')
902 FORMAT(I7,' D',I7,' C',I7,' T',I7,' A')
903 FORMAT(F6.1,'% D',F6.1,'% L',F6.1,'% T',F6.1,'% A')
904 FORMAT(F6.1,'% D',F6.1,'% C',F6.1,'% T',F6.1,'% A')
905 FORMAT( ',I4,' ('I4,' CIRC,'I4,' LONG-DOT'))
906 FORMAT( ',I4,' ('I4,' LONG,'I4,' CIRC-DOT'))
907 FORMAT( ',I4,' ('I4,' CIRC,'I4,' CIRC-DOT','
          > ',I4,' LONG,'I4,' LONG-DOT'))
908 FORMAT( ',I4,' ('I4,' CIRC,'I4,' LONG))
909 FORMAT( ',F4.1,'% ('F4.1,'% CIRC,'F4.1,'% LONG-DOT'))
910 FORMAT( ',C',F4.1,'% ('F4.1,'% LONG,'F4.1,'% CIRC-DOT'))
911 FORMAT( ',T',F4.1,'% ('F4.1,'% CIRC,'F4.1,'% CIRC-DOT','
          > ',F4.1,'% LONG,'F4.1,'% LONG-DOT'))
912 FORMAT( ',A',F4.1,'% ('F4.1,'% CIRC,'F4.1,'% LONG'))
913 FORMAT( ' RATIO L/C: ',F6.2)
914 FORMAT( ' RATIO L/T: ',F6.2)
915 FORMAT( ' RATIO L/A: ',F6.2)

WRITE(6,*)'NAME OF THE INPUT FILE:'
READ(5,*) INPFILE
OPEN (UNIT=9,FILE=INPFILE)
READ(9,*)
READ(9,*)
READ(9,*)
READ(9,*)
READ(9,*)
10 WRITE(6,*)'WHICH SECTION'S DATA FIRST (C/L) ?'
READ(5,*) ANSI
IF (ANS1.EQ.'C'.OR.ANS1.EQ.'c') THEN
  INDC1=1
ELSE IF (ANS1.EQ.'L'.OR.ANS1.EQ.'l') THEN
  INDC1=0
ELSE
  GO TO 10
END IF
WRITE(6,*)'NUMBER OF DATA POINTS FOR THE CIRCUMFERENTIAL SECTION:'
READ(5,*) NC
WRITE(6,*)'NUMBER OF DATA POINTS FOR THE LONGITUDINAL SECTION:'
READ(5,*) NL
NTOT=NL+NC
CNST=REAL(100)/REAL(NTOT)
C
IF (INDC1.EQ.1) THEN
  DO I=1,NC
    READ(9,*) J,X,L_L(I),T_L(I),A_L(I),B_L(I)
  END DO
  DO I=1,NL
    READ(9,*) J,X,L_L(I),T_L(I),A_L(I),B_L(I)
  END DO
END DO ELSE
DO I=1,NL
READ(9,* J,X,L_L(I),T_L(I),A_L(I),B_L(I)
END DO
DO I=1,NC
READ(9,* J,X,L_C(I),T_C(I),A_C(I),B_C(I)
END DO
END IF
CLOSE(9)
CIRC_D=0 ! NUMBER OF D-TYPE IMAGES, CIRCUMFERENTIAL SECTION
CIRC_L=0 ! NUMBER OF L-TYPE IMAGES, CIRCUMFERENTIAL SECTION
CIRC_T=0 ! NUMBER OF T-TYPE IMAGES, CIRCUMFERENTIAL SECTION
CIRC_A=0 ! NUMBER OF A-TYPE IMAGES, CIRCUMFERENTIAL SECTION
LONG_D=0 ! NUMBER OF D-TYPE IMAGES, LONGITUDINAL SECTION
LONG_C=0 ! NUMBER OF C-TYPE IMAGES, LONGITUDINAL SECTION
LONG_T=0 ! NUMBER OF T-TYPE IMAGES, LONGITUDINAL SECTION
LONG_A=0 ! NUMBER OF A-TYPE IMAGES, LONGITUDINAL SECTION
C
DO I=1,NC
IF (L_C(I)/T_C(I).LE.LT_CRIT) THEN
CIRC_D=CIRC_D+1
ELSE IF (A_C(I)/B_C(I).GT.AB_CRIT) THEN
CIRC_L=CIRC_L+1
ELSE IF (A_C(I)/B_C(I).LT.1./AB_CRIT) THEN
CIRC_A=CIRC_A+1
ELSE
CIRC_T=CIRC_T+1
END IF
END IF
END DO
DO I=1,NL
IF (L_L(I)/T_L(I).LE.LT_CRIT) THEN
LONG_D=LONG_D+1
ELSE IF (A_L(I)/B_L(I).GT.AB_CRIT) THEN
LONG_C=LONG_C+1
ELSE IF (A_L(I)/B_L(I).LT.1./AB_CRIT) THEN
LONG_A=LONG_A+1
ELSE
LONG_T=LONG_T+1
END IF
END IF
END DO
CIRC_LD=NINT(REAL(LONG_D*CIRC_L)/REAL(CIRC_L+CIRC_T))
CIRC_TD=NINT(REAL(LONG_D*CIRC_T)/REAL(CIRC_L+CIRC_T))
LONG_CD=NINT(REAL(CIRC_D*LONG_C)/REAL(LONG_C+LONG_T))
LONG_TD=NINT(REAL(CIRC_D*LONG_T)/REAL(LONG_C+LONG_T))
COMB_L=CIRC_L+CIRC_LD
COMB_C=LONG_C+LONG_CD
COMB_T = CIRC_T + CIRC_TD + LONG_T + LONG_TD
COMB_A = CIRC_A + LONG_A

* OUTPUT RESULTS *

WRITE(6,'*') 'CIRCUMFERENTIAL SECTION:
WRITE(6,901) CIRC_D, CIRC_L, CIRC_T, CIRC_A
WRITE(6,903)
> REAL(100*CIRC_D)/REAL(NC),
> REAL(100*CIRC_L)/REAL(NC),
> REAL(100*CIRC_T)/REAL(NC),
> REAL(100*CIRC_A)/REAL(NC)
WRITE(6,'*') 'LONGITUDINAL SECTION:
WRITE(6,902) LONG_D, LONG_C, LONG_T, LONG_A
WRITE(6,904)
> REAL(100*LONG_D)/REAL(NL),
> REAL(100*LONG_C)/REAL(NL),
> REAL(100*LONG_T)/REAL(NL),
> REAL(100*LONG_A)/REAL(NL)
WRITE(6,'*') 'COMBINED SECTIONS:
WRITE(6,905) COMB_L, CIRC_L, CIRC_LD
WRITE(6,906) COMB_C, LONG_C, LONG_CD
WRITE(6,907) COMB_T, CIRC_T, LONG_TD, LONG_T, CIRC_TD
WRITE(6,908) COMB_A, CIRC_A, LONG_A
WRITE(6,909)
> CNST*REAL(COMB_L), CNST*REAL(CIRC_L), CNST*REAL(CIRC_LD)
WRITE(6,910)
> CNST*REAL(COMB_C), CNST*REAL(LONG_C), CNST*REAL(LONG_CD)
WRITE(6,911)
> CNST*REAL(COMB_T), CNST*REAL(CIRC_T), CNST*REAL(LONG_TD),
> CNST*REAL(LONG_T), CNST*REAL(CIRC_TD)
WRITE(6,912)
> CNST*REAL(COMB_A), CNST*REAL(CIRC_A), CNST*REAL(LONG_A)

C
OPEN (8, FILE = 'OUTFILE')
WRITE(8,'*') 'INPUT FILE:', INPFILE
WRITE(8,'*') 'CIRCUMFERENTIAL SECTION:
WRITE(8,901) CIRC_D, CIRC_L, CIRC_T, CIRC_A
WRITE(8,903)
> REAL(100*CIRC_D)/REAL(NC),
> REAL(100*CIRC_L)/REAL(NC),
> REAL(100*CIRC_T)/REAL(NC),
> REAL(100*CIRC_A)/REAL(NC)
WRITE(8,'*') 'LONGITUDINAL SECTION:
WRITE(8,902) LONG_D, LONG_C, LONG_T, LONG_A
WRITE(8,904)
> REAL(100*LONG_D)/REAL(NL),
> REAL(100*LONG_C)/REAL(NL),
> REAL(100*LONG_T)/REAL(NL),
> REAL(100*LONG_A)/REAL(NL)
WRITE(8,*)
WRITE(8,*)'COMBINED SECTIONS:
WRITE(8,905) COMB_L,CIRC_L,CIRC_LD
WRITE(8,906) COMB_C,LONG_C,LONG_CD
WRITE(8,907) COMB_T,CIRC_T,LONG_TD,LONG_T,CIRC_TD
WRITE(8,908) COMB_A,CIRC_A,LONG_A
WRITE(8,909)
> CNST*REAL(COMB_L),CNST*REAL(CIRC_L),CNST*REAL(CIRC_LD)
WRITE(8,910)
> CNST*REAL(COMB_C),CNST*REAL(LONG_C),CNST*REAL(LONG_CD)
WRITE(8,911)
> CNST*REAL(COMB_T),CNST*REAL(CIRC_T),CNST*REAL(LONG_TD),
> CNST*REAL(LONG_T),CNST*REAL(CIRC_TD)
WRITE(8,912)
> CNST*REAL(COMB_A),CNST*REAL(CIRC_A),CNST*REAL(LONG_A)

* ASPECT RATIOS

* ASPECT RATIOS (COMBINED SECTIONS):
WRITE(6,*)'ASPECT RATIOS (COMBINED SECTIONS):
WRITE(8,*)
WRITE(8,*)'ASPECT RATIOS (COMBINED SECTIONS):
IF (COMB_C.NE.0) THEN
   RATIO_LC=REAL(COMB_L)/REAL(COMB_C)
WRITE(6,913) RATIO_LC
WRITE(8,913) RATIO_LC
ELSE
   WRITE(6,*)'RATIO_LC: INFINITY'
   WRITE(8,*)'RATIO_LC: INFINITY'
END IF
IF (COMB_T.NE.0) THEN
   RATIO_LT=REAL(COMB_L)/REAL(COMB_T)
WRITE(6,914) RATIO_LT
WRITE(8,914) RATIO_LT
ELSE
   WRITE(6,*)'RATIO_LT: INFINITY'
   WRITE(8,*)'RATIO_LT: INFINITY'
END IF
IF (COMB_A.NE.0) THEN
   RATIO_LA=REAL(COMB_L)/REAL(COMB_A)
WRITE(6,915) RATIO_LA
WRITE(8,915) RATIO_LA
ELSE
   WRITE(6,*)'RATIO_LA: INFINITY'
   WRITE(8,*)'RATIO_LA: INFINITY'
END IF

344
CLOSE(8)

C

END

END IF
Appendix 4B: Results of Morphometrical Measurements

1. *Table Appendix 4B.1 The average thickness (diameter) of elastic fiber bundles*

<table>
<thead>
<tr>
<th>Age (month)</th>
<th>Thickness (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>0.00154</td>
</tr>
<tr>
<td>23.5</td>
<td>0.00159</td>
</tr>
<tr>
<td>1.75</td>
<td>0.00165</td>
</tr>
<tr>
<td>20</td>
<td>0.00149</td>
</tr>
<tr>
<td>8.5</td>
<td>0.00147</td>
</tr>
<tr>
<td>2.25</td>
<td>0.00155</td>
</tr>
<tr>
<td>2.25</td>
<td>0.00139</td>
</tr>
<tr>
<td>19</td>
<td>0.00148</td>
</tr>
<tr>
<td>11</td>
<td>0.00174</td>
</tr>
<tr>
<td>2.75</td>
<td>0.00153</td>
</tr>
<tr>
<td>14</td>
<td>0.00168</td>
</tr>
<tr>
<td>2.5</td>
<td>0.00172</td>
</tr>
<tr>
<td>2.5</td>
<td>0.0015</td>
</tr>
<tr>
<td>2.5</td>
<td>0.00149</td>
</tr>
<tr>
<td>2</td>
<td>0.00133</td>
</tr>
<tr>
<td>2.63</td>
<td>0.00141</td>
</tr>
<tr>
<td>2.63</td>
<td>0.00159</td>
</tr>
<tr>
<td>2.63</td>
<td>0.0016</td>
</tr>
<tr>
<td>2.63</td>
<td>0.00158</td>
</tr>
<tr>
<td>3.75</td>
<td>0.00164</td>
</tr>
<tr>
<td>2.75</td>
<td>0.00144</td>
</tr>
<tr>
<td>14</td>
<td>0.00162</td>
</tr>
<tr>
<td>12</td>
<td>0.0018</td>
</tr>
<tr>
<td>-----</td>
<td>--------</td>
</tr>
<tr>
<td>19.5</td>
<td>0.00158</td>
</tr>
<tr>
<td>19</td>
<td>0.00173</td>
</tr>
<tr>
<td>19</td>
<td>0.00187</td>
</tr>
<tr>
<td>29</td>
<td>0.00197</td>
</tr>
<tr>
<td>30</td>
<td>0.00138</td>
</tr>
<tr>
<td>30</td>
<td>0.00135</td>
</tr>
<tr>
<td>2.5</td>
<td>0.0014</td>
</tr>
<tr>
<td>2.5</td>
<td>0.00121</td>
</tr>
<tr>
<td>9</td>
<td>0.00167</td>
</tr>
<tr>
<td>9</td>
<td>0.00169</td>
</tr>
<tr>
<td>9</td>
<td>0.00138</td>
</tr>
<tr>
<td>8.5</td>
<td>0.00157</td>
</tr>
<tr>
<td>2.5</td>
<td>0.00183</td>
</tr>
<tr>
<td>8.5</td>
<td>0.00166</td>
</tr>
<tr>
<td>12</td>
<td>0.00178</td>
</tr>
<tr>
<td>1.75</td>
<td>0.00194</td>
</tr>
<tr>
<td>4.5</td>
<td>0.00181</td>
</tr>
</tbody>
</table>
2. *Table Appendix 4B.2 The distribution of four types of elastic fiber orientation*

<table>
<thead>
<tr>
<th>Age (month)</th>
<th>Type C (%)</th>
<th>Type L (%)</th>
<th>Type T (%)</th>
<th>Type A (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>0.09</td>
<td>0.64</td>
<td>0.25</td>
<td>0.02</td>
</tr>
<tr>
<td>23.5</td>
<td>0.12</td>
<td>0.52</td>
<td>0.32</td>
<td>0.04</td>
</tr>
<tr>
<td>1.75</td>
<td>0.21</td>
<td>0.61</td>
<td>0.18</td>
<td>0</td>
</tr>
<tr>
<td>20</td>
<td>0.1</td>
<td>0.46</td>
<td>0.41</td>
<td>0.03</td>
</tr>
<tr>
<td>8.5</td>
<td>0.13</td>
<td>0.68</td>
<td>0.19</td>
<td>0</td>
</tr>
<tr>
<td>2.25</td>
<td>0.13</td>
<td>0.65</td>
<td>0.2</td>
<td>0.02</td>
</tr>
<tr>
<td>2.25</td>
<td>0.15</td>
<td>0.52</td>
<td>0.3</td>
<td>0.03</td>
</tr>
<tr>
<td>19</td>
<td>0.09</td>
<td>0.51</td>
<td>0.37</td>
<td>0.03</td>
</tr>
<tr>
<td>11</td>
<td>0.03</td>
<td>0.66</td>
<td>0.29</td>
<td>0.02</td>
</tr>
<tr>
<td>2.75</td>
<td>0.02</td>
<td>0.67</td>
<td>0.31</td>
<td>0</td>
</tr>
<tr>
<td>14</td>
<td>0.06</td>
<td>0.54</td>
<td>0.38</td>
<td>0.02</td>
</tr>
<tr>
<td>2.5</td>
<td>0.03</td>
<td>0.52</td>
<td>0.43</td>
<td>0.02</td>
</tr>
<tr>
<td>2.5</td>
<td>0.17</td>
<td>0.61</td>
<td>0.21</td>
<td>0.01</td>
</tr>
<tr>
<td>2.5</td>
<td>0.14</td>
<td>0.62</td>
<td>0.23</td>
<td>0.02</td>
</tr>
<tr>
<td>2</td>
<td>0.09</td>
<td>0.69</td>
<td>0.21</td>
<td>0.02</td>
</tr>
<tr>
<td>2.63</td>
<td>0.07</td>
<td>0.56</td>
<td>0.37</td>
<td>0</td>
</tr>
<tr>
<td>2.63</td>
<td>0.02</td>
<td>0.62</td>
<td>0.32</td>
<td>0.04</td>
</tr>
<tr>
<td>2.63</td>
<td>0.06</td>
<td>0.55</td>
<td>0.35</td>
<td>0.04</td>
</tr>
<tr>
<td>2.63</td>
<td>0.04</td>
<td>0.63</td>
<td>0.23</td>
<td>0.1</td>
</tr>
<tr>
<td>3.75</td>
<td>0.04</td>
<td>0.75</td>
<td>0.21</td>
<td>0</td>
</tr>
<tr>
<td>2.75</td>
<td>0.01</td>
<td>0.67</td>
<td>0.31</td>
<td>0.01</td>
</tr>
<tr>
<td>14</td>
<td>0.1</td>
<td>0.58</td>
<td>0.27</td>
<td>0.05</td>
</tr>
<tr>
<td>12</td>
<td>0.04</td>
<td>0.54</td>
<td>0.4</td>
<td>0.02</td>
</tr>
<tr>
<td>19.5</td>
<td>0.1</td>
<td>0.37</td>
<td>0.51</td>
<td>0.02</td>
</tr>
<tr>
<td>19</td>
<td>0.1</td>
<td>0.56</td>
<td>0.34</td>
<td>0</td>
</tr>
<tr>
<td>19</td>
<td>0.02</td>
<td>0.66</td>
<td>0.31</td>
<td>0.01</td>
</tr>
<tr>
<td>29</td>
<td>0.05</td>
<td>0.76</td>
<td>0.16</td>
<td>0.03</td>
</tr>
<tr>
<td>30</td>
<td>0.08</td>
<td>0.51</td>
<td>0.39</td>
<td>0.02</td>
</tr>
<tr>
<td>30</td>
<td>0.09</td>
<td>0.51</td>
<td>0.37</td>
<td>0.03</td>
</tr>
<tr>
<td>2.5</td>
<td>0.04</td>
<td>0.69</td>
<td>0.25</td>
<td>0.02</td>
</tr>
<tr>
<td>2.5</td>
<td>0.13</td>
<td>0.49</td>
<td>0.35</td>
<td>0.03</td>
</tr>
<tr>
<td>9</td>
<td>0.14</td>
<td>0.53</td>
<td>0.34</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>0.11</td>
<td>0.51</td>
<td>0.37</td>
<td>0.01</td>
</tr>
<tr>
<td>9</td>
<td>0.11</td>
<td>0.58</td>
<td>0.3</td>
<td>0.01</td>
</tr>
<tr>
<td>8.5</td>
<td>0.06</td>
<td>0.35</td>
<td>0.56</td>
<td>0.03</td>
</tr>
<tr>
<td>2.5</td>
<td>0.03</td>
<td>0.58</td>
<td>0.36</td>
<td>0.03</td>
</tr>
<tr>
<td>8.5</td>
<td>0.08</td>
<td>0.54</td>
<td>0.33</td>
<td>0.05</td>
</tr>
<tr>
<td>12</td>
<td>0.05</td>
<td>0.46</td>
<td>0.48</td>
<td>0.01</td>
</tr>
<tr>
<td>1.75</td>
<td>0.02</td>
<td>0.49</td>
<td>0.49</td>
<td>0</td>
</tr>
<tr>
<td>4.5</td>
<td>0.09</td>
<td>0.4</td>
<td>0.49</td>
<td>0.02</td>
</tr>
</tbody>
</table>
### Table Appendix 4B.3 Result of point counting: the numbers of total and hit points

<table>
<thead>
<tr>
<th>Age (month)</th>
<th>Total (circ.)</th>
<th>Hit (circ.)</th>
<th>Total (long.)</th>
<th>Hit (long.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>755</td>
<td>300</td>
<td>808</td>
<td>250</td>
</tr>
<tr>
<td>23.5</td>
<td>651</td>
<td>161</td>
<td>1558</td>
<td>162</td>
</tr>
<tr>
<td>1.75</td>
<td>501</td>
<td>117</td>
<td>1102</td>
<td>234</td>
</tr>
<tr>
<td>20</td>
<td>1331</td>
<td>266</td>
<td>458</td>
<td>226</td>
</tr>
<tr>
<td>8.5</td>
<td>922</td>
<td>110</td>
<td>488</td>
<td>87</td>
</tr>
<tr>
<td>2.25</td>
<td>897</td>
<td>360</td>
<td>1931</td>
<td>306</td>
</tr>
<tr>
<td>2.25</td>
<td>1314</td>
<td>309</td>
<td>2335</td>
<td>381</td>
</tr>
<tr>
<td>19</td>
<td>1617</td>
<td>333</td>
<td>1324</td>
<td>280</td>
</tr>
<tr>
<td>11</td>
<td>1049</td>
<td>279</td>
<td>2336</td>
<td>297</td>
</tr>
<tr>
<td>2.75</td>
<td>1050</td>
<td>331</td>
<td>922</td>
<td>342</td>
</tr>
<tr>
<td>14</td>
<td>1220</td>
<td>235</td>
<td>907</td>
<td>224</td>
</tr>
<tr>
<td>2.5</td>
<td>1275</td>
<td>382</td>
<td>909</td>
<td>232</td>
</tr>
<tr>
<td>2.5</td>
<td>1255</td>
<td>227</td>
<td>1234</td>
<td>243</td>
</tr>
<tr>
<td>2.5</td>
<td>813</td>
<td>254</td>
<td>1632</td>
<td>362</td>
</tr>
<tr>
<td>2</td>
<td>716</td>
<td>151</td>
<td>1026</td>
<td>286</td>
</tr>
<tr>
<td>2.63</td>
<td>785</td>
<td>219</td>
<td>606</td>
<td>186</td>
</tr>
<tr>
<td>2.63</td>
<td>742</td>
<td>170</td>
<td>820</td>
<td>216</td>
</tr>
<tr>
<td>2.63</td>
<td>946</td>
<td>196</td>
<td>686</td>
<td>195</td>
</tr>
<tr>
<td>2.63</td>
<td>809</td>
<td>210</td>
<td>1119</td>
<td>230</td>
</tr>
<tr>
<td>3.75</td>
<td>899</td>
<td>139</td>
<td>659</td>
<td>197</td>
</tr>
<tr>
<td>2.75</td>
<td>1316</td>
<td>262</td>
<td>1041</td>
<td>239</td>
</tr>
<tr>
<td>14</td>
<td>914</td>
<td>364</td>
<td>1391</td>
<td>255</td>
</tr>
<tr>
<td>12</td>
<td>915</td>
<td>180</td>
<td>871</td>
<td>256</td>
</tr>
<tr>
<td>19.5</td>
<td>1152</td>
<td>227</td>
<td>1063</td>
<td>207</td>
</tr>
<tr>
<td>19</td>
<td>1498</td>
<td>204</td>
<td>968</td>
<td>150</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>19</td>
<td>1276</td>
<td>186</td>
<td>917</td>
<td>249</td>
</tr>
<tr>
<td>29</td>
<td>1482</td>
<td>254</td>
<td>966</td>
<td>227</td>
</tr>
<tr>
<td>30</td>
<td>1382</td>
<td>228</td>
<td>1825</td>
<td>209</td>
</tr>
<tr>
<td>30</td>
<td>976</td>
<td>144</td>
<td>977</td>
<td>135</td>
</tr>
<tr>
<td>2.5</td>
<td>1151</td>
<td>218</td>
<td>2016</td>
<td>303</td>
</tr>
<tr>
<td>2.5</td>
<td>1191</td>
<td>182</td>
<td>1840</td>
<td>159</td>
</tr>
<tr>
<td>9</td>
<td>1456</td>
<td>182</td>
<td>1512</td>
<td>179</td>
</tr>
<tr>
<td>9</td>
<td>1353</td>
<td>271</td>
<td>1095</td>
<td>254</td>
</tr>
<tr>
<td>9</td>
<td>1020</td>
<td>209</td>
<td>1599</td>
<td>252</td>
</tr>
<tr>
<td>8.5</td>
<td>1881</td>
<td>403</td>
<td>1643</td>
<td>389</td>
</tr>
<tr>
<td>2.5</td>
<td>949</td>
<td>249</td>
<td>1156</td>
<td>253</td>
</tr>
<tr>
<td>8.5</td>
<td>804</td>
<td>64</td>
<td>1131</td>
<td>139</td>
</tr>
<tr>
<td>12</td>
<td>1600</td>
<td>79</td>
<td>2393</td>
<td>211</td>
</tr>
<tr>
<td>1.75</td>
<td>1061</td>
<td>252</td>
<td>1201</td>
<td>297</td>
</tr>
<tr>
<td>4.5</td>
<td>1840</td>
<td>316</td>
<td>1171</td>
<td>236</td>
</tr>
<tr>
<td>Age (month)</td>
<td>$A_V$ (circ.)</td>
<td>$A_V$ (long.)</td>
<td>$V_V$ (circ.)</td>
<td>$V_V$ (long.)</td>
</tr>
<tr>
<td>------------</td>
<td>---------------</td>
<td>---------------</td>
<td>---------------</td>
<td>---------------</td>
</tr>
<tr>
<td>7.5</td>
<td>0.397</td>
<td>0.309</td>
<td>0.09131</td>
<td>0.07107</td>
</tr>
<tr>
<td>23.5</td>
<td>0.247</td>
<td>0.104</td>
<td>0.05681</td>
<td>0.02392</td>
</tr>
<tr>
<td>1.75</td>
<td>0.234</td>
<td>0.212</td>
<td>0.05382</td>
<td>0.04876</td>
</tr>
<tr>
<td>20</td>
<td>0.2</td>
<td>0.493</td>
<td>0.046</td>
<td>0.11339</td>
</tr>
<tr>
<td>8.5</td>
<td>0.119</td>
<td>0.178</td>
<td>0.02737</td>
<td>0.04094</td>
</tr>
<tr>
<td>2.25</td>
<td>0.401</td>
<td>0.158</td>
<td>0.09223</td>
<td>0.03634</td>
</tr>
<tr>
<td>2.25</td>
<td>0.235</td>
<td>0.163</td>
<td>0.05405</td>
<td>0.03749</td>
</tr>
<tr>
<td>19</td>
<td>0.206</td>
<td>0.212</td>
<td>0.04738</td>
<td>0.04876</td>
</tr>
<tr>
<td>11</td>
<td>0.266</td>
<td>0.127</td>
<td>0.06118</td>
<td>0.02921</td>
</tr>
<tr>
<td>2.75</td>
<td>0.315</td>
<td>0.371</td>
<td>0.07245</td>
<td>0.08533</td>
</tr>
<tr>
<td>14</td>
<td>0.193</td>
<td>0.247</td>
<td>0.04439</td>
<td>0.05681</td>
</tr>
<tr>
<td>2.5</td>
<td>0.3</td>
<td>0.255</td>
<td>0.069</td>
<td>0.05865</td>
</tr>
<tr>
<td>2.5</td>
<td>0.181</td>
<td>0.197</td>
<td>0.04163</td>
<td>0.04531</td>
</tr>
<tr>
<td>2.5</td>
<td>0.312</td>
<td>0.222</td>
<td>0.07176</td>
<td>0.05106</td>
</tr>
<tr>
<td>2</td>
<td>0.211</td>
<td>0.279</td>
<td>0.04853</td>
<td>0.06417</td>
</tr>
<tr>
<td>2.63</td>
<td>0.279</td>
<td>0.307</td>
<td>0.06417</td>
<td>0.07061</td>
</tr>
<tr>
<td>2.63</td>
<td>0.229</td>
<td>0.263</td>
<td>0.05267</td>
<td>0.06049</td>
</tr>
<tr>
<td>2.63</td>
<td>0.207</td>
<td>0.284</td>
<td>0.04761</td>
<td>0.06532</td>
</tr>
<tr>
<td>2.63</td>
<td>0.26</td>
<td>0.206</td>
<td>0.0598</td>
<td>0.04738</td>
</tr>
<tr>
<td>3.75</td>
<td>0.155</td>
<td>0.299</td>
<td>0.03565</td>
<td>0.06877</td>
</tr>
<tr>
<td>2.75</td>
<td>0.199</td>
<td>0.23</td>
<td>0.04577</td>
<td>0.0529</td>
</tr>
<tr>
<td>14</td>
<td>0.398</td>
<td>0.183</td>
<td>0.09154</td>
<td>0.04209</td>
</tr>
<tr>
<td>12</td>
<td>0.197</td>
<td>0.294</td>
<td>0.04531</td>
<td>0.06762</td>
</tr>
<tr>
<td>19.5</td>
<td>0.197</td>
<td>0.195</td>
<td>0.04531</td>
<td>0.04485</td>
</tr>
<tr>
<td>19</td>
<td>0.136</td>
<td>0.155</td>
<td>0.03128</td>
<td>0.03565</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>19</td>
<td>0.146</td>
<td>0.272</td>
<td>0.03358</td>
<td>0.06256</td>
</tr>
<tr>
<td>29</td>
<td>0.171</td>
<td>0.235</td>
<td>0.03933</td>
<td>0.05405</td>
</tr>
<tr>
<td>30</td>
<td>0.165</td>
<td>0.115</td>
<td>0.03795</td>
<td>0.02645</td>
</tr>
<tr>
<td>30</td>
<td>0.148</td>
<td>0.138</td>
<td>0.03404</td>
<td>0.03174</td>
</tr>
<tr>
<td>2.5</td>
<td>0.189</td>
<td>0.15</td>
<td>0.04347</td>
<td>0.0345</td>
</tr>
<tr>
<td>2.5</td>
<td>0.153</td>
<td>0.086</td>
<td>0.03519</td>
<td>0.01978</td>
</tr>
<tr>
<td>9</td>
<td>0.125</td>
<td>0.118</td>
<td>0.02875</td>
<td>0.02714</td>
</tr>
<tr>
<td>9</td>
<td>0.2</td>
<td>0.232</td>
<td>0.046</td>
<td>0.05336</td>
</tr>
<tr>
<td>9</td>
<td>0.205</td>
<td>0.158</td>
<td>0.04715</td>
<td>0.03634</td>
</tr>
<tr>
<td>8.5</td>
<td>0.214</td>
<td>0.237</td>
<td>0.04922</td>
<td>0.05451</td>
</tr>
<tr>
<td>2.5</td>
<td>0.262</td>
<td>0.219</td>
<td>0.06026</td>
<td>0.05037</td>
</tr>
<tr>
<td>8.5</td>
<td>0.08</td>
<td>0.123</td>
<td>0.0184</td>
<td>0.02829</td>
</tr>
<tr>
<td>12</td>
<td>0.049</td>
<td>0.088</td>
<td>0.01127</td>
<td>0.02024</td>
</tr>
<tr>
<td>1.75</td>
<td>0.238</td>
<td>0.247</td>
<td>0.05474</td>
<td>0.05681</td>
</tr>
<tr>
<td>4.5</td>
<td>0.172</td>
<td>0.202</td>
<td>0.03956</td>
<td>0.04646</td>
</tr>
</tbody>
</table>
### Appendix 5A: Calculated Additional Morphometrical Parameters

1. Table Appendix 5A.1 Result of TotV, TotN, TotL, TotC, and TotT calculated from circumferential samples

<table>
<thead>
<tr>
<th>Age</th>
<th>V(sample)</th>
<th>V_circ</th>
<th>TotV</th>
<th>TotN</th>
<th>C_percent</th>
<th>TotC</th>
<th>T_percent</th>
<th>TotT</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>1.8907</td>
<td>0.0913</td>
<td>0.1726</td>
<td>954338</td>
<td>0.09</td>
<td>858904</td>
<td>0.25</td>
<td>23858</td>
</tr>
<tr>
<td>23.5</td>
<td>2.9584</td>
<td>0.0568</td>
<td>0.1680</td>
<td>929058</td>
<td>0.12</td>
<td>111487</td>
<td>0.32</td>
<td>29729</td>
</tr>
<tr>
<td>1.75</td>
<td>1.015</td>
<td>0.0538</td>
<td>0.0546</td>
<td>301975</td>
<td>0.21</td>
<td>634147</td>
<td>0.18</td>
<td>54355</td>
</tr>
<tr>
<td>20</td>
<td>10.7594</td>
<td>0.046</td>
<td>0.4949</td>
<td>273594</td>
<td>0.1</td>
<td>273594</td>
<td>0.41</td>
<td>11217</td>
</tr>
<tr>
<td>8.5</td>
<td>4.37928</td>
<td>0.0273</td>
<td>0.1198</td>
<td>662580</td>
<td>0.13</td>
<td>861355</td>
<td>0.19</td>
<td>12589</td>
</tr>
<tr>
<td>2.25</td>
<td>1.27368</td>
<td>0.0922</td>
<td>0.1174</td>
<td>649372</td>
<td>0.13</td>
<td>844184</td>
<td>0.2</td>
<td>12987</td>
</tr>
<tr>
<td>2.25</td>
<td>3.05844</td>
<td>0.0540</td>
<td>0.1653</td>
<td>913812</td>
<td>0.15</td>
<td>137071</td>
<td>0.3</td>
<td>27414</td>
</tr>
<tr>
<td>19</td>
<td>2.81568</td>
<td>0.0473</td>
<td>0.1334</td>
<td>737462</td>
<td>0.09</td>
<td>663716</td>
<td>0.37</td>
<td>27286</td>
</tr>
<tr>
<td>11</td>
<td>1.7408</td>
<td>0.0611</td>
<td>0.1065</td>
<td>588734</td>
<td>0.03</td>
<td>176620</td>
<td>0.29</td>
<td>17073</td>
</tr>
<tr>
<td>2.75</td>
<td>1.8095</td>
<td>0.0724</td>
<td>0.1311</td>
<td>724700</td>
<td>0.02</td>
<td>144940</td>
<td>0.31</td>
<td>22465</td>
</tr>
<tr>
<td>14</td>
<td>2.47896</td>
<td>0.0443</td>
<td>0.1100</td>
<td>608297</td>
<td>0.06</td>
<td>364978</td>
<td>0.38</td>
<td>23115</td>
</tr>
<tr>
<td>2.5</td>
<td>1.90332</td>
<td>0.069</td>
<td>0.1313</td>
<td>725976</td>
<td>0.03</td>
<td>217792</td>
<td>0.43</td>
<td>31216</td>
</tr>
<tr>
<td>2.5</td>
<td>1.9975</td>
<td>0.0416</td>
<td>0.0831</td>
<td>459678</td>
<td>0.17</td>
<td>781454</td>
<td>0.21</td>
<td>96532</td>
</tr>
<tr>
<td>2.5</td>
<td>1.25875</td>
<td>0.0717</td>
<td>0.0903</td>
<td>499325</td>
<td>0.14</td>
<td>699055</td>
<td>0.23</td>
<td>11648</td>
</tr>
<tr>
<td>2</td>
<td>2.45802</td>
<td>0.0485</td>
<td>0.1192</td>
<td>659412</td>
<td>0.09</td>
<td>593471</td>
<td>0.21</td>
<td>13847</td>
</tr>
<tr>
<td>2.63</td>
<td>0.9216</td>
<td>0.0641</td>
<td>0.0591</td>
<td>326915</td>
<td>0.07</td>
<td>228841</td>
<td>0.37</td>
<td>12095</td>
</tr>
<tr>
<td>2.63</td>
<td>1.65838</td>
<td>0.0526</td>
<td>0.0873</td>
<td>482846</td>
<td>0.02</td>
<td>96569</td>
<td>0.32</td>
<td>15451</td>
</tr>
<tr>
<td>2.63</td>
<td>1.53239</td>
<td>0.0476</td>
<td>0.0729</td>
<td>403300</td>
<td>0.06</td>
<td>241980</td>
<td>0.35</td>
<td>14115</td>
</tr>
<tr>
<td>2.63</td>
<td>2.13355</td>
<td>0.0598</td>
<td>0.1275</td>
<td>705286</td>
<td>0.04</td>
<td>282114</td>
<td>0.23</td>
<td>16221</td>
</tr>
<tr>
<td>3.75</td>
<td>1.63017</td>
<td>0.0356</td>
<td>0.0581</td>
<td>321257</td>
<td>0.04</td>
<td>128503</td>
<td>0.21</td>
<td>67464</td>
</tr>
<tr>
<td>2.75</td>
<td>2.0704</td>
<td>0.0457</td>
<td>0.0947</td>
<td>523837</td>
<td>0.01</td>
<td>52383</td>
<td>0.31</td>
<td>16238</td>
</tr>
<tr>
<td>14</td>
<td>2.03826</td>
<td>0.0915</td>
<td>0.1865</td>
<td>103141</td>
<td>0.1</td>
<td>103141</td>
<td>0.27</td>
<td>27848</td>
</tr>
<tr>
<td>Age (in months)</td>
<td>Volume (mm³)</td>
<td>Age (in months)</td>
<td>Volume (mm³)</td>
<td>Age (in months)</td>
<td>Volume (mm³)</td>
<td>Age (in months)</td>
<td>Volume (mm³)</td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td>-------------</td>
<td>----------------</td>
<td>-------------</td>
<td>----------------</td>
<td>-------------</td>
<td>----------------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2.0907</td>
<td>0.0453</td>
<td>0.0947</td>
<td>523657</td>
<td>0.04</td>
<td>209462</td>
<td>0.4</td>
<td>209461</td>
</tr>
<tr>
<td>19.5</td>
<td>2.38995</td>
<td>0.0453</td>
<td>0.1082</td>
<td>598610</td>
<td>0.1</td>
<td>598610</td>
<td>0.51</td>
<td>30529.6</td>
</tr>
<tr>
<td>19</td>
<td>2.2754</td>
<td>0.0312</td>
<td>0.0711</td>
<td>393446</td>
<td>0.1</td>
<td>393446</td>
<td>0.34</td>
<td>13377.4</td>
</tr>
<tr>
<td>19</td>
<td>3.33053</td>
<td>0.0335</td>
<td>0.1118</td>
<td>618237</td>
<td>0.02</td>
<td>123647</td>
<td>0.31</td>
<td>19165.6</td>
</tr>
<tr>
<td>29</td>
<td>2.49075</td>
<td>0.0393</td>
<td>0.0979</td>
<td>541521</td>
<td>0.05</td>
<td>270760</td>
<td>0.16</td>
<td>86643.2</td>
</tr>
<tr>
<td>30</td>
<td>1.96512</td>
<td>0.0379</td>
<td>0.0745</td>
<td>412251</td>
<td>0.08</td>
<td>329801</td>
<td>0.39</td>
<td>16077.2</td>
</tr>
<tr>
<td>30</td>
<td>1.9747</td>
<td>0.0340</td>
<td>0.0672</td>
<td>371579</td>
<td>0.09</td>
<td>334421</td>
<td>0.37</td>
<td>13748.1</td>
</tr>
<tr>
<td>2.5</td>
<td>3.157</td>
<td>0.0434</td>
<td>0.1372</td>
<td>758622</td>
<td>0.04</td>
<td>303448</td>
<td>0.25</td>
<td>18965.5</td>
</tr>
<tr>
<td>2.5</td>
<td>2.9548</td>
<td>0.0351</td>
<td>0.1039</td>
<td>574789</td>
<td>0.13</td>
<td>747226</td>
<td>0.35</td>
<td>20117.3</td>
</tr>
<tr>
<td>9</td>
<td>2.0817</td>
<td>0.0287</td>
<td>0.0598</td>
<td>330839</td>
<td>0.14</td>
<td>463175</td>
<td>0.34</td>
<td>11248.0</td>
</tr>
<tr>
<td>9</td>
<td>2.99052</td>
<td>0.046</td>
<td>0.1375</td>
<td>760441</td>
<td>0.11</td>
<td>836485</td>
<td>0.37</td>
<td>28136.1</td>
</tr>
<tr>
<td>9</td>
<td>1.5544</td>
<td>0.0471</td>
<td>0.0732</td>
<td>405140</td>
<td>0.11</td>
<td>445654</td>
<td>0.3</td>
<td>12154.0</td>
</tr>
<tr>
<td>8.5</td>
<td>2.05479</td>
<td>0.0492</td>
<td>0.1011</td>
<td>559075</td>
<td>0.06</td>
<td>335445</td>
<td>0.56</td>
<td>31308.2</td>
</tr>
<tr>
<td>2.5</td>
<td>1.65429</td>
<td>0.0602</td>
<td>0.0996</td>
<td>551064</td>
<td>0.03</td>
<td>165319</td>
<td>0.36</td>
<td>19838.0</td>
</tr>
<tr>
<td>8.5</td>
<td>3.56283</td>
<td>0.0184</td>
<td>0.0655</td>
<td>362388</td>
<td>0.08</td>
<td>289910</td>
<td>0.33</td>
<td>11958.0</td>
</tr>
<tr>
<td>12</td>
<td>2.11328</td>
<td>0.0112</td>
<td>0.0238</td>
<td>131656</td>
<td>0.05</td>
<td>65828.0</td>
<td>0.48</td>
<td>63195.1</td>
</tr>
<tr>
<td>1.75</td>
<td>1.09272</td>
<td>0.0547</td>
<td>0.0598</td>
<td>330655</td>
<td>0.02</td>
<td>66131.0</td>
<td>0.49</td>
<td>16202.1</td>
</tr>
<tr>
<td>4.5</td>
<td>4.70844</td>
<td>0.0395</td>
<td>0.1862</td>
<td>102966</td>
<td>0.09</td>
<td>926695</td>
<td>0.49</td>
<td>50453.2</td>
</tr>
</tbody>
</table>

*Age in month, volume in mm³*
2. Table Appendix 5A.2 Result of TotV, TotN, TotL, TotC, and TotT calculated from longitudinal samples

<table>
<thead>
<tr>
<th>Age</th>
<th>V(sample)</th>
<th>V_{long}</th>
<th>TotV</th>
<th>TotN</th>
<th>\text{L}_{\text{percent}}</th>
<th>TotL</th>
<th>\text{T}_{\text{percent}}</th>
<th>TotT</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.5</td>
<td>1.57595</td>
<td>0.07107</td>
<td>0.112</td>
<td>619141</td>
<td>0.64</td>
<td>396250</td>
<td>0.25</td>
<td>1547</td>
</tr>
<tr>
<td>23.5</td>
<td>2.6659</td>
<td>0.02392</td>
<td>0.0637</td>
<td>352505</td>
<td>0.52</td>
<td>183303</td>
<td>0.32</td>
<td>1128</td>
</tr>
<tr>
<td>1.75</td>
<td>2.0732</td>
<td>0.04876</td>
<td>0.1010</td>
<td>558812</td>
<td>0.61</td>
<td>340875</td>
<td>0.18</td>
<td>1005</td>
</tr>
<tr>
<td>20</td>
<td>2.02215</td>
<td>0.11339</td>
<td>0.2292</td>
<td>126750</td>
<td>0.46</td>
<td>583052</td>
<td>0.41</td>
<td>5196</td>
</tr>
<tr>
<td>8.5</td>
<td>1.38592</td>
<td>0.04094</td>
<td>0.0567</td>
<td>313651</td>
<td>0.68</td>
<td>213283</td>
<td>0.19</td>
<td>5959</td>
</tr>
<tr>
<td>2.25</td>
<td>1.958</td>
<td>0.03634</td>
<td>0.0711</td>
<td>393331</td>
<td>0.65</td>
<td>255665</td>
<td>0.2</td>
<td>7866</td>
</tr>
<tr>
<td>2.25</td>
<td>3.38625</td>
<td>0.03749</td>
<td>0.1269</td>
<td>701771</td>
<td>0.52</td>
<td>364921</td>
<td>0.3</td>
<td>2105</td>
</tr>
<tr>
<td>19</td>
<td>2.0636</td>
<td>0.04876</td>
<td>0.1006</td>
<td>556225</td>
<td>0.51</td>
<td>283674</td>
<td>0.37</td>
<td>2058</td>
</tr>
<tr>
<td>11</td>
<td>3.57487</td>
<td>0.02921</td>
<td>0.1044</td>
<td>577235</td>
<td>0.66</td>
<td>380975</td>
<td>0.29</td>
<td>1673</td>
</tr>
<tr>
<td>2.75</td>
<td>1.34875</td>
<td>0.08533</td>
<td>0.1150</td>
<td>636201</td>
<td>0.67</td>
<td>426254</td>
<td>0.31</td>
<td>1972</td>
</tr>
<tr>
<td>14</td>
<td>1.78</td>
<td>0.05681</td>
<td>0.1011</td>
<td>558992</td>
<td>0.54</td>
<td>301856</td>
<td>0.38</td>
<td>2124</td>
</tr>
<tr>
<td>2.5</td>
<td>1.3916</td>
<td>0.05865</td>
<td>0.0816</td>
<td>451173</td>
<td>0.52</td>
<td>234610</td>
<td>0.43</td>
<td>1940</td>
</tr>
<tr>
<td>2.5</td>
<td>1.27455</td>
<td>0.04531</td>
<td>0.0577</td>
<td>319236</td>
<td>0.61</td>
<td>194734</td>
<td>0.21</td>
<td>6703</td>
</tr>
<tr>
<td>2.5</td>
<td>1.67717</td>
<td>0.05106</td>
<td>0.0856</td>
<td>473390</td>
<td>0.62</td>
<td>293501</td>
<td>0.23</td>
<td>1088</td>
</tr>
<tr>
<td>2</td>
<td>1.28656</td>
<td>0.06417</td>
<td>0.0825</td>
<td>456376</td>
<td>0.69</td>
<td>314899</td>
<td>0.21</td>
<td>9583</td>
</tr>
<tr>
<td>2.63</td>
<td>0.9828</td>
<td>0.07061</td>
<td>0.0694</td>
<td>383612</td>
<td>0.56</td>
<td>214823</td>
<td>0.37</td>
<td>1419</td>
</tr>
<tr>
<td>2.63</td>
<td>1.36245</td>
<td>0.06049</td>
<td>0.0824</td>
<td>455580</td>
<td>0.62</td>
<td>282460</td>
<td>0.32</td>
<td>1457</td>
</tr>
<tr>
<td>2.63</td>
<td>1.76456</td>
<td>0.06532</td>
<td>0.1152</td>
<td>637153</td>
<td>0.55</td>
<td>350434</td>
<td>0.35</td>
<td>2230</td>
</tr>
<tr>
<td>2.63</td>
<td>2.1239</td>
<td>0.04738</td>
<td>0.1006</td>
<td>556276</td>
<td>0.63</td>
<td>350454</td>
<td>0.23</td>
<td>1279</td>
</tr>
<tr>
<td>3.75</td>
<td>1.87824</td>
<td>0.06877</td>
<td>0.1291</td>
<td>714021</td>
<td>0.75</td>
<td>535516</td>
<td>0.21</td>
<td>1499</td>
</tr>
<tr>
<td>2.75</td>
<td>1.696</td>
<td>0.0529</td>
<td>0.0897</td>
<td>495955</td>
<td>0.67</td>
<td>332290</td>
<td>0.31</td>
<td>1537</td>
</tr>
<tr>
<td>14</td>
<td>2.8718</td>
<td>0.04209</td>
<td>0.1208</td>
<td>668181</td>
<td>0.58</td>
<td>387545</td>
<td>0.27</td>
<td>1804</td>
</tr>
<tr>
<td>12</td>
<td>1.9872</td>
<td>0.06762</td>
<td>0.1343</td>
<td>742810</td>
<td>0.54</td>
<td>401117</td>
<td>0.3</td>
<td>2971</td>
</tr>
<tr>
<td>19.5</td>
<td>1.92606</td>
<td>0.04485</td>
<td>0.0863</td>
<td>477522</td>
<td>0.37</td>
<td>176683</td>
<td>0.51</td>
<td>2435</td>
</tr>
<tr>
<td>Age (in month)</td>
<td>Volume (in mm³)</td>
<td>Growth Rate</td>
<td>Weight (in g)</td>
<td>Age (in month)</td>
<td>Volume (in mm³)</td>
<td>Growth Rate</td>
<td>Weight (in g)</td>
<td></td>
</tr>
<tr>
<td>---------------</td>
<td>----------------</td>
<td>-------------</td>
<td>--------------</td>
<td>---------------</td>
<td>----------------</td>
<td>-------------</td>
<td>--------------</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>1.6482</td>
<td>0.03565</td>
<td>0.0587</td>
<td>324811</td>
<td>181894</td>
<td>0.34</td>
<td>1104</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td>1.85415</td>
<td>0.06256</td>
<td>0.116</td>
<td>641214</td>
<td>423201</td>
<td>0.31</td>
<td>1987</td>
<td></td>
</tr>
<tr>
<td>29</td>
<td>1.81986</td>
<td>0.05405</td>
<td>0.0983</td>
<td>543744</td>
<td>413246</td>
<td>0.16</td>
<td>8699</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>2.98826</td>
<td>0.02645</td>
<td>0.0790</td>
<td>436923</td>
<td>222831</td>
<td>0.39</td>
<td>1704</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>3.74976</td>
<td>0.03174</td>
<td>0.1190</td>
<td>657918</td>
<td>335538</td>
<td>0.37</td>
<td>2434</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>2.05</td>
<td>0.0345</td>
<td>0.0707</td>
<td>390961</td>
<td>269763</td>
<td>0.25</td>
<td>9774</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>2.63704</td>
<td>0.01978</td>
<td>0.0521</td>
<td>288339</td>
<td>141286</td>
<td>0.35</td>
<td>1009</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2.4309</td>
<td>0.02714</td>
<td>0.0659</td>
<td>364702</td>
<td>193292</td>
<td>0.34</td>
<td>1239</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>1.52368</td>
<td>0.05336</td>
<td>0.0813</td>
<td>449439</td>
<td>229214</td>
<td>0.37</td>
<td>1662</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>2.1413</td>
<td>0.03634</td>
<td>0.0778</td>
<td>430153</td>
<td>249489</td>
<td>0.3</td>
<td>1290</td>
<td></td>
</tr>
<tr>
<td>8.5</td>
<td>2.56045</td>
<td>0.05451</td>
<td>0.1395</td>
<td>771531</td>
<td>270036</td>
<td>0.56</td>
<td>4320</td>
<td></td>
</tr>
<tr>
<td>2.5</td>
<td>2.22762</td>
<td>0.05037</td>
<td>0.1122</td>
<td>620261</td>
<td>359751</td>
<td>0.36</td>
<td>2232</td>
<td></td>
</tr>
<tr>
<td>8.5</td>
<td>1.4484</td>
<td>0.02829</td>
<td>0.0409</td>
<td>226507</td>
<td>122314</td>
<td>0.33</td>
<td>7474</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>2.55816</td>
<td>0.02024</td>
<td>0.0517</td>
<td>286219</td>
<td>131661</td>
<td>0.48</td>
<td>1373</td>
<td></td>
</tr>
<tr>
<td>1.75</td>
<td>1.14289</td>
<td>0.05681</td>
<td>0.0649</td>
<td>358914</td>
<td>175867</td>
<td>0.49</td>
<td>1758</td>
<td></td>
</tr>
<tr>
<td>4.5</td>
<td>1.34478</td>
<td>0.04646</td>
<td>0.0624</td>
<td>345375</td>
<td>138150</td>
<td>0.49</td>
<td>1692</td>
<td></td>
</tr>
</tbody>
</table>

*Age in month, volume in mm³*
Appendix 5B: Graphical Presentation of the Correlations at 20% Strain

1. Longitudinal samples, at 20% strain

Figure 5B1 Correlation between Stiffness $\mu_2$ and Volume Fraction (Longitudinal Samples, at 20% Strain)

Steady-state stiffness $\mu_2$ (kPa)

Volume fraction of elastic fibers

$R = 0.301$

$P = 0.059$

$1-P = 0.941$
Figure 5B2 Correlation between Stiffness $\mu_2$ and ($V_{\text{long}} \times L_{\text{percent}}$)
(Longitudinal Samples, at 20% Strain)

Steady-state stiffness $\mu_2$ (kPa)

$V_{\text{long}} \times L_{\text{percent}}$

$R = 0.363$
$P = 0.021$
$1-P = 0.979$
Figure 5B3 Correlation between Stiffness $\mu_1$ and $L_{\text{percent}}$
(Longitudinal Samples, at 20% Strain)

$R = 0.309$

$P = 0.052$

$1-P = 0.948$
Figure 5B4 Correlation between Stiffness $\mu_1$ and $T_{\text{percent}}$ (Longitudinal Samples, at 20% Strain)

$R = 0.408$
$P = 0.01$
$1 - P = 0.99$
Figure 5B5 Correlation between Stiffness $\mu_1$ and $(T_{\text{percent}} / L_{\text{percent}})$
(Longitudinal Samples, at 20% Strain)

R = 0.453
P = 0.033
1-P = 0.997
Figure 5B6 Correlation between Viscosity $\eta$ and Volume Fraction (Longitudinal Samples, at 20% Strain)

![Graph showing correlation between viscosity and volume fraction.](image)

- $R = 0.388$
- $P = 0.019$
- $1-P = 0.981$
Figure 5B7 Correlation between Viscosity $\eta$ and $L_{\text{percent}}$ (Longitudinal Samples, at 20% Strain)

$R = 0.262$
$P = 0.077$
$1-P = 0.923$
Figure 5B8 Correlation between Viscosity $\eta$ and ($V_{long} \times L_{percent}$) (Longitudinal Samples, at 20% Strain)

$R = 0.42$
$P = 0.007$
$\bar{P} = 0.993$
2. Circumferential samples, at 20% strain
Figure 5B10 Correlation between Stiffness $\mu_2$ and Volume Fraction (Circumferential Samples, at 20% Strain)

Figure 5B11 Correlation between Stiffness $\mu_2$ and $C_{\text{percent}}$ (Circumferential Samples, at 20% Strain)
Figure 5B12 Correlation between Stiffness $\mu_2$ and $(V_{circ} \times C_{\text{percent}})$
(Circumferential Samples, at 20% Strain)

$R = 0.378$
$P = 0.016$
$1-P = 0.984$

Figure 5B13 Correlation between Stiffness $\mu_1$ and Volume Fraction
(Circumferential Samples, at 20% Strain)

$R = 0.349$
$P = 0.027$
$1-P = 0.973$
Figure 5B14 Correlation between Stiffness $\mu_1$ and $(V_{\text{circ}} \times C_{\text{percent}})$

(Circumferential Samples, at 20% Strain)

$R = 0.323$

$P = 0.042$

$1-P = 0.958$
Figure 5B15 Correlation between Viscosity $\eta$ and Volume Fraction
(Circumferential Samples, at 20% Strain)

\[ R = 0.346 \]
\[ P = 0.029 \]
\[ 1-P = 0.971 \]
Figure 5B16 Correlation between Viscosity $\eta$ and $(V_{\text{circ}} \times C_{\text{percent}})$
(Circumferential Samples, at 20% Strain)

$R = 0.341$

$P = 0.031$

$1 - P = 0.983$
Figure 5B17 Correlation between Viscosity $\eta$ and $T_{\text{percent}}$
(Circumferential Samples, at 20% Strain)

$R = 0.34$
$P = 0.029$
$1 - P = 0.971$
Figure 5B18 Correlation between Viscosity $\eta$ and $L_{\text{percent}}$
(Circumferential Samples, at 20% Strain)

$R = 0.273$
$P = 0.088$
$1-P = 0.912$
Figure 5B19 Correlation between Viscosity $\eta$ and $(T_{\text{percent}} / L_{\text{percent}})$
(Circumferential Samples, at 20% Strain)

Figure 5B20 Correlation between $\tau_o$ and $L_{\text{percent}}$
(Circumferential Samples, at 20% Strain)
Figure 5B21 Correlation between \( \tau_0 \) and \( (V_{\text{long}} \times L_{\text{percent}}) \)
(Circumferential Samples, at 20% Strain)

Figure 5B22 Correlation between \( \tau_0 \) and \( (T_{\text{percent}} / L_{\text{percent}}) \)
(Circumferential Samples, at 20% Strain)
Appendix 5C: Graphical Presentation of the Correlations at 10% Strain

1. Longitudinal samples, at 10% strain

Figure 5C1 Correlation between Viscosity $\eta$ and Volume Fraction (Longitudinal Samples, at 10% Strain)
Figure 5C2 Correlation between Viscosity $\eta$ and $L_{\text{percent}}$
(Longitudinal Samples, at 10% Strain)

- $R = 0.46$
- $P = 0.003$
- $1-P = 0.997$
Figure 5C3 Correlation between Viscosity $\eta$ and $(V_{\text{long}} \times L_{\text{percent}})$
(Longitudinal Samples, at 10% Strain)

$R = 0.42$
$P = 0.007$
$1-P = 0.993$
Figure 5C4 Correlation between Viscosity $\eta$ and $T_{\text{percent}}$
(Longitudinal Samples, at 10% Strain)

$R = 0.458$
$P = 0.003$
$t,P = 0.957$
Figure 5C5 Correlation between Viscosity $\eta$ and $T_{\text{percent}} / L_{\text{percent}}$

(Longitudinal Samples, at 10% Strain)

$R = 0.49$
$P = 0.001$
$1-P = 0.999$

Figure 5C6 Correlation between $\tau_e$ and $L_{\text{percent}}$

(Longitudinal Samples, at 10% Strain)

$R = 0.30931$
$P = 0.052$
$1-P = 0.948$
Figure 5C7 Correlation between $\tau_c$ and $T_{\text{percent}}$ (Longitudinal Samples, at 10% Strain)

![Graph showing the correlation between $\tau_c$ and $T_{\text{percent}}$]

Figure 5C8 Correlation between $\tau_c$ and $(T_{\text{percent}} / L_{\text{percent}})$ (Longitudinal Samples, at 10% Strain)

![Graph showing the correlation between $\tau_c$ and $(T_{\text{percent}} / L_{\text{percent}})$]
2. Circumferential samples, at 10% strain

Figure 5C9 Correlation between Stiffness $\mu_2$ and $(V_{\text{circ}} \times C_{\text{percent}})$
(Circumferential Samples, at 10% Strain)

<table>
<thead>
<tr>
<th>$V_{\text{circ}} \times C_{\text{percent}}$</th>
<th>Steady-state stiffness $\mu_2$ (kPa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.000</td>
<td>0</td>
</tr>
<tr>
<td>0.005</td>
<td>4</td>
</tr>
<tr>
<td>0.010</td>
<td>8</td>
</tr>
<tr>
<td>0.015</td>
<td>12</td>
</tr>
</tbody>
</table>

$R = 0.302$
$P = 0.037$
$1-P = 0.963$
Figure 5C10 Correlation between Stiffness $\mu_1$ and $(V_{\text{circ}} \times C_{\text{percent}})$
(Circumferential Samples, at 10% Strain)

$R = 0.283$
$P = 0.075$
$1/P = 0.925$
Figure 5C11 Correlation between Viscosity $\eta$ and $C_{\text{percent}}$
(Circumferential Samples, at 10% Strain)

$R = 0.427$
$P = 0.006$
$1-P = 0.994$
Figure 5C12 Correlation between Viscosity $\eta$ and ($V_{\text{circ}} \times C_{\text{percent}}$)  
(Circumferential Samples, at 10% Strain)
Figure 5C13 Correlation between Viscosity $\eta$ and $T_{\text{percent}}$
(Circumferential Samples, at 10% Strain)

$R = 0.37411$
$P = 0.017$
$1-P = 0.983$
Figure 5C14 Correlation between Viscosity $\eta$ and $(T_{\text{percent}} / L_{\text{percent}})$
(Circumferential Samples, at 10% Strain)

$R = 0.388$
$P = 0.013$
$1-P = 0.987$

Figure 5C15 Correlation between $\tau_e$ and $L_{\text{percent}}$
(Circumferential Samples, at 10% Strain)

$R = 0.388$
$P = 0.013$
$1-P = 0.987$
Figure 5C16 Correlation between $\tau_e$ and $T_{\text{percent}}$
(Circumferential Samples, at 10% Strain)

Figure 5C17 Correlation between $\tau_e$ and $(T_{\text{percent}}/L_{\text{percent}})$
(Circumferential Samples, at 10% Strain)
Figure 5C18 Correlation between $\tau_o$ and $L_{\text{percent}}$ (Circumferential Samples, at 10% Strain)

$R = 0.264$

$P = 0.0097$

$1-P = 0.9$