Optical remote sensing of hypertensive blood vessels

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Optical Remote Sensing of Hypertensive Blood Vessels

by

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A Doctoral Thesis

Submitted in partial fulfilment of the requirements for the award of the degree of Doctor of Philosophy of Loughborough University.

September 1997

Dedicated to

Mukand Singh Dheensay
The aim of this research is to investigate the effects of hypertension on small artery structure by extending the instrumentation which is available for optical analysis of vessels in-vitro. Conventionally, arterial vessels are pressurised in a perfusion myograph and are analysed by video microscopy. A key objective of this research is to enable the examination of vessels by absorption spectroscopy.

For this purpose, a new device, the fibermyograph, suitable for the pressurisation and perfusion of small blood vessels in-vitro is developed. Segments of rat mesenteric arteries are dissected and mounted in the fibermyograph onto a pair of identical cannulae in a controlled environment. Set at constant perfusion pressure, light is transmitted through the vessel via a fiber optic arrangement coupled to a photodiode array spectrometer. Simultaneously, the dimensions of the perfused vessels are monitored and recorded using video microscopy. Errors in the dimensional analysis are investigated and a model based explanation is derived.

An experimental protocol is developed to acquire the transmission spectra of vessels undergoing morphological changes due to pressurisation or tensioning. Features of the averaged normalised transmission spectra are extracted by means of a non-linear function fit and these features are correlated to the morphological parameters blood pressure, vessel wall cross sectional area and the vessel wall thickness to lumen diameter ratio. Spectroscopic studies are also carried out using the conventional wire-myograph and on tissue samples mounted on slides.

The standard clinical indicator of hypertension is blood pressure levels. All of the arterial specimens chosen for this study are from two rat strains, one with induced hypertension and the other, normal rats as controls. In past clinical studies, blood pressure was found to correlate with the arterial wall thickness to lumen ratio and the wall cross sectional area. The results in this thesis support these observations.

The new spectroscopic features measured in this work are found to correlate with blood pressure, the wall to lumen ratio and the cross sectional area of the arterial vessel. This suggests that hypertensive disease can produce structural changes of the arterial wall, measurable by a remote optical method. Given these present results, direct in vivo diagnosis of hypertension may be feasible in the near future.
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Chapter 1. Introduction.

In sustained hypertension, the small arterial vessels’ wall experiences some structural changes\(^1,2\) that affect the elasticity\(^3,4,5\) and the composition\(^6,7,8,9\) of the vascular wall. These structural changes motivated a study to find a means to differentiate hypertensive vessels from normal vessels through optical remote sensing methods. The approach this study takes in obtaining spectroscopy information of the vessel state is based on transmission spectroscopy techniques. The obtained spectra would indicate any apparent structural changes between the normal and hypertensive vessels through the shape of the spectral profile.

The path to solving the problem of obtaining specific spectroscopic features in relation to the wall structure was carried out in 3 major stages. First, arterial tissue samples were examined in specially designed hardware equipment incorporating transmission spectroscopy techniques. Second, the features of the obtained normalised spectra were extracted and the results were statistically correlated to the morphological and physiological parameters of the arterial vessels. Finally, conclusions were drawn from the significant correlation values of the spectroscopic features in relationship to arterial blood pressure and media to lumen ratio. The morphological parameters obtained in this study were examined and compared to previous published results to support the findings of this thesis.

An increase in arterial resistance (blood pressure) is the most obvious indicator of hypertensive disease. The cause or the consequence of the hypertensive state of the vascular wall is difficult to determine, but research into hypertension shows that small arteries are important determinants of peripheral resistance in hypertensive disease\(^10,\)
Furthermore, the evidence in clinical research on hypertensive disease suggests that the vessel media to lumen size ratio of small arteries is increased in comparison to a healthy normal artery.

A great deal of information concerning the physiological properties of arterial tissue is found through studies of isolated vessels, where the influences of surrounding tissue is eliminated. The in-vitro studies in this thesis are based on such isolated arterial specimens, obtained from the mesentery beds of hypertensive and normal rats. The mesentery provides resistance arteries with various lumen diameters that are easily accessible.

In clinical studies, animal models are used to mimic diseases affecting humans, because it is relatively easy to control the effects of the environment. In the case of a hypertensive model, environmental effects such as smoke, salt intake and stress could play a part in the degree of hypertensive disease in humans. Using animal models these external effects can be eliminated in a controlled environment and a more basic level of cause and consequence of hypertensive disease can be examined. In animal models, hypertensive disease is induced or inbred. In this thesis the rat breed used is the adult Wistar rats with induced hypertension and normal adult Wistar rats are used as controls.

Hypertension was induced through a model based on the Goldblatt one kidney one clip model. This particular model is developed by removing the right kidney of the rat and placing a constricting clip on the left kidney. The advantage in this model is that the control derives from the same strain but has undergone a sham operation. Therefore the mechanisms that underline the development and sustained hypertension in both strains is the same. It represents renal artery stenosis in humans which is a secondary form of hypertension where activation of the renin-angiotensin system plays a major part in bringing about the elevated blood pressure. In this thesis we examine the physiological or mechanical aspects of this type of hypertensive disease and not the biochemical aspect of it.
Previous spectroscopic research studies into diseased arteries has concentrated solely on atherosclerosis and was mostly focused on the larger conduit arteries. These studies are based on fluorescence spectroscopy because the larger arteries contain large amounts of elastin and this substance fluoresces well. There have been studies into small arteries with fluorescence techniques and detection with a photo multiplier tube, but these concentrate on the myogenic responses of arterial vessels. To date, there has never been a spectroscopic study performed on the effects of hypertensive disease on small arterial vessels (lumen diameter range between 100μm to 300μm).

This thesis documents for the first time a transmission spectroscopy study in the wavelength region of 340 to 840nm on the effects of hypertensive disease on small arterial vessels. The research work starts with a basic examination of arterial specimens from the 1st, 2nd and 3rd order branch arteries of the mesentery. The arterial samples are dissected and placed on slides. This simple examination of a slab of passive tissue on a slide, provided a means to obtain basic transmission spectra profiles for further study.

The slide study exposed some disadvantages. First it examined only passive tissue illuminated by an unfiltered light source. Ultraviolet light may have damaged the tissue and it was not contained in a controlled environment of 37°C, gassed at 95% O₂ and 5% CO₂. Furthermore, the morphological parameter measurements of the media to lumen ratio and the media cross sectional area were never obtained for the 1st and 2nd order arteries and are given only as approximates for the 3rd order arteries.

The study into small arterial vessels was taken to a more physiological level by applying transmission spectroscopy techniques to the conventional clinical wiremyograph system. The wiremyograph is a common device used in the study vessels in-vitro. In the wiremyograph, ring preparations of small arteries were mounted on specially structured mounts in the bath. The bath provides a controlled environment that is similar to in-vivo conditions. The artery is threaded with stainless steel wires (40μm) and tied on the mounts to a stationary position. This apparatus
enabled the mounted arterial vessel to be stretched (range 100μm to 350μm) and the transmission spectra were obtained through specially designed optical probes.

The conventional wiremyograph set-up posed three fundamental problems. First, the morphological parameters obtained with this system were only estimates of the true vessel wall and the values were obtained only at the beginning of each experiment. Furthermore, it was not possible to measure the vessel dimensions during experimentation. Second, the wire attachments to the artery do not allow the vessel to function in an in-vivo manner because of the intervening tensions introduced by the mounting wires. Third, the arterial vessel edges are damaged by the mounting wires and morphological measurements for each stretch would never have represented the true vessel wall. Besides, it was impossible to determine exactly what the optical probe was measuring because of the location of the probe with respect to the suspended vessel.

A more physiological system was then built based on the concepts of a perfusion myograph system, to overcome the shortcomings of the wiremyograph system. The new device, termed “fibermyograph”, enabled for the first time simultaneous video microscopy and spectroscopy.

In the fibermyograph system, ring preparations of the arterial specimens were mounted onto two identical glass cannulae in the bath and the vessels’ edges tied with a nylon thread (20μm) to hold it stationary. The arterial vessel’s lumen was dilated through connections to an external perfusion pressure system (range of 30mmHg to 110mmHg). Built in optical tweezers enabled the transmission spectra measurements and the vessels’ morphological parameters were obtained through the recorded images.

The transmission spectra obtained from the slide, wiremyograph and fibermyograph systems were normalised and averaged. The averaged normalised spectra were fitted to a logistic function to extract the main features of the spectral profiles. These parameters describe the shape of the normalised transmission spectra. The extracted
parameters are correlated and statistically analysed with the wall to lumen size ratio, wall cross sectional area of the sample vessel and arterial blood pressure.

1.1 : Layout of the thesis.

This document is divided into 11 chapters. The first chapter covers a general introduction to the research work carried out in the study presented in this thesis. Chapter 2 concentrates on the results of the physiological aspects in hypertensive disease obtained by clinical studies and reviews the equipment currently used in small vessel in-vitro investigations. This chapter includes a brief overview on spectroscopy techniques. Chapter 3 examines the overall hardware equipment used in the experimental stage of the study other than the specific hardware designed for the slide, wiremyograph and fibermyograph systems.

Chapters 4, 5 and 6 concentrate more on the specific hardware integrating spectroscopic techniques to the available hardware. Chapter 4 describes the specific hardware and provides transmission spectra results for the slide study. Chapter 5 covers the wiremyograph apparatus and the fibermyograph system is described in Chapter 6.

In Chapter 7 a model is described which is used to extract the spectroscopic features of the spectra. The performance of the model is evaluated and a comparison of the fitted parameters to the normalised transmission spectra is examined. Chapter 8 covers the correlation relationships between the morphological parameters of the arterial vessels for the slide, wiremyograph and fibermyograph systems to arterial blood pressure and compares the results with previously published results.

Chapter 9 examines the relationship of the extracted transmission spectra features to the morphological parameters and blood pressure for the first time. Chapter 10 provides the overall discussion and is based on the results and discusses the complications involved in obtaining the normalised transmission spectra. Final conclusions for the work presented in this thesis are stated in Chapter 11.
Chapter 2. Physiological aspects of hypertension and the equipment used to investigate them in-vitro.

2.1: What is Hypertension?

Hypertension is described as a condition where a person has a consistently high blood pressure in relation to their age. However, it is important to remember that everyone can have a temporary high blood pressure reading at some time or another, resulting from excitement, nervousness, exertion, anger, fatigue, cold or even smoking. What's more, blood pressure levels tend to rise with age. Hypertension is a condition where that high blood pressure is sustained over a period of time. The symptoms of high blood pressure are usually unreliable. Although it may cause headaches, visual problems, dizziness or shortness of breath, these could also be due to other conditions. Even those with dangerously elevated blood pressure can feel perfectly normal. The limit of hypertension is usually defined as the systolic blood pressure more than 160 mmHg and the diastolic blood pressure more than 95 mmHg.

Literature proves that sustained hypertension above certain levels results in diminished life expectancy. For example in the US, hypertensive related diseases cause approximately 890,000 deaths each year. Hypertension is associated with diabetes mellitus, coronary artery disease and arteriosclerosis. Complications of hypertension include hypertensive heart disease, renal impairment, haemorrhage of the brain (stroke), ischemic heart disease and eye disease (retinal haemorrhage).
There are a number of theories to the cause of hypertension such as family history, renal disease, endocrine diseases, pregnancy related, oral contraceptive pills, diet, environment factors, lack of exercise and obesity, hormonal imbalance, certain kinds of stress or smoking. What causes hypertension may be a combination of many factors or just a prominent one and that can vary among individuals. Research into the underlying causes of hypertension is unable to give a conclusive cause but most cases are family related.

As hypertension is a slowly developing and progressing disease, a long term treatment program is usually prescribed by doctors to control this disease. Non-drug treatment of hypertension includes weight loss, optimal exercise, quit smoking and alcohol, low salt diet, decrease stressful life style, treatment of other associated diseases like diabetes, coronary heart disease and renal disease. Drug treatments include various combination of beta blockers, diuretics, angiotensin converting enzyme inhibitors (ACEI), calcium channel blockers, alpha blockers and vasodilators. The current treatment of hypertension is constantly improving such as longer lasting drugs, drugs with fewer side effects, and new forms of non-drug therapy such as biofeedback and relaxation training, but the best solution is still preventive measures.

2.2 : The structure of the artery.

The arterial wall plays a major role in the understanding of the cause and consequence of hypertensive disease. Therefore it is important to understand its material content as is briefly described in this section.

All mammal small arteries are composed of the same basic substance and the artery structure is very similar. Figure 2.1 shows an image of an isolated mesentery artery in a perfusion system and figure 2.2 is a photomicrograph of the traverse section of a typical small artery obtained from literature. The arterial wall is made up of three layers: an internal tunica intima, surrounded by a tunica media, and then an external tunica adventitia.
Figure 2.1  An example of an image of an isolated mesentery artery in a perfusion system.

![Arterial wall and Lumen](image)

Figure 2.2  The wall composition of a mesentery artery of the guinea pig. $L =$ lumen, $e =$ endothelium, $I =$ internal elastic lamina, $m =$ media, $a =$ adventitia.

![Wall composition](image)

The tunica intima has two components. The innermost, which is in contact with the blood is the endothelium. This is a single layer of cells, which extends as a continuous lining, covering all the surfaces which come in contact with blood. It is mechanically rather fragile but has considerable powers of regeneration and growth. Surrounding
the endothelium is a thin subendothelial layer, containing a few collagen-generating cells and collagen fibres. Then comes a layer of branching elastic fibres which forms the inner boundary to the next wall layer, the tunica media. The tunica media is made up of multiple layers. Elastic tissue is separated by thin layers of connective tissue, collagen fibres, and sparse smooth muscle cells which cross-link in a mesh.

Smooth muscle cells are long (25-50 µm) and thin (5 µm), with tentacle like extensions, and contain contractile filaments of muscle. Small arteries with an internal diameter of approximately 300 microns have up to 6 layers of smooth muscle cells, whereas arterioles with a diameter of 30-50 microns have only a single layer of smooth muscle cells. Smooth muscle cells are arranged circumferentially around the lumen of the vessel, and during contraction these cells assume a spiral conformation.

The outer most layer of the arterial wall is the tunica adventitia. In some places it may be as thick as or even thicker than the media. This is because it is composed of loose connective tissue, containing relatively sparse elastin and collagen fibres formed in a longitudinal direction. It has a poorly defined edge as it merges into the surrounding tissues. The vascular nerve and blood supply to the artery is located within the tunica adventitia and does not penetrate into the media of the vessel.

2.3 : A review of the physiological aspects of arterial hypertension.

An increase in arterial resistance is the most obvious indicator of hypertensive disease. Meyer in his book, proposes three major hypothesis to explain the increased arteriolar resistance. Firstly, that the arterial contraction is secondary to the action of vasoconstrictor agents, such as angiotensin and catecholamines. The second underlines the importance of mechanical factors, such as a decreased arterial lumen diameter, to the peripheral resistance. Thirdly, he proposes the existence of a functional anomaly in the arteriolar wall which provokes excessive contraction. In other terms, the abnormality responsible for the maintenance of hypertension could lie, not at the level
of the regulating system (nervous system and hormones), but at the level of the regulated systems itself (intrinsic properties of the vessel wall itself).

As far as research into hypertension is concerned, it is difficult to know whether the above hypothesis are the cause or the consequence of the hypertensive state of the vascular wall. Because the subject of hypertension is so wide, in this section the intention is to draw focus on some conclusions into the physiological or mechanical aspects of arterial hypertension in clinical research. Today, most of the available evidence suggests, both in human and in animal models of hypertension, that the media (wall) to lumen ratio of small arteries increases. An increased media to lumen ratio has been reported in mesenteric 7,8, renal 13, hepatic 14 and cerebral 15 resistance vessels.

Heargerty 1 and Baumbach 2 concluded that this increase in the media to lumen ratio is caused either by the addition of new material onto the lumen of the vessel or by a rearrangement of the same material around a smaller lumen. They explain that the structural alterations based on vascular growth are caused by hypertrophy (change in size) or hyperplasia (change in number) of smooth muscle cells. In hyperplasia, since rearrangement of cells does not involve a growth process the media cross sectional area of the vessel is unchanged. Whereas in hypertropy, the media is visibly of greater thickness, making the cross sectional area different, between the hypertensive and normal specimens.

Aalkjaer 27, Korsgaard 28 and Schiffrin 29 researched into the subcutaneous resistance arteries in humans with mild essential hypertension, and found an increase in the media to lumen ratio, but the cross sectional area of the walls remained similar to the normal group. This suggests that a remodelling of existing tissue had occurred rather than growth. They concluded that in human essential hypertension, since cell volume is normal, the increased media to lumen ratio is brought about by remodelling and not growth in the vessel wall.

On the other hand, using a three dimensional dissector technique, Mulvany 9 showed that medial hypertrophy of the Simultaneously Hypertensive Rat (SHR)’s mesenteric
arteries is due to smooth muscle cell hyperplasia. Owens confirmed these findings with morphometric, biochemical and immunological studies of isolated smooth muscle cells. Walker's examination on the structural proteins, such as collagen, elastin and tenacin, failed to show any difference between SHR and Wistar Kyoto Rat (WKY) arteries. Any increase in the amount of smooth muscle in resistance arteries from genetically hypertensive rats appears therefore to be due to hyperplasia rather than hypertrophy, which is supported by the increased number of cell layers as observed by Mulvaney and Lee.

Goldblatt discovered that most aspects of the hypertensive disease in man could be mimicked in dogs if renal blood pressure and flow were lowered by means of clamps around the renal arteries. One of the safest way to produce high blood pressure in experimental animals is to interfere with their renal blood supply. Renovascular hypertension is the prototype of renin-dependant hypertension in humans. An obstructing lesion located on the renal arterial tree may cause, beyond a critical degree of stenosis, a pressure gradient and a blood flow reduction, thereby triggering the release of renin from the ischemic kidney. This increase in release renin induces renovascular hypertension.

The pattern seen in secondary models of hypertension such as those used to model renovascular hypertension in rats is somewhat different. Using the dissector technique as well, studies by Korsgaard showed that the increase in media thickness seen in one kidney, one clip Goldblatt hypertensive rat model is due to smooth muscle cell hypertrophy rather than hyperplasia. The one kidney, one clip Goldblatt model is truly a classic example of induced renovascular hypertension.

Meyer, on the other hand proposed that the two kidney Goldblatt model is very different from the one kidney model and is not mainly dependent upon the renin-angiotensin system. This is due to the plasma noradrenalin concentrations, which are normal in two kidney Goldblatt hypertension of the rat. This suggests that neurogenic mechanisms, if they exist, are certainly not of great importance in true renovascular hypertension in this species.
An interesting aspect into understanding the hypertensive artery is where and how the affects of hypertensive disease are observed the most. Furuyama\textsuperscript{10} discovered that the increase in media to lumen ratio became less pronounced in the pre-capillary vessels suggesting that it is the small arteries which are the important determinants of peripheral resistance for hypertensive vessel. Short\textsuperscript{11} and Suwa\textsuperscript{12} came to the same conclusion. Mulvaney and Halpern\textsuperscript{34} investigated the contractile properties of small arteries of SHR and WKY rats and concluded that in having greater smooth muscle cells, the SHR model had greater contractility.

Using pulse contour analysis techniques, McVeigh\textsuperscript{5} identified a striking change in the oscillatory component of the diastolic waveform, consistent with alterations in the compliance characteristics of the small arteries. Mulvaney\textsuperscript{3, 4} however, rationalised that a reduction in the elastic modulus would serve to normalise arterial compliance, despite an increase in wall thickness. A decrease in the elastic modulus of the wall materials is demonstrated in small arteries in experimental hypertension. This is a means whereby the vessel can functionally maintain its distensibility characteristics despite the relatively increased wall thickness required by the increased pressure.

Meyer\textsuperscript{6} claims that there is an increase in the synthesis of collagen in the arterial media which accumulates between the arterial smooth muscle cells. With these two observations, increase in callogen components and decrease in elastin modulus, could suggests that hypertension is, if not determined, at least partially maintained by the increased rigidness of peripheral arteries and arterioles.

The build up of callogen and reduction of elastin modulus are generally known to be age-related changes. Older arteries have higher contents of these two substances. Li and Schiffer\textsuperscript{35} found results showing the rapid development of functional and structural changes in small resistance arteries in renal hypertensive rats to be within 4-6 weeks of hypertension, with significant increase in media width in the arterial walls. Falloon\textsuperscript{36} found that in addition to altered structural properties of the vessel wall, functional modifications in the endothelium can influence arteriolar tone in hypertensive vascular disease.
2.4: The mechanics of arterial blood vessels.

The walls of blood vessels are elastic, and can change their size or shape when different forces are applied to them. These forces include both the pressures and shear stress exerted by the blood, and the constraints imposed by the surrounding tissue. All blood vessels in vivo are bound to the surrounding tissue, and are subjected to a considerable longitudinal stretch all the time. When a segment of artery is excised and released, its length decreases by 30-40%. In other words, in vivo the arteries are naturally in a state of radial distension and longitudinal stretch.

The walls of blood vessels are visco-elastic. About 70% to 80% of the walls of arteries consists of water, which is not elastic, but is incompressible. The rest of the wall material consists of a mesh of fibres which do have elastic properties. There are three sorts of fibres which determine the elastic properties of vessel walls as a whole. They are elastin, collagen, and smooth muscle cell.

Elastin is a rubber-like substance, with a Young's modulus of approximately $3 \times 10^5$ Nm$^{-2}$. Collagen is much stiffer than elastin, with a Young's modulus of approximately $10^8$ Nm$^{-2}$. Smooth muscle has a Young's modulus similar to that of elastin; however, the exact value measured in an experiment depends on the level of physical activity. It varies from about $1 \times 10^5$ Nm$^{-2}$ when the muscle is completely relaxed, to about $2 \times 10^6$ Nm$^{-2}$ in the active state. Therefore it is difficult to say what the Young's modulus is in vivo for the smooth muscle cell, since the degree of activity is not usually known.
2.5 : In vitro studies of isolated small arteries.

Myographs are the major devices used for the study of small arteries. The advantage of using myographs is that arteries can be isolated and studied in-vitro under in-vivo conditions by methods of a controlled surrounding environment. Blood vessels with internal diameters of around 300 microns and 1-2 mm in length can be mounted as ring preparations into the myograph. There are two major types of myograph used in clinical study today and these are briefly described in sections 2.5.1 and 2.5.2. Section 2.5.3 discusses some of spectroscopic techniques used in the study of arterial blood vessels.

2.5.1 : The wiremyograph.

The Mulvany wiremyograph comprises of an organ bath of 12 ml capacity in which two separate pairs of mounting heads are housed. One of the mounting heads is connected to a micrometer screw which enables it to be moved in a horizontal plane. The other is connected by a pin through the body of the myograph to a pre-calibrated force transducer (see figure 2.3). The signal from the force transducer is amplified and recorded on a two channel flat bed chart recorder. The stainless steel organ bath is heated to 37 °C by water circulating through a pair of heating blocks positioned on either side of the organ bath chamber. The myograph has ports which allow for oxygenation of the bathing medium together with an inlet tube for replacing the bath fluid.

The Mulvany wiremyograph permits simultaneous study of two small arteries. Each artery is carefully threaded on two 40 µm stainless steel wires which were secured to the mounting heads by a pair of screws. One of the wires is attached to the mounting head connected to the force transducer and the other wire attached to the mounting head connected to the micrometer. This myograph is mainly used for the assessment of isometric and isotonic forces. Light microscopy is used for measurement of vessel dimensions.
2.5.2: The pressure myograph.

In the pressure myograph the arterial vessel is threaded onto perfusion pipette connected to pressure transducers. The vessel is held in place by a holding pipette and collecting pipette. Vessel diameter is determined from light microscopy via a video camera, displaying the imaged vessel on a monitor. Smaller vessels can be mounted with this technique than with the wiremyograph and because this set-up allows the perfusion of vessel under pressure, it is more physiological than a wire myograph. Figure 2.4 shows a schematic of the perfusion myograph.
2.5.3: Spectroscopy.

2.5.3.1: Brief introduction to the concept of spectroscopy.

Light has both particle-like and wave-like properties. The fact that radiation consists of a stream of energy packets, moving in the direction of the beam with the same velocity as that of light, is very important, in understanding how molecules of organic substances absorb radiation. This phenomenon of absorption or emission of radiation by the molecules of samples through which the radiation passes, is the basis of spectroscopy.
The relation of $E=\hbar \nu$ is also very important. It states that the energy ($E$) of a monochromatic radiation depends only on the frequency ($\nu$) or wavelength of its waves and not on the intensity of its beam, where $\hbar$ is the Plank constant. A beam of radiation is more or less intense depending on the number of photons per unit time, per unit area, but the energy per photon (quantum energy) is always the same for a definite frequency of the radiation. Light waves can be reflected, refracted or diffracted when they interact with matter.

The two most frequently used types of spectroscopic investigations are as follows:

1. Ultraviolet and visible spectroscopy, in which the instruments use a source which can produce radiation of wavelengths between 200 and 750 nm.
2. Near infrared spectroscopy, in which a source is capable of producing waves 750 nm to 2 microns.
3. Infrared spectroscopy, in which a source capable of producing waves 2 to 16 microns long is used.

2.5.3.2: Fluorescence spectroscopy.

In fluorescence spectroscopy, a small area of sample is irradiated with light pulses and after a lapse of set time which is dictated by the emission and absorption characteristics of the sample, the sample fluorescence is measured. Fluorescence spectroscopy is the major optical technique used in determining vessel characteristics. Most of the fluorescence applications are based on the big arteries such as the aorta, primarily because catheters using this type of spectroscopy technology are used to detect atherosclerotic plaque in the aorta. The catheters are usually inserted inside the lumen to enable a better reception of the fluorescence of the inner vessel wall. This techniques works well in the major arteries because here there are larger quantities of elastin and this substance fluoresces well.
Oraevsky $^{19}$ used frozen and saline kept samples of human aorta and found a qualitative difference between normal and atherosclerotic plaque in the 530nm-630nm region using fluorescence spectroscopy techniques. Keijzer$^{18}$ used only frozen samples of the aorta, sliced approximately into the different individual layers of adventitia, media and intima. She found that the media layer of the aorta fluoresces far more than either intima or adventitia layers. This suggests that there is more elastin in the media of the aorta than in either of the other two layers. Kittrel$^{21}$, used frozen aorta samples obtained from patients with very early stages of atherosclerosis and found that there is a major difference between normal and plaque wall at 600nm.

The pressure myograph and fluorescence techniques have been developed to work with coronary resistance artery by VanBavel$^{23}$. His group used a PhotoMultiplier Tube to measure the emitted fluorescence of the arterial vessels (1st order arterial vessels) and studied the fluctuations of the cross sectional area of the vessel. They assume a linear relationship between the emitted fluorescence value to the cross sectional area of the vessel. Further work on mesenteric arteries have also been carried out by the same group$^{22}$ to analyse pressure induced changes on the vessel. Their conclusion is that the myogenic responses are induced by wall stress, rather than by distension of the vascular wall.

2.5.3.3: Absorption spectroscopy.

In absorption spectroscopy a measurement is made of the light passing through a given material and the resulting spectrum is the absorption spectrum. The absorption spectrum expresses the degree of absorption of a material as a function of wavelength. Absorption spectroscopy is used mainly in the study of tissue and blood tissue. The maximum absorption for hemoglobin is at the wavelength 550nm$^{37}$. Soret, β and α oxyhemoglobin absorption bands are in the regions of 418nm, 544nm and 578nm respectively$^{19}$. Absorption spectroscopy research of tissue such as adipose, gallstone, bladder, whole blood, brain, breast tissue, heart, kidney, liver, lung, skin, muscle, uterus and tumors utilise the wavelength range of 650 to 1100 nm$^{38}$. Pure distilled water has a maximum absorption value at 745nm$^{39}$.
2.6 : Conclusions.

This chapter has highlighted some of the consequences of hypertensive disease where there are obvious structural changes in the wall of small arteries. These structural alterations are based on vascular growth either by addition of new material onto the lumen of the vessel or by a rearrangement of the same material around a smaller lumen. This added new material is assumed to be smooth muscle cells. Furthermore, there is a decrease in the elastin modulus and a build up of collagen synthesis in the arterial wall of a hypertensive artery as compared to the normal artery.

The aim of the study presented in this thesis is to define a spectroscopic parameter that differentiates the hypertensive artery from the normal artery and this is to be achieved through transmission spectroscopy. To use fluorescence techniques would be unfeasible because we know that the small arteries don’t build up in elastin content, the substance that fluorescence well, but only experience changes in the elastin modulus. Arteries become more rigid in hypertension and are more responsive than normal arteries mostly because there is build up of collagen tissue.

Collagen and elastin are substances in arteries which cement the muscle cells. They are called connective tissue and are in every layer of the arterial wall. Muscle cells are protein based structures and are exclusively in the media layer of the arterial wall. From this chapter we know that the arterial wall is composed of muscle cells, collagen, elastin, water, nerve cells, and blood supply cells which exist in the 3 layers of the wall. In section 2.5.3, we determined that there are interesting discoveries in the wavelength range 450nm to 600nm and we know that the artery is approximately 80% of water and that water absorbs in the wavelength region of 745nm. So, a study into arterial vessel must explore the transmission responses in the range of 450nm to 750nm.

In this thesis, in order to differentiate hypertensive and normal arterial vessels, the transmission responses in the range of 340nm to 840nm is considered to be sufficient to obtain an indication of the condition of the arterial vessel under examination.
Chapter 3. General overview of experimental hardware.

3.1 : Introduction.

This chapter is dedicated to highlighting the overall equipment, available commercially, and not the specialized hardware pieces used in this study. The specialized pieces have been designed specific to the needs of the spectroscopic study to enable in-vitro examination of tissue samples and are described in detail in chapters 4, 5 and 6. To give a better understanding of the commercial equipment's performance and integration into the entire set-up the facts are presented here. An overview of the basic hardware structure layout is shown in the block diagram in figure 3.1. The hardware represented by block 2 is the specialized hardware equipment manufactured in the mechanical workshop. The remaining sections of this chapter are based on blocks 1, 3 and 4.

![Block diagram showing the general hardware layout](image)

Figure 3.1 A basic block diagram showing the general hardware layout of the experiments in this thesis.
3.2 : Description of the basic hardware set-up.

Light (block 1 of figure 3.1) from a 150W tungsten quartz lamp supplied by Dolan-Jenner Industries, High Density Illumination Series 180 reaches the sample tissue (block 2 of figure 3.1) via a fused silica fiber link. The light then passes through the tissue and is picked up by a probe with a glass fiber core. This glass fiber is positioned and clamped to the slit, which passes the light into a spectrograph (block 3 of figure 3.1). In the spectrograph the measured light is dispersed by the grating and projected as a continuous band of wavelengths onto a photodiode array, placed in the focal plane.

The array surface covers an area of 62.5 mm$^2$ with 1024 array elements and is contained in the detector head. Array based spectrometers have better stability and reproducibility since they contain no moving parts. The signal from the detector head is then amplified and digitized by an analog to digital converter (65536 levels) before being transferred via a controller to a computer (block 4 of figure 3.1) for processing.

The detector head is stabilized with cooling fins and has thermoelectric coolers to maintain the temperature of the array and can maintain a predefined temperature despite small changes in the local ambient temperature. The controller card used for the transfer of data into the computer's memory operates at speeds of 8MHz. The host processor used during experimentation was a 486 Compaq running on DOS version 6.2 with a memory capacity of 8 Megabytes.

The entire process of acquiring the raw spectroscopic data is computer initiated and controlled using a language structure, similar to BASIC. The software programs incorporate executable driver routines, developed to support electronics hardware and control the many aspects of system data acquisition and processing. The program code contains a function which holds 9 parameters that are required by the interface
card control logic to set the system to internal triggering. Each measurement is taken after a predefined exposure time and the scan mode is set to single scan.

In the single scan mode, data are acquired and stored in the active signal store. The maximum number of stores available is 30 and each store is independent. The amount of data that can be stored in individual stores is dependent on the host computer’s memory capacity. Each store has a structure similar to the array and it stores the 1024 pixel values with the corresponding wavelength values. Finally, the raw transmission spectra data are extracted from the stores into a tab delimited text file.

The optical cables are liquid retardant, lightweight and have a wide bandwidth. Links are secured against signal leakage using specially manufactured devices in the lab. Cable distorted by bending or kinks can also easily attenuate the transmission, therefore the fragile glass fiber was placed in a special plastic tubing to make it more mechanically strong. To reduce the amount of light loss from scattering and angular losses, all fiber surfaces were made vertical to their horizontal axis and were polished to be of mirror-like smoothness. The ends of the fibers were also checked to be free of dust particles and stain, which can cause absorption losses during experimental measurements. For cleaning purposes, optical tissue, ethyl or isopropyl alcohol on a soft swab was used sparingly to clean the fiber’s tip.

3.3 : System resolution.

The resolution of the measured spectra is dependent on the entrance slit width, the dispersion, and the element width of the diode array. Spectral resolution for a diode array spectrograph is usually accepted as being limited by the bandpass over at least two elements. The observed values from all the experimental data in this thesis gives the system resolution value as 0.5nm.
3.4: The non-uniformities in the light source.

The light source used is a 150 W quartz tungsten halogen and this section examines its performance over time. This is important because in this thesis we are doing a comparative study and a standard light source or a light source which is constant over time is important.

A simple setup was made to couple the light source to the spectrograph using a multimode glass fiber and leaving the apparatus switched on for long periods of time (8 to 10 hours). Spectroscopy measurements were done after a duration of half hour for exposure times of 3 seconds. During the measurements, a power meter was connected directly to the light source and the observed power readings were noted.

Figures 3.2 and 3.3 give the transmission spectra of the light source in the wavelength range of 630 to 680 nm which is approximately situated in the peak of the spectra. In both figures, the positive point about the plots is the fact that minor fluctuations are consistent and stable over time. The negative point is that the whole spectrum shifts by approximately $\pm 2\%$ (figure 3.2). Nevertheless the source is reproducible by normalizing the spectrum to shifts to an accuracy of approximately $\pm 0.7\%$ as shown in figure 3.3. The light source spectras are normalized by taking the anti log of the power readings (dBm value) and obtaining a ratio relative to one initial power reading value.

In figure 3.3, the normalized spectra still retain the tiny characteristic fluctuations of the original since every spectrum is multiplied by a constant factor. Nevertheless after a number of other observations, it was concluded that a shift of $\pm 0.2$ dBm on the power meter reading could be ignored since it resulted in a light source spectral shift of approximately $\pm 1\%$ only. Furthermore the spectroscopy scans in this thesis ranged from 3 to 0.8 second exposures and the effects seen in this section would not have been significant.
Figure 3.2  Raw transmission intensity $[I \text{ (arbitrary units)}]$ spectra showing the variations of the white light source for intervals of half hourly scans obtained using a glass fiber guide coupled to the light source directly. [exposure time of 3 seconds].

Figure 3.3  Raw transmission intensity $[I \text{(arbitrary units)}]$ variations of white light source after figure 3.2 normalization.
3.5 : The detector head.

The detector head houses the diode array and has a built in cooling system. There is leakage current in the detector called dark current which is present even if there is no light falling on the detector. Dark current sets a limit on the exposure time for measurements, since charge saturation eventually occurs. It varies with temperature and can cause baseline or background changes over time. An example of the transmission intensity profiles of tissue, source and background is shown in figure 3.4. Figure 3.4 shows that the background reading shifts prominently in the near infrared region.

![Figure 3.4](image-url) The raw transmission intensity profiles of arterial specimen on slide, light source (I) and the background = noise + dark current (BkI) for exposures of 3 seconds.
After numerous experimentation, I observed the background error shifts are within ±0.3% of the light source readings, which is insignificant. Nevertheless, background noise adds to the signal and this has important consequences beyond the simple requirement to subtract the value from every signal reading. Background noise could include noise from electronics including readout noise and dark current and stray radiation from ambient light.

3.6: The slit.

The slit determines the attainable resolution and not the grating. Figure 3.5 shows the slit used for all the experiments in this thesis which was supplied by Oriel Inc. The figure shows that the slit has debris at the entrance which could cause some non-linearity in the measurements. The slit width is 25 μm with a height of 3mm.

![Image of the slit used in experiments obtained with video microscopy (75x) showing debris from the laser cut out.](image)

3.7: Raw transmission spectra of the solutions used in all experimental set-ups.

In chapter 2, we explained that the arterial vessel is composed of approximately 80% water. The detailed profile and precise absorption value vary depending on the type and purity of water. A comparison of the transmission profiles of distilled water and
Krebbs solution, which is widely used in all the experiments in this thesis, is shown in figure 3.7. Here there is an approximate shift of ±1.1% between the two different samples of solutions. The composition of the Krebbs solution is given in Appendix 2.

![Apparatus diagram for the measurement of Krebbs solution and distilled water placed in a glass cuvette. The raw transmitted light is measured through a glass fiber probe as are all measurements in this thesis.](image)

**Figure 3.6** Apparatus diagram for the measurement of Krebbs solution and distilled water placed in a glass cuvette. The raw transmitted light is measured through a glass fiber probe as are all measurements in this thesis.

![Raw transmission spectra of Krebbs solution compared to pure distilled water using the apparatus configured in figure 3.6 for exposure times of 2 seconds. I is the intensity for the Krebbs and water and Diff is the difference between the two intensity profiles. I, Diff in arbitrary units.](image)

**Figure 3.7** Raw transmission spectra of Krebbs solution compared to pure distilled water using the apparatus configured in figure 3.6 for exposure times of 2 seconds. I is the intensity for the Krebbs and water and Diff is the difference between the two intensity profiles. I, Diff in arbitrary units.
The absorption profile in figure 3.8 was obtained by Sullivan. He used a Nielsen spectrometer, placed distilled water sample in a glass cuvette and illuminated the cuvette with a tungsten source. The transmitted light was measured with a photomultiplier detector. The absorption profile in figure 3.8, obtained from his results, clearly shows a maximum absorption at wavelengths of 725 to 775 with a peak at 745 nm. The absorption coefficient has a minimum close to 450 nm.

![Absorption Coefficient of Distilled Water](image)

**Figure 3.8** Absorption coefficient of distilled water in the visible region.

We can conclude that the Krebbs solution used during the experiments in this thesis has a similar profile as distilled water from figure 3.7 and that the absorption of Krebbs is maximum in the range of 750 nm.

3.8 : Ambient temperature monitoring.

The room temperature was checked for all experiments in this thesis and it did not vary by more than 3°C. The mean temperature for all the observations was 23.0±0.5°C and the ambient temperature was noted before each scan with a lab
thermometer with a precision of ±0.1°. This temperature monitoring is important for the slide studies because the tissue examined in this instance is not contained in a controlled environment. Monitoring the ambient temperature is a precautionary step. Temperature can affect the state of a vessel only at high temperatures but at room temperatures there isn't any serious defect to the tissue.

3.9 : Calibration.

Calibration is necessary to compensate for misalignment of the photodiode array or wavelength drive. For calibrating purposes, a standard source is needed. Placement of a narrow band interference filter from Ealing in block 2 of figure 3.1, gives a standard source with a bandwidth of approximately 10 nm with the 3dB points at 652.1nm and 663.0nm. The measured normalised transmission spectra profiles are then used to find the new 3dB points. Any offset from the original 3dB points is saved in a calibration ASCII file and loaded prior to conducting all measurements. Every equipment is calibrated to a protocol given in Appendices 3, 4 and 5.

3.10 : The protocol.

The experimental protocol must include steps to counter the problems posed by the hardware equipment as highlighted in earlier sections. First, addressing the problem of the light source intensity shifts. By using a power meter to measure the output light intensity at the source enables normalisation of the spectra. Second, the accumulation of background noise other than the dark current could cause a limit to the exposure times. The lower the exposure times the less the readout noise. Experiments are more reliable if conducted in a controlled environment such as a dark room. Third, the slit is not perfect and therefore positioning the point source from the detection probe is important. Once the probe is set in position at the slit entrance, it should be clamped.
3.11: Obtaining the normalised transmission spectra.

The task here is to extract the data from these signals and noise in the most efficient manner. The array is capable of correcting for drifts of 0.5°C for ambient temp of ±10°C. All the raw data from the experiments were saved into tab delimited text files. These files were then loaded into MATLAB running in the UNIX environment and analysed to find the normalised transmission spectra values. The MATLAB routine loads the numerically listed files and performs the mathematical expression given in equation (3.1) to calculate the normalised transmission $T$,

$$T = \frac{TL - BL}{WL} \quad (3.1)$$

where $TL$ is the measured intensity with vessel and source, $BL$ is the measured intensity with vessel and without source and $WL$ is the measured intensity without vessel but with source.


One way to produce high blood pressure in experimental lab rats is to interfere with their renal blood supply. All specimens used in the experiments of this thesis had induced renovascular hypertension which is a prototype of renin-dependant hypertension in humans. The model used is the Goldblatt one kidney, one clip model. Female Wistar normotensive rats were obtained from the Biomedical Services Unit at Leicester University and were used since they gain less weight at any age. Weight increases can cause fluctuations in blood pressure levels.

Hypertension is induced by placing a constricting clip around the left renal artery of a kidney under anaesthesia after removing the right kidney. The constricting clip is made from annealed silver ribbon of about 2.30 mm wide, 0.15 mm thick and 15mm long. All the rats were killed by stunning followed by cervical dislocation. The
mesentery was then removed and immediately placed in a beaker of cold (4°C) Krebbs.

In order to make a valid comparative study it is important to ensure that all vessels were obtained from the same point in the mesenteric vascular bed. This was achieved by pinning the mesentery out to display and locating the correct site by selecting the arteries on the basis of branching pattern. Third order branch resistance arteries were dissected from the animals and mounted in the myograph. All vessel dissections and vessel mounting were carried out in freshly prepared cold (4°C) Krebbs solution. The Krebbs solution was gassed with 95% oxygen and 5% carbon dioxide to achieve a pH of 7.4 and heated to 37°C. This environment is similar to the in-vivo conditions. After mounting, the bath temperature is raised to 37°C and the arteries left to equilibrate for 30 minutes prior to morphological measurements.
Chapter 4. Arterial tissue on slides.

4.1: Introduction.

A lot of the work reported previously in literature is based on tissue kept in different conditions (such as frozen and in saline solutions) and placed on glass slides. The motivation to carry out the experiments in this section was to examine the effects of hypertensive disease on the branch orders of the mesenteric artery bed. This work gave an opportunity to obtain basic optical transmission spectra of the arterial wall where the tissue under examination was kept in saline solution. These experiments, conducted by placing samples of arteries on slides, gave some optical indication as to the effects of external environment to tissue especially the effects of the light source. Furthermore, the arterial tissue is not homogenous since the endothelium, media and adventitia layers of the artery are still intact when flattened out on slides. The spectra therefore would be a good approximation of one side of the arterial wall.

4.2: Preparation.

Guts of numbered, weighed and blood pressured specimens are sustained in krebbs saline solution at temperatures of 5-6 degrees Celsius. The solution prevents the contraction and sealing up of the lumens of the gut arteries and the low temperature reduces tissue metabolism. Sections of arteries are then dissected from 1st, 2nd and the 3rd order branch of the mesenteric bed. Figure 4.1 gives an idea of the positioning of these branches in the mesentery bed system. The vessel shaped like a ring is sliced on
one side, opened and flattened gently onto a slide. Tissue is placed with the endothelium facing up (the endothelium faces the probe point, see figure 4.2) with a drop of krebbs solution to enable the tissue to remain moist on the glass slide.

Figure 4.1 A sketch of the mesentery bed layout showing the position of the 1\textsuperscript{st}, 2\textsuperscript{nd} and 3\textsuperscript{rd} order arteries.

To prevent squashing and moisture loss in the tissue, it is encased in grease. This is done by etching the edges of the slide in grease and covering the specimen with a thin cover glass. The slides are 0.8 to 1.0 mm thick with the covers approximately 0.13 to 0.16 mm thick. The slides are cleaned with lens paper prior to placing the samples on them. The process of slicing and placing the tissue on the slide took about 2 minutes for each slide.

The branch samples are not similar in length when dissected from the mesentery but the surface areas of all the artery samples were approximately the same. Furthermore there were some blood stains on a few of the arteries. Flushing these slight traces of blood stains out could have affected or removed some of the fragile endothelial tissue that line the inside lumen wall of the artery. Therefore, the tissue samples were left as they were dissected and it was tried instead to position the probe head onto a lesser affected area.
4.3 : Experimental set-up.

Figures 4.2 and 4.3 show the specialised apparatus used for the measurement of transmission spectra of tissue samples on slide. This apparatus represents block 2 of figure 3.1 on page 20. The distance of the light source from the specimen is approximately 10cm. The probe head, 1mm diameter, sits directly above the specimen. Light transmitted through the tissue is measured through a 50micron core/125micron cladding multimode glass fibre and channelled to the spectrometer.

![Diagram of the set-up](image)

Figure 4.2  Schematic diagram of the set-up clamped onto a portable optical bench.

![Photograph of the experimental equipment](image)

Figure 4.3  A photograph of the experimental equipment showing the placing of the slide on the slide holder.
4.4 : Experimental protocol.

Each transmission spectrum was obtained from measurements with an exposure time of 3 seconds. The measurements were conducted in a sequence starting with the sample slide with tissue, then the background with the source covered and finally the light source with the sample removed and slide in position. All experiments were done to the slide protocol in Appendix 3, including the calibration of the equipment. These experiments were conducted in a dark room with the ambient temperature monitored and fluctuations noted as $24.0 \pm 0.5 \, ^{\circ}\text{C}$.

4.5 : Normalised transmission spectra results.

A total number of 15 adult Wistar rats clipped for periods of 8 and 4 weeks, along with the paired controls were examined. Paired controls are rats about the same age which undergo renal operations together to create a hypertensive strain and a normal strain. From the observed specimens, 6 developed arteriolar hypertension with a mean blood pressure of 206.5 mmHg and 9 were normotensive with a mean blood pressure of 121.5 mmHg.

Figures 4.4 and 4.5 are examples of the normalised transmission spectra profiles of 8 week old group of a hypertensive and a normal rat. The normalised transmission spectra in figure 4.4 and 4.5 show that there are no obvious differences between the normal and the hypertensive specimen in the position of the orders of the mesenteric branches. This trend is observed in the remaining 8 week group specimens' normalised transmission spectra. All 3rd ordered arteries have relatively thinner walls compared to the 1st and 2nd order for the normal and hypertensive specimens.

In figure 4.4 for the hypertensive vessel, the differences between 1st, 2nd and 3rd order arteries is less compared to the normal vessel in figure 4.5. This suggests that the branching orders of the hypertensive vessel are similar in thickness as compared to the normal arteries.
Figure 4.4  Normalised transmission spectra (T) of a 8 week clipped hypertensive rat (G247), BP=219mmHg, showing the 1\textsuperscript{st}, 2\textsuperscript{nd} and 3\textsuperscript{rd} order branch. [11.10.96]

Figure 4.5  Normalised transmission spectra (T) of a 8 week normal control rat (G265), BP= 106mmHg, showing the 1\textsuperscript{st}, 2\textsuperscript{nd} and 3\textsuperscript{rd} order branch. [7.11.96]
Figures 4.6 and 4.7 are examples of the normalised transmission spectra of the 4 week group of a hypertensive and a normal rat. Figures 4.6 and 4.7 show a very obvious difference in the profiles of the normalised transmission spectra. This trend is clearly repeated in all the remaining 4 week specimens. The normalised transmission spectra in figure 4.6 and 4.7 are different from the normalised transmission spectra in figure 4.4 and 4.5. The order of the arteries have changed in figures 4.6 and 4.7 for the 4 week group as compared to figures 4.4 and 4.5 for the 8 week group. In figures 4.6 and 4.7 the 3rd order artery is much thicker artery for the 4 week olds than the one in figures 4.4 and 4.5 for the 8 week olds.

There is a difference in the normalised transmission spectra in figure 4.6 and 4.7 where clearly the profile shapes of the arteries are significantly different. The hypertensive specimen has a sharper profile as compared to the normal specimen and the 3rd order artery has thickened for the hypertensive rat. Furthermore, there is a great deal of absorption in the ranges of 400 to 540 for the hypertensive in figure 4.7.

Figure 4.6 Normalised transmission spectra (T) of the 1st, 2nd and 3rd order mesenteric of a 4 week normal control rat (G292), BP= 135.4mmHg. [12.11.96]
Figure 4.7 Normalised transmission spectra (T) of the 1st, 2nd and 3rd order mesenteric of a 4 week clipped rat (G274), BP=222mmHg. [17.10.96]

4.6 : Discussion.

The study with the 1st, 2nd and 3rd order arteries is very important because it can be conclusive to the effects of hypertension as suggested by Furuyama 18, Short 19 and Suwa 20. These authors came to the conclusions that it is the small arteries that are the most important determinants of peripheral resistance for hypertensive disease. Therefore, it was worthwhile to carry out experimentation with the technique outlined in this chapter because of these clinical findings. Furthermore, this was a simple technique to obtain arterial tissue spectra.

In all the observations of the transmission spectra in this chapter, there is very minimal absorption in the 550nm range for most of the arteries suggesting that the effects of the blood stains is very minimal if any. In acquiring the transmission spectra, there were some discrepancies in the transmission profiles of the specimens
in this section. One possibility was the fact that the tissue facing the probe was not from a homogenous sample and thus errors can be caused by bits of adipose tissue, still attached to the external wall of the arterial wall itself. Surgical instruments could easily have caused damage when slicing open the artery for mounting on the slides. Furthermore, arteries consist of approximately 80% water, thus the storage method of placing the mesentery in Krebb's solution would inevitably affect the state of arteries.

Exposure to strong light sources can kill the tissue samples, but since all exposure times were over short periods of time (3 sec), this couldn't have caused much damage. Furthermore the slides are placed at a distance of 10 cm from the light source, making this effect rather small. But there was drying out of the tissue, especially for the thinner samples.

Despite all the precautions during the experimentation, I feel the normalised transmission spectra obtained with the technique presented in this chapter could have been improved on a great deal. Taking different positional scans and averaging the spectra would have been of great advantage. Another disappointment in the study with the slides is that I could only obtain approximate 3rd order mesenteric lumen and wall thickness morphological values. Therefore the statistical analysis of the spectra can only be done to the morphological readings of the 3rd order artery and is presented in chapter 8 and 9.

The arterial tissue examined in this chapter was a passive slab, placed on glass slides and exposed to the light source. A more physiological method would be to contain the arterial specimen in a controlled environment and to make the arterial tissue respond to tensions similar to in-vivo conditions. This is possible through the application of the conventional wiremyograph system described in chapter 2 section 2.5.1. This system is integrated into the optical set-up and the method is described next in chapter 5.
Chapter 5. Arterial vessels mounted in the wiremyograph.

5.1: Introduction.

The wiremyograph is a device used by researchers to find the mechanical and physiological properties of vessels in vitro and it is an automated system. Segments of arteries are dissected and mounted into the mounting heads in the wiremyograph bath. The mounting heads enable the wall tension to be measured while the internal circumference is controlled directly. The main usage of this equipment is to find the changes in active wall tension induced by inputting drugs into the bath and comparing the responses to a control artery specimen. Results obtained by clinicians with this method supports the hypothesis that in hypertension there is structural change in the resistance arteries 1, 2, 3, 4, 6, 7, 8, 9, 13, 14, 15, 40.

The experiments in this section were done on a limited, but precise to protocol, number of specimens. No mechanical changes were necessary to the wiremyograph system to integrate the optical equipment. A simple optical probe structure is introduced into the bath, a few millimetres above the artery and the transmitted light measured. This study is based on 16 adult Wistar arterial specimens, obtained from 8 paired specimens. Paired specimens are adult wistar rats aged approximately the same, which have undergone renal constriction surgery simultaneously to create the hypertensive model and the control. The normalised transmission spectra obtained in this study are further analysed statistically to find the correlation to the morphological characteristics of the arterial tissue and that is detailed in Chapter 8.
5.2: Arterial preparation.

The preparation were segments of 1 to 1.5 mm long arterial vessels taken from the 3rd order mesenteric branch of adult hypertensive Wistar rats and as the controls, normal Wistar rats. All the rats were adult females and weighed 265±25 grams with the mean blood pressure for the two stocks being 196 mmHg and 121.5 mmHg. The model used is the one kidney one clip Goldblatt model. Paired rats from the two stocks were culled after periods of 4 and 8 weeks of induced hypertension. The dissected arterial segments were kept in krebbs saline solution at temperatures of 5-6°C prior to mounting onto the wiremyograph.

5.3: Mounting onto the wiremyograph.

The ring preparations were mounted onto the wiremyograph in pairs since the Mulvany myograph permits simultaneous study of two small arteries. Each vessel is held in position by two wires (40mm in diameter) threaded inside the vessel lumen and suspended in clear Krebbs solution as seen in figure 5.1.

![Diagram of a vessel mounted in the wiremyograph organ bath chamber](image)

Figure 5.1: Diagram of a vessel mounted in the wiremyograph organ bath chamber onto a mounting head and seen from above. The wires are stainless steel (40 microns in diameter) and are tied to the mounting heads (A and B). A is attached to a micrometer and B is mounted to a pre-calibrated transducer and is stationary.
The wires are then secured onto the myograph mounting heads to enable the internal diameter of the vessel to be controlled directly through an external setting of the micrometer. The stainless steel organ bath was constantly supplied with oxygen funnelled through a tiny rubber tube outside the myograph and heated to 37° C by a pair of heating blocks positioned on either side of the organ bath chamber.

5.4: Morphology measurements.

After the vessels have been mounted in the wiremyograph bath, it is placed under a light microscope, fitted with a lens and a calibrated micrometer eyepiece. The arterial segment's wall thickness and lumen diameter is measured at 3 points along the length of the vessel and an average is taken to be the final value. The morphological measurements were carried out with both wires barely touching the vessel's internal wall, thus no tension was applied to the walls. This means that the morphology measurements made in this study are only estimates of the true vessel dimensions under no tension. The following measurements were taken along with the length of the vessel under no tension:

1. media thickness (m) is taken as the average of 3 readings
2. wire thickness (d) taken as an average of 3 readings.
3. distance between the inner edges of the wires (f) as an average of three readings.

Figure 5.2 gives a sketch of the measured values m, d and f.

![Figure 5.2 Sketch to illustrate the measured values for the morphological measurements.](image-url)
With the above morphological measurements the internal circumference (IC), lumen diameter (L) and media cross sectional area (MED_CSA) can be calculated as follows:

Internal circumference, $IC = (\pi + 2)d + 2f$  \hspace{1cm} (5.1)

Lumen diameter, $L = \frac{IC}{\pi}$  \hspace{1cm} (5.2)

Media cross sectional area, $MED_{CSA} = \pi m(L + m)$  \hspace{1cm} (5.3)

5.5: Experimental Apparatus.

A schematic diagram of the entire experimental apparatus is given in figure 5.3. The equipment is set-up, clamped onto a portable optical bench and calibrated according to the calibration protocol in Appendix 4. The light source was channelled to the vessel through a pair of small glass windows situated directly below each mounting head via a fiber link. A Bi-convex lens is used to collimate the light and increase the intensity of the light reaching the sample.

The wiremyograph bath was mounted directly above the light source and the probe was carefully positioned a few millimetres above the vessel. The probe’s positioning was a delicate procedure and is done according to protocol. The wiremyograph bath held two vessels simultaneously, usually from paired specimens of hypertensive and normotensive rats. This would enable a better comparison between vessels, since the spectroscopy and morphological results can be obtained under similar physiological circumstances.
Figure 5.3: Schematic diagram of the overall experiment apparatus. The myograph is placed above the light source and held in place by square blocks. The horizontal and vertical stage is clamped vertical to the portable optical bench.

Figure 5.4: A photograph of the experimental apparatus showing the probe placing in the myograph bath. The tip of the probe is submerged in the wiremyograph bath.
To enable spectroscopy measurements to be carried out, a specially designed probe head, shown in figure 5.3, was positioned above the vessel and submerged in Krebbs solution of the wiremyograph bath. The 1 mm diameter probe head tip contained a glass fiber approximately 50μm in diameter. The light transmitted through the vessel is measured with the spectrometer and finally saved as tab delimited text files. Figure 5.4 is a photograph of the experimental set-up described in the diagram of figure 5.3. Each experiment was conducted precisely to the protocol and the apparatus was calibrated only once prior to taking the first measurements. All equipment was kept in a secure dark room at all times.

5.6 : Raw transmission intensity measurements.

The restrained vessels are scanned for periods of 2 and 3 seconds. The vessel's internal lumen was stretched from 100 μm to 350 μm in steps of 50 μm through a external controller to the micrometer setting. The collected raw transmission intensity profiles were saved as text delimited files. Each vessel was scanned to the precise protocol procedures. All transmission spectra were obtained from two different probe positions. One set of data is collected with the probe head placed in a stationary position and the other when the probe head position is moved 20μm for each 50 μm stretch in a horizontal direction. The probe head was clamped to the horizontal and vertical stages, with resolutions of 0.01mm and 0.05 mm

The arterial vessels were dissected from groups with extreme hypertension, borderline hypertension and normal cases. All arterial preparations were from the same mesenteric bed position with some arteries obtained from sister branching. All vessel transmission measurements were repeated twice with different exposure times with the ambient temperature noted as 24°C with fluctuations of ±0.5°C. Furthermore the experiments were conducted on vessels sustained in a temperature controlled environment of 37° C. A series of 680 individual transmission spectra were obtained
for the 16 specimens over the stretch range of 100 μm to 350 μm. Presented in figures 5.5 and 5.6 are the normalized transmission spectra of the 8 week group of a hypertensive and a normal rat specimen taken from a stationary position of the probe. The normalized transmission spectra were obtained for an exposure time of 3 seconds. All the vessels are from paired specimens, mounted simultaneously in the same wiremyograph bath, under similar physiological conditions.

Figures 5.5 and 5.6 show the normalized transmission spectra for a paired 8 week normal and 8 week hypertensive specimen. From these figures we cannot clearly distinguish the effect of the stretch on the disease vessel in comparison to the normal vessel. This trend is repeated in other normalized transmission observations of the 8 week pairs used in this study. This result is similar to the result for the slide study where there were no significant changes in spectra shape for the 8 week group.

Figure 5.5  Normalised transmission spectra (T) of specimen G262 (BP = 106 mmHg, media thickness = 27.23 μm, control = 8week (normotensive)) The spectra is for the stretch range of 100 μm to 350 μm in steps of 50 μm. [ Experiment on : 31.10.96]
Figures 5.6 and 5.7 show the normalized transmission spectra for a 4 week normal and a 4 week hypertensive arterial specimen. A comparison between figures 5.6 and 5.7 clearly shows that the hypertensive artery has a much steeper normalized transmission profile. This trend is repeated in the remaining normalized transmission spectra of the 4 week pairs in this study. The steep trend in figure 5.8 suggests a high absorption in the range of 400nm to 540nm. This is the blue-green range of the whitelight spectrum. This steep trend in figure 5.8 is not noticeable in figure 5.7 of the normal artery.

Furthermore, it is noted from observations of the normalized transmission spectra of figures 5.5, 5.6, 5.7, and 5.8 that the 4 week pairs all have very steep profiles in comparison to the 8 week pairs. Li and Schiffin noted that most of the effects of arterial structural changes happen in the early stages of hypertensive disease.
Figure 5.7 Normalised transmission spectra (T) of specimen G292 (BP = 135.4 mmHg, media thickness = 25.32 μm, control = 4week (normotensive)). The spectra is for the stretch range of 100 μm to 350μm in steps of 50 μm. [Experiment on : 12.11.96]

Figure 5.8 Normalised transmission spectra (T) of specimen G295 (BP = 231 mmHg, media thickness = 46.7 μm, clipped = 4week (hypertensive)). The spectra is for the stretch range of 100 μm to 350μm in steps of 50 μm. [Experiment on : 12.11.96]
The normalised transmission spectra of the 4 week group of vessels shown in figure 5.7 and 5.8 show similar results to the findings in the slide study for the 4 week group, where the spectra shape is consistent but the experimental methods are very different. For the tissue samples, arterial vessels were slabs of passive material and for the wiremyograph study the vessels are sustained in a controlled environment with tensions applied to mimic in-vivo conditions.

5.7 : Discussion.

One advantage of the wiremyograph is obtaining the morphological measurements which can be carried out using a light microscope. These measurements would greatly help in enabling a better understanding of the normalised transmission spectra because comparisons can be made between the two strains based on these measurements. Unfortunately, since the morphological measurements are done under minimum tension conditions, it means that these measurements are only estimates of the true vessel dimensions. Furthermore these measurements were carried out only once before conducting the transmission measurements. This was due to the probe positioning in the wiremyograph set-up, making it impossible to carry out microscopic morphological measurements during the stretching of the vessel.

Figure 5.9 is a micrograph of a hypertensive vessel clearly showing the smooth muscle cells orientation, which is circumferential, around the previous position of the wires (marked X in figure 5.9). During experimentation the probe would be positioned somewhere between points a and b along the centre of the vessel away from the wires. In this arterial example the transmission scan could possibly contain spectroscopic information for the normal arterial tissue, bits of fat tissue and even another small artery. This is a major disadvantage of this set up. There is little chance of positioning the probe tip at a location and then having micrographs of the same location taken later. Furthermore, the opportunity to accumulate morphological information through micrographs was not made available to me.
A micrograph of a hypertensive vessel 1μm thick, sliced from a stored arterial tissue sample after mounting on the wiremyograph.

The vessels in the wiremyograph are scanned from above, across the lumen, stretched by 50 μm and the procedure is repeated. I estimate that the vessel is in continuous tension, longitudinally and circumferentially, therefore the possibility of tissue moving is very slight, unless the wires used to mount the vessels themselves moved. Clearly in figure 5.9, we can see the wall at the edges has become abnormally thin because of the tension produced during stretching and perhaps mainly due to the effects of the steel wires.

By observing figure 5.9 again, we can observe the condition of the artery edge, upon which all the morphological measurements on the vessel dimensions are based. This suggests that any improvement into the optical design for the wiremyograph would not result in a practical benefit because of the abnormal thinning of the vessel as it is stretched. This means, the wiremyograph technique would never give a good morphological measurement of the vessels dimensions.

A profoundly better solution is to use a more physiological method such as the perfusion myograph system, where the arterial preparation’s lumen is pressurised to dilate and contract. This would eliminate the effects of the wires on the edges of the
vessels. Another sought after improvement is the ability to track the position of optical probes in relation to the artery. This would help in positioning the detection fiber tip to a clear and clean section of the artery, away from pieces of attached tissue. The new device constructed to provide these improvements is the fibermyograph system and is described next in Chapter 6.
Chapter 6. Arterial vessels in the fibermyograph system.

6.1 : Introduction.

The development of the fibermyograph described in this chapter allowed for the first time spectroscopy and video microscopy of small blood vessels in vitro possible. This new device is built on the concept of a perfusion myograph system described in section 2.5.2 of chapter 2. The pressure myograph system in chapter 2 is expensive, but the fibermyograph system in this chapter cost about £30 to build and it worked relatively well during experimentation.

The structure of the fibermyograph is similar to the wiremyograph, where a bath is used to provide a controlled environment. The vessel is mounted and suspended, held in position with a pair of cannulae placed in the bath. The fibermyograph has external connections to an automated perfusion pressure system. As the perfusion pressure is increased, the changes in vessel dimension are recorded with video microscopy almost synchronously to taking the transmission spectra. A frame grabber is used to obtained image frames which are analysed by applying programs written in MATLAB and run in a UNIX environment.

The advantage of the fibermyograph system is that it allows the monitoring of the condition of the vessel's walls and helps in the positioning of the fibers used to measure the transmission spectra. Furthermore, this system allows the vessel to perform in a more physiological environment than that of the wiremyograph or slide system.
6.2 : The fibermyograph.

The basic design of the fibermyograph is illustrated in figure 6.1. It is made of perspex to make it inert to the chemicals used in experimentation and is also easy to clean. The fibermyograph bath houses a single pair of identical glass cannulae (1.2 mm in diameter) with sharp tips, used to hold and enable perfusion of the arterial sample in suspension. One of the cannulae is attached to a micrometer to enable movement of the vessel in the horizontal plane and the other is stationary. The stationary cannula is attached to an automated perfusion system through a 2mm diameter tygon plastic tubing and the other cannula end of the tube is plugged.

There is a glass window directly below the cannulae to enable light microscopy to be carried out on the vessel. The bath is covered with a cover to protect the arterial sample. This cover has a protruding window which is placed into the krebbs solution in the bath, preventing image distortion caused by the movement of the krebbs liquid.

The fibers (glass and plastic) are attached to perspex tweezers with Araldite glue and the ends are polished. The D-tweezer is the detection tweezer and has a stainless steel tip 1.5mm in diameter fitted with a glass fiber with a 50µm core. The stainless steel tip is used because it helps to protect the fragile glass fiber and it is removable to allow cleaning of the fiber tip. The LS-tweezer is the light source tweezer and it is fitted with a 1mm diameter core polymer fiber.

The tweezers are clamped onto the system and are moved using conventional helical grooved nuts, 3mm in diameter with each complete turn giving approximately 1.5 mm in horizontal height. The micrometer is a helical nut with similar hardware specifications as the grooved nuts. The only purpose these nuts were used in the prototype fibermyographer, is due to cost, where precision micrometers are expensive. Therefore the system could be improved if these nuts are replaced with precision devices for future application.
The fibermyograph is fitted with inlets and outlets to enable the flow of heated water to circulate around the bath and the water is heated continuously by an external heating system. The heated water is used to sustain a constant temperature of 37 °C of the fibermyograph bath containing the krebbs solution. The bath was gassed with 95% O₂ and 5% CO₂ to achieve a pH of 7.4, which is similar to in-vivo conditions. Figure 6.2 is a photograph of the completed fibermyograph jig with all the external tube fittings.

Figure 6.1 Schematic diagram with a top view of the fibermyograph jig. A technical drawing is given in Appendix 6.
6.3 : The preparation.

In this study, 5 arterial vessels were obtained from 2 hypertensive rats and a single normal rat. The mean blood pressures from the two strains were 202mmHg and 135.5mmHg and the mean weight for the adult female wistar rats was 338.5 grams for the hypertensive and 275 grams for the normal. The 3 rats had blood pressure ranging from a high level of 212mmHg, a middle level of 192mmHg and a normal level of 135.5mmHg. The arterial preparations were 1 to 2 mm in length and dissected from the 3rd order branch of the mesenteric resistance artery. The dissected arterial vessels had the attached adipose tissue removed with a dissection forcep and this procedure was done using a microscope. The isolated artery is finally placed into the fibermyograph bath, submerged in room temperature Krebs saline solution.
6.4: Mounting the vessel into the fibermyograph jig.

Before the vessel is mounted, the fibermyograph jig is placed under a light microscope and the cannulae tips are checked to make sure they are intact. The fibermyograph bath comprises of a 119ml bath chamber which houses a pair of identical cannulae. The cannulae are first pumped with Krebbs solution and checked to make sure they are not blocked. The fibermyograph bath is filled with Krebbs saline solution and the stored arterial preparation is placed in the bath. One end of the vessel is slipped onto a cannula using a dissection forcep and tied onto the tip of the cannula with a single hairlike surgical nylon thread (20μm). The same procedure is followed at the other end. Figure 6.3 shows one end of the cannula where the vessel has been secured.

![Nylon thread (20μm)](image)

**Figure 6.3** One end of the arterial vessel shows the nylon thread (20μm) tied to secure the vessel to the sharp glass cannula.

6.5: Experimental Apparatus.

Figure 6.4 shows a block representation of the entire apparatus used to obtain the video microscopy and transmission spectra of the arterial vessel contained in the fibermyograph jig. The vessel is mounted into the fibermyograph bath of the jig and
placed on a microscope with a black and white CCD camera attached to the eyepiece. The Sony CCD camera has a 0.66 inch CCD array with a line resolution of 756 by 581. The CCD camera is attached to a monitor and during experimentation the images are recorded with a JVC video recorder.

The end of the stationary cannula is attached to an automated perfusion control system. This perfusion system allows external control of the perfusion to the arterial vessel. The other cannula which is attached to the micrometer, is blocked to create a continuous set pressure and disallow flow across the vessel. As the perfusion pressure increases, the vessel lumen expands and experiences circumferential tension. This pressure causes the arterial walls to expand and this in turn, deforms the vessel’s straight formation. The micrometer attached to one end of the vessel is moved to stretch the vessel and enable it to return to a straight formation.

The vessel is then perfused to check if it is well secured and intact. If the vessel is not secured or contains fractures, the increase in perfusion pressure will not dilate the lumen of the vessel at all. The water inlet and outlet of the fibermyograph was connected to an external heating system which contains a pump and heating blocks. The pump is to enable the circulation of heated water around the outside of the bath. The bath temperature was kept to an approximate of 37°C during experimentation and monitored with a digital lab thermometer.

The detection tweezer (D-tweezer) end is fitted with an SMA connector which was then attached to the spectrometer. The light source tweezer (LS tweezer) of the fibermyograph was coupled to the unfiltered white light source. The light source was monitored with a power meter and noted to be 13.2 with fluctuations of ± 0.1 dBm during all the experiments. The entire set-up from the initial checks to calibration is performed to the protocol in Appendix 5. Figure 6.5 is a photograph of the entire apparatus.

Figure 6.6 is a photo of the side view of the fibermyograph jig.
Figure 6.4 Basic block diagram of the entire experimental apparatus used in the fibermyograph study.

Figure 6.5 A photograph of the entire experimental set-up
6.6: Obtaining the transmission and morphological measurements.

The microscope lens used in the experiments was a high performance lens manufactured by Bausch and Lomb. The lens magnification was set at 8x1.5x1 and it had a numerical aperture of 0.15. The lens was positioned approximately to the centre of the suspended vessel held in the fibermyograph bath. The fiber tweezers were then carefully positioned in the bath next to the vessel according to protocol. Figure 6.7 shows an image of a vessel with the D-tweezer contained in the stainless steel sheath to protect the glass fiber.
After the positioning of the fiber tweezers, the vessel is made to undergo morphological changes induced through the external perfusion system producing pressures shifts in the range of 30mmHg to 80mmHg. When these changes have settled, the vessel image is obtained and transmission measurements are carried out on the vessel.

Figure 6.8A is an example of a vessel prior to the spectroscopic scan and figure 6.8B is an example of the same vessel shown in figure 6.8A being illuminated. The transmission spectra is measured on the vessel in figure 6.8B and the morphological measurements are obtained from information stored in the image in figure 6.8A obtained through video microscopy. These images are analysed, by applying a simple algorithm to find the outer and inner edge positions of the blood vessel and the method is detailed in section 6.8.

Figure 6.8B shows that the illuminated vessel looks slightly larger than the vessel in figure 6.8A. This is because of light being scattered by the circular vessel and this creates a blooming effect.
Figure 6.8  

A An image of vessel h13, hypertensive vessel, at pressure 50mmHg just prior to the transmission measurement as seen in figure 6.8B.

B Vessel h13 as seen in figure 6.8A, seen illuminated with the light source as the transmission spectra is obtained.

6.7: Normalised transmission spectra.

An example of the normalised transmission spectra for the fibermyograph system for the vessel N1 (BP=135.5mmHg) is given in figure 6.9 and vessel H1 (BP=212mmHg) is given in figure 6.10. All the vessels in the fibermyograph study are from the 8 week group of hypertensive and normal specimens. The profile of the normalised spectra for both figures is broadly similar. This is coherent to the results obtained in the slide and wiremyograph study for the 8 week group. In the wavelength region of 695nm to 795nm of both figures there is more detail than either the wiremyograph or the slide normalised transmission spectra. The exposure time to obtain the raw transmission spectra for the vessels in the fibermyograph system was in the region of 0.6 to 1 second.
Figure 6.9  Normalised transmission spectra (T) of G384 (BP=135.5mmHg, weight=275grms, normal. Spectra is shown for perfusion pressure range of 30 to 80 mmHg in 10mmHg steps. Experiment on 22.4.97

Figure 6.10  Normalised transmission spectra (T) of specimen G378 (BP=212mmHg, weight=353grms, hypertensive. Perfusion pressure range of 30 to 80mmHg in steps of 10mmHg. Experiment on 18.4.97
6.8: Morphological measurement of the arterial vessels.

Figure 6.11 is an example of an image obtained from the fibermyograph system apparatus. Since the vessels lie horizontally in the image, a column of the image is equivalent to a vertical profile of the vessel at a pixel point. Figure 6.12 is a column profile of the image in figure 6.11. The edges found by applying the algorithm described in section 6.9 is equivalent to edges found in this perpendicular profile of the vessel. This profile is W-like as seen in figure 6.12, and it is very consistent for all of the images. In these experiments all the vessels have well defined lumen and wall edges. The profile in figure 6.12 indicates that the lumen is circular because it is symmetrical in shape.

From figure 6.11, it is clear that the surrounding external background is of very great contrast with the external vessel walls making it relatively easy to find the external wall edge. The difficulty in these images lies in finding the correct internal wall edge rather than the possible impurities in the lumen. The algorithm presented in section 6.9 works on one frame of image at a given time. Each frame is obtained using an image grabber and saved as a 8 bit .TIFF (Tagged Image File Format) image file. For
each vessel there is a sequence of 6 frames, each taken from a different perfusion pressure value ranging from 30mmHg to 100 mmHg. Every image is a individualistic profile of the vessel.

![Graph showing intensity profile I](image)

Figure 6.12 Column number 153 of vessel n23 showing the distinctive W-like intensity profile I (arbitrary units), under perfusion pressure of 50mmHg

Five vessels were studied and a compilation of approximately 260 individual images with the corresponding transmission spectra were obtained. The entire processing time for each frame is approximately 30 seconds and is performed using MATLAB in a UNIX environment.

6.9 : The workings of the algorithm to detect vessel edges.

It is computationally expensive to apply an image processing algorithm to an entire image. In this thesis, the algorithm is applied to only one fifth the whole image and is centred to the image. This area was selected because it sufficiently covers the detection fiber tip surface and location of the fiber tip during experimentation. The algorithm begins by reading the selected image area into MATLAB and enhances it
with a look up table routine. The enhanced image is then thresholded to separate the image into different categories with similar common characteristics. The threshold image is derived to define the vessel edges and the edges are located. The location points are used to calculate the morphological parameter values such as wall thickness and lumen diameter. Finally all the results are stored in text format. Sections 6.9.1 to 6.9.6 describe the working of the algorithm with respect to one example.

6.9.1 : Reading the image into matlab.

The saved tiff format file is loaded into a MATLAB environment to apply functions to read and store the value of each pixel in an image as a grey level, in a matrix formation of 256 by 256. A smaller matrix of 51 by 250 pixels, is selected from the range between pixel 103 and pixel 153 in the j column. This pixel range is centred to the image and this range was selected based on the assumption that it would cover the area of the detection fiber tip sized at 50μm. The selected image area for vessel n23 is displayed in figure 6.13.

Figure 6.13 A slice of the image (vessel n23), displaying the area used for the algorithm extracted from pixel 103 to 153 of image 6.11.
6.9.2: Look Up Table (LUT) routine.

A look up table routine is a means to enhance the image without adding new information. In this thesis the LUT transformation is a linear transformation. When applied to an image, it changes the grey levels of all the pixels in the image extending it from the interval of maximum pixel value and minimum pixel value to the interval 0 to 255 for each pixel as seen in figure 6.14. This transformation will enhance the contrast and the brightness of the image. Furthermore it makes all the frames obtained from the images of different vessels to have the same pixel intensity in comparison.

![LUT values vs Pixel number](image)

Figure 6.14 Shows the typical intensity profiles after the LUT is performed on the intensity profile in figure 6.12 for vessel n23.

6.9.3: Thresholding.

Thresholding is used to separate the pixels of an image into different categories which share some common characteristic. If the pixel is greater than the threshold it is
assigned a 'one' (in this algorithm it is the background) and if it's lower than the threshold it is assigned a 'zero' (wall tissue). The threshold limit is set to 85 which is approximately 1/3 of 255 and this is a good estimate of the tissue pixel value. Here the image is divided into two categories 'one' and 'zero'. Thresholding transforms an image into a binary image. Figure 6.15 gives the result of the image with the thresholded edge values (in white).

Figure 6.15 The image in figure 10 after thresholding showing the edges distinctively (Y1,Y2,Y3,Y4).

6.9.4 : Derivation.

Taking a derivative of the image after thresholding, enhances the current image edge by acting like a high frequency filter. Since the vessel edges are well defined by the thresholding process this is a relatively easy task to do. A comparison of the edges is done to find the maximum and minimum values and this information allows the edges to be detected. The first minimum is the first edge and the first maximum is the second edge. To find the other two edges, the algorithm goes to the other end of the
derived result and does the same comparison to obtain the third edge and fourth edge as seen in figure 6.16.

![Graph showing derivation points](image)

**Figure 6.16** The derivation points for the thresholded image in figure 6.13 of vessel n23.

### 6.9.5: Calculate.

Examining the shape in figure 6.12 for the intensity profile of vessel n23, we can deduce that it has a circular shape because it is centred to the lumen since the profile is symmetrical. With the position of the 4 edges found as shown in figure 6.15 the morphological measurements are calculated per unit length as follows:

- **Wall thickness**
  \[
  \frac{(Y_4 - Y_3) + (Y_2 - Y_1)}{2}
  \]  \[(6.1)\]

- **Lumen diameter**
  \[
  Y_3 - Y_2
  \]  \[(6.2)\]

- **Wall cross sectional area**
  \[
  \pi \left( \frac{Y_3 - Y_2}{2} \right)^2
  \]  \[(6.3)\]
The calculated wall thickness results for vessel n23 shown as an image in figure 6.15 are given in figure 6.17.

![Graph showing wall thickness results](image)

**Figure 6.17** The calculated wall thickness values given in relative pixel values for the image in figure 6.15 for each pixel in the j-column.

**6.9.6 : Store**

All the data after analysis of the vessel’s morphological parameters obtained by applying equation 6.1, 6.2, and 6.3 are stored into a text format file. This is a very flexible format for it allows data to be transferred into other software applications with minimum hassle. This algorithm is sufficient enough to be used because it finds the edges for each column and this corresponds to one point in 51 points. Finding an average at the end result gives a good indication of the vessel wall thickness.
6.10 : Discussion.

The fibermyograph made possible for the first time, the study of isolated sections of resistance arteries in vitro by transmission spectroscopy conducted simultaneously with video microscopy. The method presented in this chapter is the preferred method since it is more physiological than the wiremyograph or the slide. The mounted vessel had no alien substances in the lumen during the measurement of the transmission spectra, other than Krebbs solution, and the measuring fiber was placed very close to the vessel's walls. The condition of the measured section of the wall could be seen on the monitor.

The vessel's wall thickness varies along the length of the vessel and since the transmission spectra were measured at an approximate centre of the vessel, it was relevant to use this section of the vessel to obtain the morphological values. Furthermore, the vessel's morphological measurements were not calibrated to a standard graticule to give an absolute measurement. This was caused by the fibermyograph jig base being too thick (12mm), which in turn made the position of the focused image of the vessel short. When the graticule was placed on the microscope stage, the lens could not focus onto the graticule. This could be easily overcome by reducing the base width of a future fibermyograph jig. To cut the perspex base in this prototype would have risked having the base of the jig crack, so this was not attempted.

The advantage of the system is that the obtained images gave more realistic wall thickness and lumen diameter values in relation to the increased pressure from the perfusion system. This was not possible with the wiremyograph system. The images analysed in this chapter have clearly defined lumens and external walls. In cases where the lumen of the vessel is hard to define such as when vessels are contracting, the algorithm described in section 6.9 would be insufficient to detect the edges. This is because as vessels contract the walls create an extremely dark, small lumen. The algorithm for a contracting vessel would have to be based on an edge tracking algorithm.
The transmission spectra of vessels contracting and held in suspension in the fibermyograph system would be of interest. Analysing vessels which contract under a standard perfusion pressure would provide some results which can give us a better understanding of the mechanisms at work during contraction. These results would help in modelling the vessel’s reactions to contractions. We have, in the past done a case study, conducted on images of a contracting vessel (drug induced) and a semi-empirical optical model of light transmission was developed. The findings and results of this case study were published in Applied Optics and Optoelectronics (Sept. 1996) and are presented in Appendix 7. They are not included in the main body of this thesis as they address a clearly separate issue and do not affect the main study.
Chapter 7. Empirical model for the normalised transmission spectra.

7.1: Preparation of the averaged normalised transmission spectra.

The normalised transmission data are averaged over a wavelength of 3nm for the range of 340 to 840 nm to reduce the data number from 1024 points to 170 points via a simple averaging program written in MATLAB. These averaged data points amount to approximately 17% of the measured normalised transmission data points. The averaged data are stored as a text file and loaded into a non-linear fitting package offered in a statistical package (STATISTICA version 5).

7.2: The model.

The averaged normalised transmission spectra generally form a bell shape, as shown in figure 7.1 and a suitable fit to the averaged normalised transmission spectra is a logistic function. By subtracting two logistic functions the expression in equation 7.1 fits to the averaged normalised transmission spectra and is expressed as:

\[ T = \frac{A1}{(1 + \exp(-Cl(x - OFFSET1)))} - \frac{A2}{(1 + \exp(-C2(x - OFFSET2)))} \]  

(7.1)
where \( x \) is wavelength, \( A_1, A_2 \) describe the height of the spectra, \( C_1, C_2 \) the two slopes of the spectra and \( \text{OFFSET}_1, \text{OFFSET}_2 \) the wavelength values where the gradient of the spectra is maximum. The positioning of these parameters is shown in figure 7.1 and in this figure it is obvious that the parameters from the model in equation 7.1 are capable of characterising 6 major properties which describe the spectra shape.

![Model: \( T=0.4/(1+\exp(-0.057*(x - 410)))-0.1/(1+\exp(-0.18*(x - 750))) \)](image1.png)

Figure 7.1 An example of the model showing the positioning of the parameters to be extracted from the transmission equation 7.1.

### 7.3: Fitting the model to normalised transmission spectra.

The averaged normalised transmission data are fitted to equation 7.1 using the Quasi-Newton non-linear estimation fit. In the Quasi-Newton algorithm the fitted parameters are obtained through 1\textsuperscript{st} and 2\textsuperscript{nd} order derivation of the raw data points and
this information is used to follow a path towards a minimum of the loss function. The loss function used for fitting is a weighted least squares estimate and is defined as the sum of squared deviations about the predicted values. The disadvantage of the Quasi Newton method is it tends to deviate if the start values are not sufficiently close to the root. Fortunately, in these fits the start parameters in table 7.1 gave extremely good fits to most of the normalised transmission spectra analysed in this thesis.

<table>
<thead>
<tr>
<th>A1</th>
<th>0.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>C1</td>
<td>0.1</td>
</tr>
<tr>
<td>OFFSET1</td>
<td>400</td>
</tr>
<tr>
<td>A2</td>
<td>0.1</td>
</tr>
<tr>
<td>C1</td>
<td>0.1</td>
</tr>
<tr>
<td>OFFSET2</td>
<td>800</td>
</tr>
<tr>
<td>convergence value</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

Table 7.1  Start parameters for the normalised transmission spectra fits

The transmission measurements for the arterial specimens for each of the studies were done repeatedly and each spectrum was fitted. Table 7.2 is a guide to the number of modelled fits for the averaged normalised spectra obtained from the slide, wiremyograph and fibermyograph experiments.

<table>
<thead>
<tr>
<th>Experiment type</th>
<th>number of arterial specimens</th>
<th>normal</th>
<th>hypertensive</th>
<th>number of individual fits to the obtained individual spectra</th>
</tr>
</thead>
<tbody>
<tr>
<td>slides</td>
<td>18</td>
<td>9</td>
<td>9</td>
<td>54</td>
</tr>
<tr>
<td>wiremyograph</td>
<td>20</td>
<td>10</td>
<td>10</td>
<td>659</td>
</tr>
<tr>
<td>fibermyograph</td>
<td>5</td>
<td>2</td>
<td>3</td>
<td>246</td>
</tr>
</tbody>
</table>

Table 7.2  The overall number of fits performed to the arterial specimens acquired under different experimental procedures.
7.4: Example fits of the averaged normalised transmission spectra of specimens.

In figure 7.1, the profile of the spectra is an ideal case fit for the logistic function in equation 7.1. The spectra in this thesis have a range of shapes and two of these spectra are selected to demonstrate the effect and quality of the fit by equation 7.1. From the two examples one spectrum was obtained from a typical spectrum that represents the quality of the fit for most of the spectra in this thesis and the other is a worst case fit that represents about 2% of the total fits. Both example spectra were obtained from normal arterial specimens, one from a 8 week group and the other from a 4 week group. Figure 7.2 is an example of the fitted averaged normalised transmission spectrum of a normal 4 week group (rat G292) arterial vessel obtained from the wiremyograph pool of data.

![Graph showing normalised transmission spectra](image)

Figure 7.2 The fitted averaged normalised transmission spectra (N30) of G292 for 350μm stretch. Specimen is a normal 4 week control. The fit is a continuous line and the data are represented as circles.
The shape of the averaged normalised transmission spectrum in figure 7.2 is a very
typical example of the transmission profiles of most young normal arterial specimen
data. Figure 7.3 shows the observed versus the predicted values of this fit from the
spectra values of specimen G292 shown in figure 7.2.

![Observed versus Predicted Values](image)

Figure 7.3 The observed versus the predicted values for the normal specimen fit.
Calculated mean standard deviation = 0.011.

From figures 7.2 and 7.3 it can been seen that the equation 7.1 fits to the averaged
normalised transmission spectra profile quite well. It is obvious that the model is
appropriate for the data since in figure 7.3, most of the data points tend to fall onto or
near a straight line. This means the predicted values are very close indeed to the
observed values. This observation is true for most of the averaged normalised
transmission spectra in this thesis.

The next step is to determine how precise the fit is in obtaining the fitted parameters
A1, A2, C1, C2, OFFSET1 and OFFSET2. To estimate this precision we consider the
repeated extraction of the fitted parameters using data obtained by adding small
random fluctuations to a given data set. This method is used to estimate precision in
the absence of a large pool of real data sets. The procedure is carried out as follows.

The mean standard deviation of the observed to the predicted values of the fit is
multiplied to a normally distributed randomly generated noise (values ranging from 0
to 1). This noise is added to the spectral values shown in figure 7.2. The new
spectrum with the noise added is fitted with the fit equation 7.1 and figure 7.4 is an
example of the noise added spectrum.

![Figure 7.4](image)

Figure 7.4  The fitted averaged normalised transmission spectra (T) with added
noise for spectra G292 in figure 7.2.

This procedure is repeated 70 times to the initial spectra values (shown in figure 7.2),
each time adding new random generated noise. The standard deviations of the fitted
parameters from the various noise added spectra are given in table 7.3.

<table>
<thead>
<tr>
<th></th>
<th>A1</th>
<th>A2</th>
</tr>
</thead>
<tbody>
<tr>
<td>OFFSET1</td>
<td>0.0009</td>
<td>0.002</td>
</tr>
<tr>
<td>C1</td>
<td>0.002</td>
<td>C2</td>
</tr>
<tr>
<td></td>
<td>OFFSET2</td>
<td>1.1</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td></td>
</tr>
</tbody>
</table>

Table 7.3  The standard deviation values of the parameters for a typical case fitted
spectra.

77
In table 7.3 the precision of the fit for the parameters is good with deviations of 1.1 nm for OFFSET2 and 0.5 nm for OFFSET1. Figure 7.5 shows an example of a type of spectral profile that is quite unique and represents a problem for the logistic function in equation 7.1. The spectral profile in figure 7.5 is for a 8 week normal artery (rat G265) and is an extreme case.

![Graph showing averaged normalised transmission spectra profile of a normal 8 week (rat G265)](image)

Figure 7.5 Averaged normalised transmission spectra profile of a normal 8 week (rat G265).

We repeat all the procedures for the example spectral profile of G292 in figure 7.2 for the spectral profile of G265 shown in figure 7.5. The observed to the predicted values plot is shown in figure 7.6 and from this figure it is obvious that the mean standard deviation of the observed to the predicted values for this particular example is larger than for figure 7.3. Figure 7.7 shows the fitted noise added transmission profile for figure 7.5 and here the data distribution in the wavelength range of 700nm to 800nm is affected relatively more than the other wavelength values.
Figure 7.6  The observed to predicted values for the example in figure 7.5. Calculated mean standard deviation value is 0.02208.

Figure 7.7  The noise added transmission profile for the spectra shown in figure 7.5.
The standard deviation values for the fitted parameters of the spectrum with the added noise for rat G265 in figure 7.5 for worst case is given in table 7.4.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0.02</td>
</tr>
<tr>
<td>C1</td>
<td>0.006</td>
</tr>
<tr>
<td>OFFSET1</td>
<td>4.4</td>
</tr>
<tr>
<td>A2</td>
<td>0.03</td>
</tr>
<tr>
<td>C2</td>
<td>1.5</td>
</tr>
<tr>
<td>OFFSET2</td>
<td>44.2</td>
</tr>
</tbody>
</table>

Table 7.4  The standard deviations of the parameters for the worst case fit spectra shown in figure 7.5.

In table 7.4 the standard deviation for the parameter OFFSET1 is 4.4nm and the parameter OFFSET2 is 44nm. The large value for OFFSET2 is due to the problem of locating this parameter. In this particular example the reconstructed spectra with noise destroy some of the spectral characteristic shape, especially in the wavelength range of 700 nm to 840 nm. Table 7.4 has highlighted the fact that OFFSET2 is a parameter which is not as reliable as parameter OFFSET1. Nevertheless, this example is a worst case and the deviations for the parameters in table 7.4 are representing a very small number of spectra in the study presented in this thesis.

7.5 : Discussion.

Section 7.4 has highlighted a problem for the model given by the expression in equation 7.1. From figure 7.5 of the 8 week grouped rat, it can be observed that there is a weakness of the model i.e. it is not capable of modelling the shape of the spectral profile for the range of 450 to 650nm. This is because in typical profiles of normal arteries the spectral shape does not slant like in this example. In order to model the spectral profile in figure 7.5, the function in equation 7.1 needs more parameters to
improve the fit. This is not a simple problem and a better solution would involve a complicated equation.

In typical situations the model (equation 7.1) does fit sufficiently to the spectra and the obtained parameters do provide the basic information of the spectral shape's individual characteristics. For example, figure 7.7 shows a severely sloped spectral profile of a 4 week hypertensive specimen (G295) fitted with the expression in equation 7.1. As seen in figure 7.7, the fit is clearly capable of modelling a spectral shape with a large positive slope as well.

![Graph showing fitted normalised transmission spectra](image)

Figure 7.7 The fitted normalised transmission spectra for G295 for stretch 200µm. Hypertensive specimen 4 week clipped. The fit is a continuous line and the data are represented as circles.

Obviously there is much more information displayed in the normalised transmission spectra than described by the six extracted parameters of equation 7.1. Nevertheless, the extracted parameters do correspond to the important features in the normalised transmission spectra and we believe these features are sufficient in this study to enable us to distinguish the difference between hypertensive and normal arterial spectra.
Chapter 8. Statistical analysis of vessel morphology.

This chapter presents the statistical analysis of the morphological data of the arterial vessels to the physiological parameter blood pressure. The morphological parameters are media cross sectional area and media to lumen ratio. All statistical comparisons are based on Pearson’s correlation formula. All parameters were obtained from the slide, wiremyograph and the fibermyograph study as presented in chapter 4, 5 and 6 of this thesis.

8.1 : Pearson’s correlation equation.

Pearson’s linear correlation measures the association between variables that are ordinal or continuous rather than nominal. The linear correlation coefficient $r$ (also called the product moment correlation coefficient, or Pearson’s $r$) for pairs of quantities $(x_i, y_i), i=1,2,\ldots,N,$ is given by the formula.

$$r = \frac{\sum (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum (x_i - \bar{x})^2} \sqrt{\sum (y_i - \bar{y})^2}}$$

where, $\bar{x}$ is the mean of the $x_i$’s, $\bar{y}$ is the mean of the $y_i$’s. The value of $r$ lies between -1 and 1, inclusive. $r$ takes on a value of 1 when the data points lie on a perfect straight line with positive slope with $x$ and $y$ increasing together. If the data
points lie on a perfect straight line with negative slope, \( y \) is decreasing as \( x \) is increasing, then \( r \) has the value -1. A value of \( r \) near zero indicates that the variables \( x \) and \( y \) are uncorrelated.

When a correlation is known to be significant, \( r \) is a conventional way of summarising its strength. The value of \( r \) can be translated into a statement about what residuals (root mean square deviations) are expected to be, if the data is fitted to a straight line by the least squares method. Unfortunately \( r \) is not a good parameter to decide whether an observed correlation is statistically significant or whether one observed correlation is significantly stronger than another. The reason is that \( r \) ignores the individual distributions of \( x \) and \( y \).

The reliability of the correlation result can be quantitatively represented by the \( p \)-level. The \( p \)-level represents the probability of error that is involved in accepting an observed result as valid. If the \( p \)-level is 0.05, this indicates that there is a 5% probability that the correlation relationship between the variables is untrue. The confidence limit is a region that contains a certain percentage of the total probability distribution. For example a confidence limit of 95% means that 95% of the normal distributed data values fall in the region of the measured value.

8.2: Listing and description of the morphological parameter abbreviation.

The morphological parameters of interest are stated in table 8.1 along with the abbreviation and units used in the correlation tables presented in the following sections.
<table>
<thead>
<tr>
<th>Morphological parameter</th>
<th>Expression in correlation table</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rat type</td>
<td>TYPE</td>
<td>describes the type of arterial specimen as hypertensive or normal.</td>
</tr>
<tr>
<td>Arterial Clip</td>
<td>CLIP</td>
<td>the duration of the insertion of the annealed clip to the kidney artery in the rat and the control. (weeks).</td>
</tr>
<tr>
<td>Blood pressure media</td>
<td>BP</td>
<td>blood pressure (mmHg)</td>
</tr>
<tr>
<td>media thickness.</td>
<td>MEDIA</td>
<td>the obtained media layer thickness of the arterial specimen. (μm)</td>
</tr>
<tr>
<td>media to lumen ratio.</td>
<td>MEDLUM</td>
<td>the media to lumen diameter ratio for the arterial specimens in the wiremyograph.</td>
</tr>
<tr>
<td>Wall cross sectional area.</td>
<td>MED_CSA</td>
<td>the calculated circular wall cross sectional area. (μm²) for the wiremyograph.</td>
</tr>
<tr>
<td>Averaged cross sectional area of vessel wall.</td>
<td>A_CSA</td>
<td>the averaged cross sectional area of the arterial specimen from the fibermyograph.</td>
</tr>
<tr>
<td>Wall to lumen ratio.</td>
<td>WAL_LUM</td>
<td>the wall to lumen diameter ratio of the arterial specimen for the fibermyograph system.</td>
</tr>
<tr>
<td>Pressure settings.</td>
<td>PRESSURE</td>
<td>the pressure settings for the fibermyograph system (mmHg)</td>
</tr>
</tbody>
</table>

Table 8.1 Description and abbreviation of the morphological parameters used in this thesis.
8.3: Blood pressure distribution of specimens.

Figure 8.1 is a histogram showing the distribution of the arterial blood pressure of the specimens from the fibermyograph and wiremyograph study. In figure 8.1, it is clear that the distribution is bimodal (have 2 peaks) which means the sample is not homogenous, but that its elements came from two different populations, each more or less normally distributed. The main reason the distribution is bimodal is because there are no elements in the blood pressure range of 140mmHg to 160mmHg since this range is considered to be a dubious hypertensive or normal.

Figure 8.1 A histogram of the wiremyograph (light grey blocks) and fibermyograph (dark grey blocks) data showing the distribution of data based on blood pressure.
The physiological parameter blood pressure is the key indicator of the state (hypertensive or normal) of a specimen. Figures 8.2 and 8.3 show changes in blood pressure levels for 4 arterial specimens over periods of 8 and 4 weeks after incision of the clip on the renal artery.

Figure 8.2   The blood pressure shifts of specimens from the 8 week group.

Figure 8.3   The blood pressure shifts of specimens from the 4 week group.
In figure 8.2 for the 8 week clipped vessel the blood pressure level tends to fall and rise after the 7th week. This looping shape in figure 8.2 for the 8 week group of hypertensive specimen suggests an adaptive process is happening over time. In figure 8.2 of the normal 8 week arterial specimen, the blood pressure levels rise at the 7th week. This is because as the artery ages the arterial blood pressure level of the specimen increases. In figure 8.3 for the 4 week hypertensive plot, the blood pressure level rises steadily. The normal 4 week specimen in figure 8.3 shows an almost constant arterial blood pressure level over time. The trends presented in figures 8.2 and 8.3 are mirrored in most of the specimens used in the study presented in this thesis.

8.4: The relationships of morphological and physiological parameters of the slide, wiremyograph and the fibermyograph.

8.4.1: Introduction.

An increased media to lumen ratio has been reported in mesenteric, renal, hepatic and cerebral resistance vessels of hypertensive vessels in comparison to normal vessels. The media to lumen ratio is a good parameter in determining the state of the arterial vessel. Research into the subcutaneous resistance artery in humans showed an increase in the media to lumen ratio, but the cross sectional area of the walls remained similar between the two strains (normal and hypertensive).

The cross sectional area of the arterial wall tissue of a vessel gives an indication on whether the increased media to lumen ratio changes are caused by an increase in the wall tissue (hypertrophy) to the same lumen size or a remodelling of the wall tissue (hyperplasia) to a smaller lumen size.
The media is a layer of tissue in the arterial wall. For the slide and wiremyograph data, the media thickness is measured using a light microscope, focused on the outer edges of the wires so that the wall was seen in a longitudinal section. By applying interference contrast optics, the media within the wall could be distinguished clearly. Therefore, media thickness is a measured morphological value and it is not the wall thickness of the arterial specimen. The media to lumen ratio is obtained by dividing the media thickness with the lumen diameter of the arterial specimen for the wiremyograph and slide data. The media cross sectional area is a calculated parameter obtained with equation 5.3 on page 43 of this thesis for the wiremyograph and the slide data.

For the fibermyograph system, the wall thickness and lumen diameter are obtained through video microscopy and is described in chapter 6 of this thesis. The wall to lumen ratio is given as the division of the wall thickness to the lumen diameter of the arterial vessel. The wall thickness includes the media, intima and adventitia layers of the arterial wall. Since the vessels studied here are small resistance vessels, the majority of the wall is composed of the media layer. The wall cross sectional area for the fibermyograph data is obtained with equation 6.3 on page 68 of this thesis.

8.4.2 : The result of the 1 kidney 1 clip Goldblatt model.

The model used to create the hypertensive and normal controls is the one kidney one clip Goldblatt model. Korsgaard 33 showed that the increase in media thickness seen in this model for the mesenteric artery is mainly due to smooth muscle cell hypertrophy. He found the lumen diameter of 15 week clipped mesenteric vessels was reduced by 16%, media thickness was increased by 58%, media to lumen ratio increased by 89% and the media cross sectional area was increased by 39% for the hypertensive vessels compared to the normal vessels. Li and Schiffrin 35 in their work with 4 to 6 week clipped mesenteric arteries, found results of lumen diameter reduced by 20%, increased media to lumen ratio by 85% and the media cross sectional area increased by 21%.
Figure 8.4 shows the media cross sectional area for the 4 week and 8 week clipped specimens of the wiremyograph pool of data. In this figure, the 8 week group shows no significant increase in the media cross sectional area suggesting that this group's media has no or little growth process. In figure 8.4, the 4 week group shows clearly that the cross sectional area has increased between the hypertensive and normal arterial specimens.

<table>
<thead>
<tr>
<th>BP</th>
<th>MED_CSA</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLIP: 4</td>
<td>CLIP: 8</td>
</tr>
<tr>
<td>TYPE: N</td>
<td>TYPE: H</td>
</tr>
<tr>
<td>80</td>
<td>120</td>
</tr>
<tr>
<td>6000</td>
<td>10000</td>
</tr>
<tr>
<td>BP vs. MED_CSA</td>
<td></td>
</tr>
</tbody>
</table>

Figure 8.4 The media cross sectional area of the two groups [8week (clip:8) and 4week (clip:4)] for the two strains [hypertensive (type:h) and normal (type:n)].
Table 8.2 gives the mean media, lumen and media cross sectional area values with the standard deviations for the data in figure 8.4. From table 8.4 the 4 week vessels have an increase of 98% of media cross sectional area and an increase of 65% in media to lumen ratio. The 8 week vessels have an increase of 20% media cross sectional area and an increase of 20% of the media to lumen ratio.

<table>
<thead>
<tr>
<th></th>
<th>4 week group</th>
<th>8 week group</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEDIA</td>
<td>44.8±3.3</td>
<td>25.6±0.7</td>
</tr>
<tr>
<td>LUMEN</td>
<td>143±13</td>
<td>135±21</td>
</tr>
<tr>
<td>MED_CSA</td>
<td>25556±2698</td>
<td>12901±1828</td>
</tr>
<tr>
<td></td>
<td>29.4±4.3</td>
<td>24.6±3.9</td>
</tr>
<tr>
<td></td>
<td>137±37</td>
<td>139±42</td>
</tr>
<tr>
<td>MED_CSA</td>
<td>15206±2961</td>
<td>12478±3681</td>
</tr>
</tbody>
</table>

Table 8.2 morphological characteristics of the mesenteric artery from the wiremyograph data.

The result in this section is similar compared to the findings of Korsgaard for 15 week clipped vessels for this model. The rats in his study were 15 weeks old and showed an increase of 39% media cross sectional area as opposed to the 20% of the 8 week clipped rats in this thesis. The trend in figure 8.4 and table 8.2 show there is media hyperplasia where there is a remodelling of the media after growth in the early stages. The changes in the early stages shown by the 4 week group are similar to the results of Li and Schiffins for the same model. This increased media cross sectional area for the 4 week clipped is a trend for media hypertrophy.

Therefore a comparative study of the effects of hypertension based on the morphological, physiological and spectroscopic parameters in this thesis for the two groups of 8 week and 4 week clipped vessels would not be conclusive unless they are separated. One group (4week clipped) shows hypertrophy trends while the other (8
week clipped) shows remodelling trends. These results are also observed for the slide pool of data for the 4 week and 8 week clipped. For the fibermyograph the specimens were 8 week clipped vessels only.

8.4.3 : Correlations for the wiremyograph and slide study.

This section looks at the relationship between the media to lumen ratio (MEDLUM) and media cross sectional area (MED_CSA) to blood pressure. Table 8.3 shows the result for the slides and wiremyograph pool of data. In this table the results for the 4 week group are quite similar for both wiremyograph and slide. The figures to follow are for the wiremyograph pool of data.

<table>
<thead>
<tr>
<th>Slide group</th>
<th>MED_CSA</th>
<th>MEDLUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 week</td>
<td>0.89</td>
<td>0.96</td>
</tr>
<tr>
<td>8 week</td>
<td>0.03</td>
<td>0.36</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Wiremyograph</th>
<th>MED_CSA</th>
<th>MEDLUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 week</td>
<td>0.82</td>
<td>0.98</td>
</tr>
<tr>
<td>8 week</td>
<td>0.42</td>
<td>0.27</td>
</tr>
</tbody>
</table>

Table 8.3 The correlation values for the relationship between media cross sectional area, media to lumen ratio to blood pressure for the slide and wiremyograph study for 4 week group and 8 week group of specimens.

Figures 8.5 and 8.6 show the relationship between arterial media cross sectional area and media to lumen ratio as a function of blood pressure for the two clipped groups (8week and 4week). Figure 8.7 shows the relationship of media cross sectional area to media to lumen ratio.
Correlation (4 week) : $r = .82$
Correlation (8 week) : $r = .42$

Figure 8.5 Media cross sectional area as a function of blood pressure. Triangles are for 4 week group and circles are for 8 week group.

Correlation (4 week) : $r = .98$
Correlation (8 week) : $r = .27$

Figure 8.6 Media to lumen ratio expressed as a function of blood pressure. Triangles are for 4 week group and circles are for 8 week group.
In figures 8.5, 8.6 and 8.7, the 4 week group shows a higher correlation than the 8 week group of clipped and control specimens. These figures support and show the distribution of the data points shown as correlation results in table 8.3. We conclude from these results that blood pressure is linearly correlated to media cross sectional area and media to lumen ratio for the 4 week group.
8.4.4: Correlations for the fibermyograph.

The correlations for the fibermyograph study are considered separately because the morphological parameters were obtained through video microscopy and are relative pixel values for each image. The morphological parameters were not calibrated to a standard value. This is not a problem as far as correlations are concerned because the correlation values show only the magnitude of a relationship.

Figure 8.8 shows the wall to lumen ratio as a function of blood pressure and there is almost no correlation. This means that the vessels have very similar wall and lumen values which is what the 8 week group in the wiremyograph showed.

![Figure 8.8](image-url)  
**Figure 8.8** The relationship of the wall to lumen ratio to blood pressure for 8 week clipped vessels.
Figure 8.9 shows the relationship of the wall cross sectional area as a function of blood pressure and it is a negative correlation. The cross sectional area of the vessel decreases by approximately 43% between the two strains. There are 3 possible reasons why the wall cross sectional area could decrease. First, this suggests that the hypertensive vessels have experienced some form of modifications which has a negative growth index as compared to the normal arterial vessel. Second, the calculated wall cross sectional area is not accurate, even if the wall to lumen ratio shows similar tendencies as the 8 week group from the wiremyograph. Third, that hypertensive disease could affect the entire wall rather than the media layer only. Taken together, figures 8.8 and 8.9 suggest that the vessels experience different morphological responses to hypertension.

Figure 8.9  The relationship between the cross sectional area of the vessel wall to blood pressure.
Figure 8.10 gives the relationship between the wall cross sectional area as a function of the wall to lumen ratio. The distribution of the data points shows that as the wall to lumen ratio increase the wall cross sectional area increases. This result is different to the 8 week group result of the wiremyograph because of the wall cross sectional area distribution shown in figure 8.9.

![Graph](image)

**Figure 8.10** The wall cross sectional area as a function of wall to lumen ratio.
Figure 8.11 gives the relationship between pressure and the mean normalised cross sectional area (CSA*) of the arterial wall for the hypertensive and normal vessels. From this figure the hypertensive vessels seems to be more responsive to the pressure changes than the normal arterial specimens. Furthermore, the hypertensive plot tends to rise in CSA* and the normal plot tends to decrease in CSA* when the pressure is increased.

In figure 8.11, there is a temporary stable CSA* period for the hypertensive plot. This pattern seems to indicate that after some pressure steps the vessel seems to settle (act passive) and as the pressure increases to a point, the artery reacts again. Furthermore the slope of the pressure-CSA* for the normal arterial plot is smoother than the hypertensive arterial plot. This is probably due to the higher modulus of elastin in the normal vessels than the hypertensive arteries. A further study is necessary to enable us to understand these special mechanisms which seem to be a property of the vessels studied here.

![Figure 8.11](image)

Figure 8.11 Mean normalised cross sectional area (CSA*) of all hypertensive (H) and normal (N) vessels for the fibermyograph pool of data as a function of perfusion pressure.
Figure 8.12 displays the relationship of the wall to lumen ratio as a function of the perfusion pressure. This figure shows a linear relationship for all the arterial vessels as the perfusion pressure increases. It is interesting that as the perfusion pressure increases both strains of vessels show fewer deviations of the wall to lumen ratio for the same amount of data. This trend suggests that vessels reach a stable distension position at relatively low perfusion pressure values for the two strains.

![Graph showing correlation](image)

Correlation: $r = -0.53$

Figure 8.12  The wall to lumen ratio as a function of perfusion pressure for all the data with a regression fit of 95% confidence.

Nevertheless, a conclusive result can only be obtained after a much more detailed study of arterial vessels in future and the tension applied to the arterial vessels must be considered. For the perfusion system the tension is both radial and longitudinal and it is important to assess the effects of both tensions on the structure of the artery. A definite future possibility would be to model the effects of the perfusion pressure mathematically and this model would give us a better understanding of arterial vessel structure.
In future, the fibermyograph system could integrate compact motorized translation stages such as those manufactured by Newport Inc. to position the micrometer attached to one end of the vessel. This would help improve the positioning of the micrometer as the vessel lengthens and shortens.

8.5 : Discussion.

The results in this section show that the specimen samples used in this study experience different morphological changes to the arterial wall which produced two main groups, the 4 week group showing media growth and the 8 week group showing media rearrangement and adaptation. The media to lumen ratio and media cross sectional area of these two groups have different increments as well. The 4 week group has higher correlation values because there are very obvious changes in the media. The 8 week group has a smaller correlation since it has adapted to the higher levels of blood pressure.

The morphological parameters from the wiremyograph and slide study were obtained prior to the actual experiment and with the fibermyograph system, the determined vessel morphological parameters are observed in-vitro at all times with video microscopy.

The results from the fibermyograph system show that the mechanisms that arise from the changes in perfusion pressure in the vessel’s wall are very complicated. The artery seems to experience several stages of responses to the shifts in the perfusion pressure. These details are very interesting, but in this thesis we are more interested in remote sensing of vessels in-vitro, being either hypertensive or normal, so the mechanical feature is not looked into in depth.
Chapter 9. Statistical comparison of the remotely sensed spectral features with vessel morphology.

9.1 : Introduction.

In this chapter the remotely sensed spectral features extracted from the normalised transmission spectra are statistically analysed to the vessels blood pressure level, media to lumen ratio (wall:lumen) and media cross sectional area (wall cross sectional area). The fitted averaged normalised transmission spectra parameters are $A_1$, $C_1$, OFFSET1, $A_2$, $C_2$ and OFFSET2.

In Chapter 7, we concluded that these spectral parameters are sufficient to describe the major characteristics of the vessel’s spectral shape. In this chapter the comparative study is based on the results in Chapter 8 which were shown to be supported by previous published studies.

All statistical comparisons in this chapter are based on Pearson’s correlation formula presented in Chapter 8 as equation 8.1. For the slide study the groups are not discussed separately since the morphological data is insufficient. The wiremyograph results are presented as two groups, 8 week and 4 week clipped and the fibermyograph results are based on only the 8 week group since all specimens were
clipped for 8 week only. The abbreviations used in this chapter are stated in table 8.1 section 8.2 of chapter 8.

9.2: The statistical results of the slide study.

Table 9.1 gives the correlation results of the slide pool of data. The morphological parameters for the 1st and 2nd order artery were not obtained and are therefore not included in table 9.1. Only the 3rd order artery results are shown in table 9.1.

<table>
<thead>
<tr>
<th>3rd order</th>
<th>BP</th>
<th>MED_CSA</th>
<th>MEDIA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>r</td>
<td>p</td>
<td>r</td>
</tr>
<tr>
<td>A1_3</td>
<td>-0.23</td>
<td>.37</td>
<td>-0.64</td>
</tr>
<tr>
<td>C1_3</td>
<td>0.03</td>
<td>.91</td>
<td>-0.37</td>
</tr>
<tr>
<td>OFFSET1_3</td>
<td>0.07</td>
<td>.77</td>
<td>0.27</td>
</tr>
<tr>
<td>A2_3</td>
<td>-0.09</td>
<td>.72</td>
<td>-0.52</td>
</tr>
<tr>
<td>C2_3</td>
<td>0.08</td>
<td>.74</td>
<td>0.43</td>
</tr>
<tr>
<td>OFFSET2_3</td>
<td>0.06</td>
<td>.82</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Table 9.1: The correlation results of the slide pool of data for the 3rd order branch of the arterial vessel for the 4week and 8 week group. Highlighted data are for p<0.05. Total number of specimens=18.

From table 9.1, the most prominent result is the correlation relationship between the parameters A1 to the cross sectional area of the arterial media. The correlation is negative, which means as the parameters A1 and A2 increase in value, the cross sectional area of the vessels is expected to decrease. This is an expected feature for the slide data because the parameters A1 and A2 are effectively indicating the tissue thickness. The transmitted light through the tissue would obviously be affected by the absorption and the scattering parameters of the tissue.
Unfortunately, actual morphological measurements were never carried out on all the branches of the mesenteric arterial specimens presented here. Nevertheless, it is still possible to find a relationship of the effects of hypertensive disease on the samples based on speculations with the sole physiological parameter, blood pressure (BP).

Figure 9.1 shows the relationship of parameter OFFSET1 with the parameter blood pressure for all 3 orders of the artery. In figure 9.1, the 1st order artery is shown as a dotted line with circular points, the 2nd order artery as a uninterrupted line with square points and the 3rd order artery as a long dashed line with triangular points.

Figure 9.1 The relationship of blood pressure to parameter OFFSET1

In figure 9.1 the parameter OFFSET1 shows a shift between the two strains of hypertensive and normal for the 1st and 2nd ordered arteries. The sloping trend of the arteries of 1st order branch is more than the 2nd and 3rd order branch. This sloping trend is also seen for the spectral parameter C1. This result means that it's not necessarily the 3rd order mesenteric artery that displays the tendencies of the hypertensive disease. The 1st order and 2nd order arteries can and do display these tendencies just as well.
9.3: Statistical results of the wiremyograph study.

In this section, we look at the results of the wiremyograph study. Here we break down the correlation results into their respective groups of 4 week clipped in table 9.2 and 8 week clipped in table 9.3.

<table>
<thead>
<tr>
<th></th>
<th>BP</th>
<th>MED_CSA</th>
<th>MEDLUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>-0.44</td>
<td>-0.19</td>
<td>-0.48</td>
</tr>
<tr>
<td>C1</td>
<td>-0.67</td>
<td>-0.38</td>
<td>-0.75</td>
</tr>
<tr>
<td>OFFSET1</td>
<td>0.78</td>
<td>0.47</td>
<td>0.84</td>
</tr>
<tr>
<td>A2</td>
<td>-0.50</td>
<td>-0.28</td>
<td>-0.55</td>
</tr>
<tr>
<td>C2</td>
<td>0.31</td>
<td>0.07</td>
<td>0.46</td>
</tr>
<tr>
<td>OFFSET2</td>
<td>0.29</td>
<td>0.14</td>
<td>0.44</td>
</tr>
</tbody>
</table>

Table 9.2 Correlations values for the 4 week clipped and control group. Highlighted correlations (r) are significant at p<0.001, N=144.

First we analyse the results in table 9.2. The parameters OFFSET1, C2 and OFFSET2 all show one trend in the correlation value which is positive to BP, MED_CSA and MEDLUM. The parameters A1, C1 and A2 all show a negative correlation relationship, which means the parameters decrease as the parameters BP, MED_CSA and MEDLUM increase.

<table>
<thead>
<tr>
<th></th>
<th>BP</th>
<th>MED_CSA</th>
<th>MEDLUM</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0.08</td>
<td>0.12</td>
<td>0.07</td>
</tr>
<tr>
<td>C1</td>
<td>-0.02</td>
<td>-0.35</td>
<td>0.22</td>
</tr>
<tr>
<td>OFFSET1</td>
<td>0.11</td>
<td>0.53</td>
<td>-0.26</td>
</tr>
<tr>
<td>A2</td>
<td>0.28</td>
<td>0.29</td>
<td>0.03</td>
</tr>
<tr>
<td>C2</td>
<td>-0.30</td>
<td>-0.29</td>
<td>-0.07</td>
</tr>
<tr>
<td>OFFSET2</td>
<td>-0.31</td>
<td>-0.35</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Table 9.3 Correlations values for the 8 week clipped and control group. Highlighted correlations (r) are significant at p<0.001, N=456.
In table 9.3, the highest correlation value is for the relationship of OFFSET1 to media cross sectional area (MED_CSA).

From table 9.2 and 9.3 it is obvious that the parameter OFFSET1 is important in the determination of the differences between hypertensive and normal arterial vessels.

A closer look at the relationship of the parameters OFFSET1 and C1 to the morphological parameters BP, MED_CSA and MEDLUM would be more interesting than the parameters OFFSET2, C2, A1 and A2 for both tables 9.2 and 9.3. The parameters A1 and A2 describe the height of the middle and tail end of the spectra respectively.

For all the figures to follow the triangular points with the dotted fit line is for 4 week clipped and control arterial specimens and the circular dots with the uninterrupted fit line is for 8 week clipped and control specimens. Clipped specimens are hypertensive and the control specimens are normal.
Figure 9.2 shows the relationship of OFFSET1 as a function of BP for both groups. In this figure, the 4 week group correlates well to OFFSET1. There is a distinct difference in OFFSET1 values between the highly hypertensive at wavelength range 480 ± 20 nm and the normal arteries at wavelength 420±20nm. The 8 week group in this figure shows a low correlation in comparison to the 4 week group and this is probably due to the distribution of the data for the 8 week group which overlaps between the two strains.

Figure 9.2 OFFSET1 as a function of blood pressure for the two clipped and control groups.
Figure 9.3 shows the relationship between OFFSET1 and media cross sectional area. In Chapter 8 we found that the media cross sectional area for the two groups increased in the hypertensive vessels in comparison with the normal vessels. The 8 week group increased by approximately 20% (mean value) and the 4 week group increased by approximately 98% (mean value). Figure 9.3 shows a positive linear correlation for both the groups. Here we see that as the shape of the transmission spectra is more sloped from the range of 380nm to 520nm, the media cross sectional area increases.

Figure 9.3  OFFSET1 as a function of media cross sectional area for the two clipped and control groups.
Figure 9.4 gives the relationship between media to lumen ratio and OFFSET1. In this figure the 4 week group shows a high correlation and there are distinctive groups similar to the distribution in figure 9.2. One group is the highly hypertensive and the other is the moderately hypertensive and normal artery. The 8 week group shows a mixed distribution and is not conclusive in differentiating the two strains.

![Figure 9.4](image-url)

Figure 9.4 OFFSET1 as a function of the media to lumen ratio for the two clipped and control groups
The parameter $C1$ describes the gradient of the slope for the averaged normalised transmission spectra. Figure 9.5 shows the relationship between blood pressure and $C1$. In this figure the 4 week group shows a good correlation and the 8 week group shows almost zero correlation. The almost zero correlation could mean two things. First, there exists no relationship between the gradient of the slope at OFFSET1 to the morphological parameter blood pressure. Secondly, the parameter $C1$ is not being detected accurately enough to describe the state of the vessel.

Figure 9.5 $C1$ as a function of the blood pressure of the arterial specimens for the two clipped and control groups.
Figure 9.6 shows the relationship between media cross sectional area to C1. Here the parameter C1 shows that as the media cross sectional area decreases the gradient of the normalised transmission spectral slope increases. This supports the relationship between OFFSET1 and media cross sectional area shown in figure 9.3.

![Graph of C1 as a function of the media cross sectional area for the two clipped and control groups](image_url)
Figure 9.7 shows the relationship between media:lumen ratio and C1. This figure shows that as the gradient of the normalised transmission spectra increases the media lumen ratio decreases for the 4 week group. The 8 week group shows an opposite relationship. This supports the findings in figures 9.4 of OFFSET1. Taken together, parameter C1 and OFFSET1 are showing similar tendencies in their relationship to blood pressure, media:lumen ratio and media cross sectional area. This means that as the normalised transmission spectra tends to have a larger slope with a higher OFFSET1 value in the wavelength region of 340nm to 540nm, it is likely that the vessel is hypertensive.

Figure 9.7 C1 as a function of the media to lumen ratio for the two clipped and control groups
9.4: The statistical result of the fibermyograph system.

The number of arteries studied in this section are only 5 with 3 different blood pressure values. It is probable that the correlation results for the blood pressure are biased, but the relationship of blood pressure to the fitted parameters is still examined here to illustrate the effects, rather than highlight the correlation value itself. Table 9.4 gives the correlation value of the fitted parameters to the morphological parameters (blood pressure, perfusion pressure, wall to lumen ratio (WAL_LUM), average cross sectional area per pixel (A_CSA)) for the entire set of data from the fibermyograph system.

In table 9.4, the correlation relationship results for the 8 week specimens are slightly different from the wiremyograph 8 week group. This is due to the fact that the morphological values in this section are obtained differently from the wiremyograph, as explained in section 8.4 of Chapter 8. In table 9.4, only parameter A1 and A2 are correlated to a significant level to perfusion pressure. This means that the sloping shape of the normalised transmission spectra is not related to the changes in perfusion pressure, but the height of the normalised transmission spectra is.

<table>
<thead>
<tr>
<th>Parameter</th>
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<th>WAL_LUM</th>
<th>A_CSA</th>
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<td>0.37</td>
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<td>-0.17</td>
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<tr>
<td>C1</td>
<td>0.32</td>
<td>0.15</td>
<td>-0.47</td>
<td>-0.43</td>
</tr>
<tr>
<td>OFFSET1</td>
<td>-0.45</td>
<td>-0.16</td>
<td>0.56</td>
<td>0.73</td>
</tr>
<tr>
<td>A2</td>
<td>-0.36</td>
<td>0.30</td>
<td>-0.25</td>
<td>0.32</td>
</tr>
<tr>
<td>C2</td>
<td>-0.56</td>
<td>-0.05</td>
<td>0.37</td>
<td>0.49</td>
</tr>
<tr>
<td>OFFSET2</td>
<td>0.51</td>
<td>-0.18</td>
<td>0.05</td>
<td>-0.55</td>
</tr>
</tbody>
</table>

Table 9.4 Correlations results for all the fibermyograph data which includes the normal and hypertensive vessels. Highlighted values are for p<0.001, N=236.
Figure 9.8 shows the highest correlation value in table 9.4 for the relationship between $A_{\text{CSA}}$ and OFFSET1. This relationship is similar to the relationship for the wiremyograph data of the 8 week group. As the normalised transmission spectra's slope increases, the wall cross sectional area also increases. Figure 9.8 shows a better distribution for the wall cross sectional area data as compared to the groupings in figure 9.3 of the media cross sectional area data of the wiremyograph.

Figure 9.8  Average wall cross sectional area as a function of OFFSET1.
Figure 9.9 shows the relationship between blood pressure (BP) and the parameter OFFSET2. In this figure, all the 5 vessels have distinct groups. A clear difference is between the hypertensive vessel (H1) at 212mmHg with a mean OFFSET2 value of 756nm and the normal (N1) vessel at 135mmHg with a mean OFFSET2 value of 734nm. Figure 9.9 shows that some vessels do have a very distinct characteristic normalised transmission spectra which are repeatedly the same shape over different perfusion pressure values.

Table 9.5 gives the mean of the averaged wall thickness, lumen diameter and the wall to lumen ratio values for two arterial specimens (N1 and H1) at the perfusion pressure of 70mmHg. The vessel H1 has approximately half the lumen size of N1 but the wall thickness is quite similar. This makes the wall to lumen ratio value for both these vessels different. These two arteries are chosen to show the effects of perfusion pressure shifts to the obtained spectra shape because the remaining arteries in this section have a similar wall to lumen ratio as N1.
Table 9.5  The mean of the averaged wall thickness, lumen diameter and the wall to lumen ratio.

<table>
<thead>
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<th>arterial specimen</th>
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<th>averaged lumen diameter</th>
<th>wall to lumen ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>H1</td>
<td>16.25</td>
<td>79.17</td>
<td>0.21</td>
</tr>
<tr>
<td>N1</td>
<td>17.15</td>
<td>152.18</td>
<td>0.11</td>
</tr>
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</table>

Figure 9.10 shows the relationship between perfusion pressure and OFFSET1 for the arteries N1 (shown as + sign) and H1 (shown as circles). OFFSET1 describes the wavelength at the point where the positive slope of the transmission spectra is at maximum gradient. In figure 9.10, artery N1 shows a larger shift in wavelength value than artery H1.

![Graph showing the relationship between perfusion pressure and OFFSET1](image)

Figure 9.10  OFFSET1 as a function of perfusion pressure. Circles are H1 and plus signs are N1.
Figure 9.11 shows the relationship for OFFSET2 and perfusion pressure. In this figure the changes in OFFSET2 wavelengths for H1 are very minimal and are centred at 757nm with fluctuations of ±1.5nm and for N1 value is centred at 747nm with fluctuations of ±3nm.

![Figure 9.11 OFFSET2 parameter shifts for artery H1 (circles) (BP=212mmHg) and N1 (plus) (BP=135.5mmHg)](image)

Taken together, figures 9.10 and 9.11 show that the normal artery (N1) and the hypertensive artery (H1) have different reacting arterial mechanisms which change to perfusion pressure increase. Figures 9.10 and 9.11 show that OFFSET2 has a more distinctive relationship with perfusion pressure than OFFSET1, which decreases steadily with increased pressure for both arteries. This leads me to believe that OFFSET2 could be an important parameter in distinguishing the state of the arterial vessel.
Van Bavel \cite{vanbavel2022} showed that when 1st order mesenteric arteries are distended, increase in perfusion pressure induced passive, then elastic effects followed by myogenic responses for normal arteries. These myogenic responses resulted in cross sectional changes which were maintained as long as the pressure level was maintained. Furthermore, he concluded that myogenic responses are induced by wall stress, rather than by distension of the vascular wall.

Figure 9.12 shows the mean wall cross sectional area of artery N1 and H2 as a function of perfusion pressure. In this figure, artery N1 has almost constant mean normalised wall cross sectional area as opposed to H1 at the pressure level range of 50mmHg to 90mmHg. This means both arteries are showing myogenic responses and that H1 is distended especially since H1 has a smaller lumen than N1. Furthermore, H1 is showing passive and elastic effects more often than myogenic instances. This in turn could mean that these two arteries are composed of different materials and that the artery H1 is more rigid than N1.

![Figure 9.12: Mean normalised cross sectional area (CSA*) for H1 and N1 as a function of perfusion pressure.](image)
9.5: Conclusion.

This chapter has shown results that correlate for the first time the arterial vessels parameters such as blood pressure, media to lumen ratio (wall:lumen) and media cross sectional area (wall cross sectional area) to the parameters obtained from the normalised transmission spectra. The parameter OFFSET1 correlated well with the morphological parameters and blood pressure throughout this chapter. In the 4 week group of data there is very obvious differences between the OFFSET1 value for hypertensive vessel and normal vessels. The hypertensive vessel had an OFFSET1 value centred at 490nm with a variation range of ±20nm and the normal arteries were grouped at 420nm with a variation range of ±20nm. For the 8 week group the OFFSET1 value overlapped and was inconclusive.

The morphological values obtained through the fibermyograph system showed distributions that are more representative of the state of the vessel during measurements than either the wiremyograph and slide study. In the fibermyograph system the correlation relationships between fitted parameters to the morphological parameters was shown to be similar to the results in the 8 week group in the wiremyograph system.

In future, it would be very interesting to see all three branch order mesentery arteries in a fibermyograph set-up, where monitoring of the arterial morphology and simultaneous spectroscopy of the vessels can be carried out in-vitro.
Chapter 10. Final Discussion.

In this thesis the model of hypertension is based on the Goldblatt one kidney one clip model. The advantage here is that the control derives from the same inbred strain but has undergone a sham operation. Thus, the mechanisms underlying the development and maintenance of hypertension in the strains should be the same. It represents renal artery stenosis in man, a secondary form of hypertension where activation of the renin-angiotensin system plays a part in bringing about blood pressure elevation.

All vessel studies, described in this thesis, were performed using isolated mesenteric resistance vessels. The mesenteric vascular bed provides an easily accessible source of resistance arteries with varying diameters. These small arteries were chosen because of their role in determining peripheral resistance where alterations in the structure and function of these arteries contributes to the elevated peripheral resistance which is a trademark of hypertension. Furthermore, a great deal of information about resistance vessel abnormalities in hypertensive disease studies have been obtained from in-vitro studies of mesenteric resistance arteries.

In Chapter 2, results from previous studies on hypertensive disease showed that an increase in the media to lumen ratio (wall:lumen) and media cross sectional area (wall cross sectional area) resulted in media growth tendencies. If there is no or little increase in the media cross sectional area but an increase in the media to lumen ratio then the tendency was towards media remodeling. The results in Chapter 8 showed that the specimens studied in this thesis have an initial stage of massive media growth.
and the followed development was towards media remodeling. This result in turn means that there are two different groups of arterial specimens derived from the same hypertensive model. One group is the 4 week clipped vessels and the other was the 8 week clipped vessels. Korsgaard 33 showed media growth tendencies in rats with 15 week clippings which resulted to 39% increase in media cross sectional area. In this thesis the rats clipped to 8 weeks showed 20% increase in media to lumen ratio and media cross sectional area. This could mean that the specimens in this thesis adapt relatively fast and efficiently to the elevated arterial pressure.

The 4 week group in this thesis showed a media to lumen ratio increase of 65% and a media cross sectional area increase of 98% as compared to the normal arteries. Li and Schiffrin 35 worked with 4 to 6 week clipped rats and showed results with increases of 83% in media to lumen ratio and 21% in the media cross sectional area. The percentage figures for the 4 week are quite high and this could be explained by the fact that there were only 7 arterial specimens in this group making the mean values relatively higher.

In Chapter 7, the presented worst case fit example showed a sloping characteristic of the transmission spectra that failed to fit sufficiently in the wavelength range of 500nm to 650nm. This case highlighted a problem in the fit where the logistic equation is unable to model a few transmission spectra (2% of total normalized transmission spectras) because of the shape of the profile. Oraevsky 19 et al. showed that there is a qualitative difference in the spectral range of 530-630nm between the normal and fibrous plaqued aorta. This is approximately in the same range as the slope in the worst case fit presented in Chapter 7. This suggests that there is a possibility that the vessels could have developed other arterial problems than hypertensive disease.

In Chapter 7, we concluded that the obtained fitted parameters were sufficient in describing the major characteristics of the normalised transmission spectra’s shape. The precision of the parameter OFFSET1 was 0.5nm and OFFSET2 was 1.1nm for a
For the worst case scenario the precision of $\text{OFFSET}_1$ was 4.4nm and $\text{OFFSET}_2$ was 44nm. This result shows that the parameter $\text{OFFSET}_1$ is a more reliable parameter in comparison to the parameter $\text{OFFSET}_2$.

Comparison studies in Chapter 9 showed that these fitted parameters are able to describe the state of an arterial vessel as either hypertensive or normal. The parameter $\text{OFFSET}_1$ was consistent throughout this thesis in the correlation results.

In Chapter 9, the results from the 4 week group of hypertensive specimens with high blood pressure values gave $\text{OFFSET}_1$ values in the wavelength range of 490nm with variations of $\pm20$nm. This is somewhere in the middle of the blue green region of the white light spectrum and closer to the yellow region. The $\text{OFFSET}_1$ value for the normal arteries is in the wavelength range of 420nm with variations of $\pm20$nm which is right in the middle of the blue region. It is therefore likely that the 4 week hypertensive artery has a different wall composition compared to the normal wall.

As reviewed in Chapter 2, Meyer $^6$ and Mulvaney $^3$, $^4$ showed that hypertension is a disease similar to the ageing of the artery where there would be an increase in collagen build up and a reduction in the elastin modulus. In Chapter 9, we showed results, based on two arteries with similar wall thickness and different wall to lumen ratios, that show different plasto-elastic behaviour to increased perfusion pressure. We concluded that these differences are based on the fact that the arterial vessels have different wall compositions.

With reference to Chapter 4, the general idea of the obtained transmission spectra profiles for the slide study is shown in figure 10.1. In this instance, the slide probe would have probably measured in-homogenous tissue, pieces of adipose tissue attached to the artery and the glass slides that provide the boundaries. One problem, the arteries in this study had, was that they tended to dry out and eventually die in these conditions. A solution to this problem was to contain the vessel in a controlled environment and this was achieved through the wiremyograph.
Figure 10.1 The style in which the slide transmission profile was obtained.

Figure 10.2 is the general outlay for the wiremyograph system, described in Chapter 5, in obtaining the transmission spectra of the arterial vessel. The probe was positioned to an approximate center of the vessel.

Figure 10.2 The transmission spectra from the wiremyograph system.
One disadvantage of the wiremyograph system was that the measurement point in relation to the vessel dimensions and condition were unknown. Another was that the tension present in the mounted vessel in the wiremyograph bath was in the longitudinal direction only since the vessel was stretched by a single wire movement attached to the micrometer. Furthermore, the wires in the vessel held it in position extremely well, but the tensions applied to the vessel seem physiologically somehow unrealistic.

The wires, threaded in the lumen of the vessel could very possibly damage the endothelium cells, which line the inner vessel wall. Endothelium is known to play a prominent part in the myogenic response of the vessel\textsuperscript{36}. Myogenic responses are important to changes of vessel contractions under in-vivo conditions and is a key factor in determining the overall subtle changes of vessel dimensions. These wires cause further damage to the vessel’s shape by deforming it. This is clearly shown in figure 5.9 on page 50 where the vessels edges thinned out.

The advantage the wiremyograph when compared to other techniques is that it allows the simultaneous study of two small arteries. The fibermyograph is only designed to hold one vessel. This is because the bath designed for the fibermyograph had to be made large to accommodate the fiber tweezers. The bath capacity is approximately 12ml for the wiremyograph and approximately 120ml for the fibermyograph.

Figure 10.3 is the conceptual outlay for the fibermyograph, described in Chapter 6, in obtaining the transmission spectra of the arterial vessel. Here the vessel wall was seen from the vertical position and the condition of the vessel could be determined in the horizontal plane of the vessel. The transmission spectra measurements were performed in the horizontal plane of the vessel and there were no foreign objects between the vessel and the fiber tweezers. There are no boundaries for the fibermyograph system and the detecting probe could be placed close to the artery. During experimentation the entire vessel’s wall could be observed and the morphological measurements of the vessel were obtained from images recorded seconds prior to the transmission measurements.
The main advantage of using the fibermyograph is that it is the most physiologically sound technique as compared with the other two described previously. In the wire myograph, the vessel is under tension during the transmission measurement but the morphological measurements and condition of arterial wall were unknown. Furthermore, the thinning of the wall by stretching can be argued as an unrealistic representation of the physical property of the vessel wall in vivo. On the other hand, the vessel in the fibermyograph experiences near in vivo conditions, where the myogenic responses are induced by the wall stress rather than by distension of the vascular wall. This is shown in the result in Chapter 9 section 9.4. Finally, the slide is totally passive, with no myogenic responses and hardly any stress applied on the tissue.

Another major advantage that the fibermyograph has over the wiremyograph is the ability to track the vessel dimensions as it is scanned. This is a real bonus as far as vessel studies in vitro are concerned.

Figure 10.3 The fibermyograph system general display in obtaining the transmission spectra. T is longitudinal tension and RT is the circumferential tension.
10.1: Future work.

The work presented in this thesis on the new fibermyograph system allows future in-vitro studies for small arterial vessels. The system is physiologically sound and as demonstrated in this thesis, is able to provide simultaneous spectroscopy and video microscopy of vessels. The work presented here should be advanced in the perfusion system’s direction. Some modifications are necessary to the current fibermyograph jig. First, the base of the jig needs to be thinned to an approximate of 0.5mm thick to enable the vessel under examination to acquire relative measurements (in μm) to a standard graticule. Secondly, the tweezers need a micrometer attachment to enable better control and ease in movement. Adding compact motorized translation stages, such as those manufactured by Newport Inc. would improve accuracy in placing the fibers close to the artery and control the micrometer.

The vessels under study should be diversified from the 3rd order mesentery to the 1st and 2nd order mesentery vessels. The main reason is because the composition of the collagen and elastin fibres changes along the branch levels. Different order arteries might show different elasto-plastic tendencies.

Different hypertension models should be studied especially the SHR and WKY model because these models show essential hypertension as opposed to the secondary hypertension studied here. I believe the results obtained through studies with a different hypertensive model such as the SHR and WKY would be very conclusive and would inevitably support the results presented in this thesis.

The fibermyograph examined vessels with set constant perfusion pressure levels increase over a period of time. It would be very interesting to have induced vessel contraction studies of diseased arterial vessels.

During spectroscopy measurement, the vessels were perfused with Krebbs only. It would be very interesting to see transmission profiles of arterial vessels with blood perfused through the lumen. This would not be very complicated because the
fibermyograph system is able to retain a layer of endothelium cells relatively better than any other system currently available. Endothelium ensures that the blood cells do not clot and stick to the vessel interior wall.

The study presented here should be supported with biochemical data as well. For example, it should be supported with studies based on the muscle cell count and results from the synthesis of the components of the vessel under examination in order to obtain the content of collagen, elastin and water percentages.

Finally, the blood pressure levels should be obtained every 5 days and the arteries under examinations should be obtained from specimens showing hypertensive tendencies at early stages. This eliminates the modifications that could happen later and the possibility that other arterial diseases which could affect the vessel.
Chapter 11. Final Conclusions.

This thesis has outlined for the first time the experimental hardware and concepts of measuring raw transmission spectra of small arterial vessel in the wavelength range of 340 to 840 nm. The arterial vessels, obtained from the mesenteric bed were examined under strict protocol and the results obtained from the transmission spectra repeated. The precise methods in obtaining these scans provided normalised transmission spectra that show spectra which are consistent in profile for every artery as shown in chapters 4, 5 and 6.

An empirical model of the normalised spectra was used to extract a set of parameters (A1, C1, OFFSET1, A2, C2 and OFFSET2). Statistical correlation analyses of the morphological and physiological data for the specimens in the slide, wiremyograph and the fibermyograph study were then compared to the fitted individual parameters.

The conclusions I have arrived for the work presented in this thesis are listed as follows:-

1. The newly designed system termed as the "fibermyograph" showed for the first time a method to acquire simultaneously transmission spectra and video microscopy is feasible.

2. It was shown experimentally, that the fibermyograph system is a more physiological adapted system than either the classical wiremyograph or the slides.
3. The classic Goldblatt one kidney one clip model for hypertension does not necessarily produce the expected results of a thickened media of the arterial wall. Older rat arteries with longer clip insertions could have adapted to conform to the increased arterial pressure.

4. The vessels under examination in most of this thesis were the 3rd order mesenteric arteries. As shown by the work with the slides, other order branches show similar responses to hypertensive disease and should be studied further.

5. The empirical model presented in this thesis is capable of describing the transmission spectra relatively well and the extracted parameters correlate well to the morphological parameters of the arterial vessel.

6. The most reliable parameter to correlate the spectroscopic parameters, A1, OFFSET1, C1, A2, OFFSET2 and C2 is the physiological parameter, arterial blood pressure, BP.

7. The wavelength range of interest for the 4 week hypertensive arterial specimen is 490±20nm and for the 4 week normal arterial specimens is 420±20nm. These are the wavelength ranges of interest for detection in the early stages of hypertension.

8. The wavelength of interest for the 8 week hypertensive and normal arterial vessel is overlapping but a further study in the wavelength region of 730nm to 760nm could be conclusive.

9. The changes in the structure of the arterial wall caused by hypertensive disease, however complicated the reasons or causes are, can be assessed remotely through the parameter OFFSET1.
References.


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Hardware specifications.

The detector head: InstaSpec II specifications.

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</tr>
<tr>
<td>max S/N (1 scan)</td>
<td>c. 10000</td>
</tr>
<tr>
<td>dynamic range</td>
<td>22 000:1</td>
</tr>
<tr>
<td>signal resolution</td>
<td>16 bit; 65536</td>
</tr>
<tr>
<td>triggering</td>
<td>internal, external, auto or transient</td>
</tr>
<tr>
<td>synchronisation</td>
<td>internal, external</td>
</tr>
<tr>
<td>typical read noise</td>
<td>c. 6000 electrons</td>
</tr>
<tr>
<td>electron sensitivity</td>
<td>c. 1900 photoelectrons/count</td>
</tr>
<tr>
<td>stabilised heads</td>
<td>106 x 81 x 64 mm</td>
</tr>
</tbody>
</table>

Electro-optical characteristics (typical values).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>dark current (25 C)</td>
<td>typical 0.1pA, maximum 0.3 pA</td>
</tr>
<tr>
<td>response non-uniformity</td>
<td>± 3%</td>
</tr>
<tr>
<td>radiant sensitivity</td>
<td></td>
</tr>
<tr>
<td>600nm</td>
<td>300 mA/W</td>
</tr>
<tr>
<td>200nm</td>
<td>50mA/W</td>
</tr>
<tr>
<td>light flux responsivity</td>
<td>187 µC/J/cm² (per flux intensity at 600 nm)</td>
</tr>
<tr>
<td>diode saturation charge</td>
<td>20 pC</td>
</tr>
<tr>
<td>saturation exposure</td>
<td>107 nJ/cm² (at 600nm)</td>
</tr>
</tbody>
</table>

Thermoelectric cooler.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>temperature settings</td>
<td>30, 25, 20, 15, 10, 5, 0, -5, -10 ° C</td>
</tr>
<tr>
<td>temperature stability</td>
<td></td>
</tr>
<tr>
<td>fluctuations</td>
<td>± 0.1 ° C</td>
</tr>
<tr>
<td>drift</td>
<td>± 0.5/10 ° C</td>
</tr>
</tbody>
</table>
Interface card.

Computer interface
analog to digital conversion
diode read rate

proprietary, full sized ISA compatible
16 bit
12.85 μs/pixel

The grating.

Model Number
Blaze Wavelength(nm)
Type
Groove Spacing (1/mm)
Array Bandpass(nm/inch)
Resolution (nm)
Spectral Range(nm)

77417
500
ruled
400
530
1.0
300-1200
Composition of the Krebbs solution.

Stock Solution

The below is diluted into 5 litres of distilled water to make 'stock solution'

<table>
<thead>
<tr>
<th>Sodium Chloride</th>
<th>138g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium Chloride</td>
<td>6.8g</td>
</tr>
<tr>
<td>Magnesium Sulphate</td>
<td>5.0g</td>
</tr>
<tr>
<td>Potassium Dihidrogen Orthophosphate ( $\text{KH}_2\text{PO}_4$)</td>
<td>2.8g</td>
</tr>
<tr>
<td>Calcium Chloride Dehydrate</td>
<td>7.4g</td>
</tr>
<tr>
<td>Distilled water</td>
<td></td>
</tr>
</tbody>
</table>

To make 2 litres of Krebbs Solution the recipe is as follows:

<table>
<thead>
<tr>
<th>Glucose</th>
<th>2.16g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium Bicarbonate</td>
<td>4g</td>
</tr>
<tr>
<td>Stock Solution</td>
<td>500ml</td>
</tr>
<tr>
<td>Distilled water</td>
<td></td>
</tr>
</tbody>
</table>

add to make 5 litres to the above.

add to make 2 litres to the above.
Protocol for the slide experiments.

1 : Calibration

1.1 Switch on the light source and leave on for at least 30 minutes prior to experimentation. Connect the power meter to the light source and observe readings.

1.2 Cover the light source with blackout material to prevent dark current build up in the array.

1.3 Run the software for data acquisition.

1.4 When light source is stabilised, remove the blackout material.

1.5 Scan for the combination below
   1.5.1. white light source only
   1.5.2. place filter and scan for white light source + filter
   1.5.3. place blackout material and then scan for background

1.6 Find the transmission spectra and find the 3dB points and compare the results with the manufacturer’s 3db point spec for the filter.

1.7 If compared points are different then change the set values in the calibration values in the Insp menu and save the file.

1.8 Run steps 1.5 to 1.7 to check before actual scans are done to the specimens.
2 : Measuring of the slides

2.1 Place the slide with the artery on it in the slide holder and position the probe head very near the encased tissue with the vertical stage.

2.2 Mark the position of the vertical stage.

2.3 With the power meter connected to the detection probe, scan across the vessel, moving the probe head with the horizontal stage.

2.4 Find the best position for the probe by getting an average position which best mirrors the dBm readings with the power meter. Mark the position.

2.5 Place the blackout material and run the data acquisition software. Set the exposure time to 3 seconds.

2.6 Scan with the tissue on the slide.

2.7 Place the blackout material and scan for the background.

2.8 Remove the slide and scan for the light source.
Protocol for the wiremyograph.

1. Calibration for the wiremyograph

1.1  The experimental apparatus is set up as in figure 5.3 on page 44 of this thesis. Switch on the light source and leave for 30 minutes for the light source to stabilise. Connect the power meter to the light source and check readings to stabilise.

1.2  Cover the detector head so that dark current doesn't accumulate before actual experimentation.

1.3  Run the software for data acquisition process [program spec.pgm]

1.4  After half hour, check the power meter to see if light source has stabilised. If so then continue.

1.5  **data acquisition**

   set scanning time to be the same figure for the combinations of 1.5.1 to 1.5.3.

   Measure the changes in light source with power meter to enable standardisation of measurements.

   1.5.1 : measure white light source only
   1.5.2 : measure white light source + filter only
   1.5.3 : measure background only but with the light source box on and covering the light source with an absorber i.e. black cardboard.

1.6  Find the normalized transmission spectra. Run the software excel program, calibrate.xls. Find the 3dB points. Compare with the 3dB points given in the manufacturers specifications.

1.7  If the compared 3dB points between the manufacturer and the transmittance spectra points are different then change the set values in the calibration menu on Insp2.

1.8  Save the new calibration values in file . Run steps 1.5 to 1.7 again and check
the new values. If there is no difference or a very small difference as in 1 nm then the system is satisfactory

2. Positioning of the probe

2.1 Calibrate the set-up in figure with the calibration protocol in section 1
2.2 Position the myograph in the set-up, approximately 10 cm above the collimated light beam centred to the myograph window
2.3 Very carefully by moving the vertical stage place the probe head directly above the vessel in the bath with the probe submerged in krebbs solution
2.4 Connect the detection probe to the power meter
2.5 Mark the location of the probe on the vertical stage
2.6 Set the stretch to 0 nm.
2.7 Find the edges of the wires by moving the probe head. This is simple since the power reading would be lower at these points.
2.8 Set the micrometer to 100 nm to stretch the vessel.
2.9 Move the horizontal stage and position the fibre head 40 \(\mu\)m inside the outer wire from the edge of the myograph head which is static.
2.10 Take the power meter reading.
2.11 Set the micrometer reading to ranges of 100 nm to 350 nm
2.12 Check the power reading and confirm that the readings show a thinning of the wall.
2.13 If not then repeat steps 2.6 to 2.12 until an appropriate position for the probe is found
2.14 Mark the position of the probe on the horizontal stage
2.15 Stretch the vessel several times and confirm readings of the power meter as to the best position for the probe.

3. Finding the saturation level
3.1 Put the vessel through the different stretches ranging from 100 to 350 nm first over a few times.

3.2 After the series of stretch the vessel to 350 nm in diameter and select exposure time at random to find the saturation levels

3.3 Through trial and error choose appropriate exposure time

4. Measurements

4.1 Set the myograph to the required stretch

4.2 Run the data acquisition software, spec2.pgm

4.3 Check that the micrometer setting on the spectrograph is set to 200 nm value, the grating wavelength.

4.4 For the measurements on the vessel, repeat till finish
   4.4.1 : Set the stretch value for the vessel
   4.4.2 : With the probe positioned stationary scan for the stretches.
   4.4.3 : Scan the background for the stretch by placing a black cardboard on the light source.
   4.4.4 : Set the stretch to 100 nm and repeat measurements[4.4.1 to 4.4.3] with the probe position moving horizontally 20 \( \mu \)m for each different stretch
   4.4.5 : Change the exposure time for the software and repeat steps 4.4.1 to 4.4.4.

4.5 Very carefully remove the vessel using a pair of forceps.

4.6 Check the probe positioning

4.7 For the measurement of the light source, set the stretch tongs to the required range of stretch and scan for the exposure times.

4.8 Cover the white light source with the cardboard and get the background data.
Appendix 5

Protocol for the measurement of the fibermyograph system set-up.

1 : Initial check of the equipment

1.1 Check if the video recorder works with the tape inserted.
1.2 Place the blackout material to cover microscope set up.
1.3 Switch on the power supply to the microscope and test the focus and set it to 8x1.5x1.0.
1.4 Use only base light on the microscope to obtain a reasonable image onto the monitor.
1.5 Check the fiber tweezers are in prime condition.
1.6 Check the fiberscope end is positioned onto the slit properly by visually verifying the positioning. Check using real time data acquisition mode and observe the fluctuations picked up when connected to the light source. It should show real time intensity to about 1200 pixels.
1.7 Use the power meter to check the white light source fiber giving approximately 3.4dBm reading.
1.8 Check the cannulae are not blocked or broken at the tips for the fibermyograph.

2 : Calibration

2.1 Switch on the light source and leave on for 30 minutes before experimenting.
2.2 Connect the power meter to the power source.
2.3 Insert the fiber tweezers into the fibermyograph jig, very carefully positioning the emitting plastic fiber to face the measuring glass fiber.
2.4 Place jig under the objective of the microscope.
2.5 Connect the emitting fiber to the light source and the measuring fiber to the
fiberscope and detector.

2.6 Clamp the jig to the microscope stage.

2.7 When source light stabilises (check the power meter) position the filter at the centre of the two tweezers.

2.8 Scan this sequence for a period of 1 sec each.
   2.8.1 : The filter + light source.
   2.8.2 : The light source only by removing the filter very carefully without touching the optical tweezers.
   2.8.3 : Block the light source with the slit and scan the background.

2.9 Find the transmittance spectra and compare the 3dB points of the spectrum to the manufacturers 3dB points.

2.10 Change the set-up in Insp2 if the calibration wavelength is different and save the calibration file and repeat steps 2.8 to 2.9 till satisfied.

3 : Initial vessel set-up.

3.1 Place artery into the myograph jig.

3.2 Place the jig on the microscope stage and clamp it.

3.3 Supply oxygen to vessel bath through oxygen tube on the jig.

3.4 Connect the pressure control motors to the jig tubing connected to the cannulae holding the vessel.

3.5 Set the temperature control systems to circulate warm water at 37 degrees.

3.6 Place the objective of the scope above the artery to be scanned.

3.7 Use the monitor to check the condition of the artery.

3.8 Step up the pressure and observe if the artery is secured to the cannulae well and if there are any ruptures in the artery if the pressure steps up.

3.9 Check the tweezers tips condition and positioning in the bath. Place the tweezers near the vessel without touching it.

3.10 The transmitting plastic fiber connected to the light source is has a slit between the two fiber and this should be closed to prevent dark current build up in the array of the spectrometer.
3.11 If there are no problems encountered then leave the vessel to familiarise to the environment for 15 minutes before experimenting.

4: Positioning of the fibers.

4.1 Cover the microscope with the blackout material and shut off all light sources from the microscope.

4.2 Connect the measuring fiber to the light source at the slit point and position the tweezer by moving to a position till the light beam illuminates the vessel wall.

4.3 Remove from the light source and connect the slit point to the transmitting fiber (plastic) and illuminate the vessel wall and clamp it.

4.4 Connect the measuring fiber to the power meter.

4.5 Increase the perfusion pressure and move the tweezer to a position where the power readings are coherent to changes of pressure.

4.6 When satisfied with the positioning of the fiber clamp it.

4.7 Check the monitor to obtain a clear image by placing the bath window on the fibermyograph jig.

4.8 Step through the perfusion pressure by varying the lumen diameter and leave for a few minutes before actual vessel measurements.

5: Actual experimental scan.

5.1 Start video recording.

5.2 Step up the pressure to the artery and observe the bending of the artery. Use the lateral micrometer connected to the cannulae to stretch it longitudinally.

5.3 Run up the pressure scale in 10mmHg steps to obtain a reasonable step size and observe the changes in the power reading.

5.4 Secure all fiber connections to the light source and the spectrometer.

5.5 Run software and check for time exposures; best bet is in the 1 to 0.8 sec
5.6 place blackout strip onto the light source entrance.
5.7 set-up the pressure to the first pressure reading, and leave the vessel at this pressure till it is settled.
5.8 off oxygen to jig and switch on the base light to the microscope.
5.9 start video.
5.10 run the data acquisition software, program pres.pgm.
5.11 off base light on the microscope.
5.12 place blackout strip and get array cleared.
5.13 obtain a scan of the vessel at that constant pressure.
5.14 place blackout strip on the light source immediately.
5.15 scan the background.
5.16 Switch on the microscope base light and observe the vessel on the monitor.
5.17 Run up pressure and wait for pressure to be stable.
5.18 Check vessel movement and correct artery positioning by moving the lateral micrometer on the fibermyograph jig prior to scan.
5.19 repeat steps 5.9 to 5.17 till end all the pressure range is completed
5.20 off video. turn on the oxygen to jig.
Technical drawing of the fibermyograph system done by Mr. Peter Barrington.

Top view of the fibermyograph.

Side view of the fibermyograph.
A model for the interpretation of imaged live blood vessels in vitro

A Kaur and P R Smith

Optical Engineering Group, Department of Electronic and Electrical Engineering, Loughborough University, Leicestershire LE11 3TU, UK.

Abstract A semi-empirical optical model for the interpretation of video images of live blood vessels in vitro is developed. The model is capable of resolving a contradiction between the measured temporal evolution of vessel dimensions and the widely held view that tissue is incompressible to first order. One possible implication of the model is that the tissue optical properties are dynamic. Data for this study are obtained from images of muscular rat arteries.

1. Introduction

Perfusion myography allows the study of the structure and function of isolated sections of resistance arteries in vitro and has been used to develop treatments for arterial disease [1,2]. Segments of the vessel are secured on two glass cannulae which are perfused with physiological salt solution with the pressure maintained constant. The pressurisation of the cannulated resistance arteries allows the vessel to assume a conformation that resembles in vivo conditions. The arteriograph is transferred to the stage of a video microscope with image contrast being achieved by the differential light absorption of the lumen with respect of the vessel wall. Vessels can be made to exhibit a type of peristaltic movement when perfused with certain drugs and it is the measurement of the internal and external dimensions of the vessel which is an issue for the optical engineer [3]. An edge-tracking algorithm [4] is used to detect, in real time, the dimensional variations of blood vessel images recorded with this technique. Although the edges detected by the algorithm are those observed in the image, there is some inconsistency between the results and those expected under the assumption of tissue incompressibility [5].

In this paper a semi-empirical optical model of light transmission through the blood vessel with circular cross-section is used to establish a framework for the relationship between the edges found by the edge-tracking algorithm and the actual edges of the vessel. The parameterisation of this model is sufficient to allow other explanations of the apparent inconsistency between tissue incompressibility and actual measurements.
2. Arterial image sequences

The arterial specimens, roughly 1cm in length and 200 microns in diameter, were taken from 12-16 week old female Wistar-Kyoto rats and details of the experimental arrangements and procedures for their analysis can be found elsewhere [4]. A typical image, selected from an instant when the vessel is contracting, is shown in Figure 1. This image has been processed in real time and the resulting vessel edges are marked.

![Image of a contracting blood vessel](image)

Figure 1: An example of a contracting blood vessel from a processed image (t = 30 s)

Changes in the lumen diameter and wall thickness for a vessel contracting and expanding in time have been compiled from a series of sampled video images of a single blood vessel. For our purposes it is sufficient that the data contain contractile events to provide samples of different states of the same vessel; the detailed dynamics of such movements, though highly interesting, are not a subject of this communication. These dimensions are recorded at 13 equi-spaced points along the x-axis and the results from a sequence of approximately 2.5 minutes are shown in Figures 2,3. There are several subtle contraction sequences seen as dark formations on the diameter plot and as light formations in the wall thickness plot with a major contraction occurring in the interval t = 21 - 112s. An estimate of the variation in total tissue volume as a function of time can be made by computing the total cross-sectional area of tissue in the vessel wall and assuming the vessel preserves a circular cross-section under morphological changes.
Figure 2: The lumen diameter of a vessel. A visible contraction at $t = 5\text{s}$ can be seen as dark formation and a major contraction between $t = 21$ and $t = 112\text{s}$ can be seen clearly. Subtle lumen changes are seen as light formations.

Figure 3: The wall thickness. Dark shades show the subtle changes in wall thickness with a contraction developed between $t = 32 - 107\text{s}$. Another clear feature can be seen in the region of $t = 5\text{s}$ showing a contraction followed by an expansion at $t = 10\text{s}$.

Figure 4 shows a measure of the cross-sectional tissue area, for the same vessel and sequence as that of Figures 2, 3. The tissue volume appears broadly to increase and decrease synchronously with contraction and expansion of the vessel; an effect reproduced in the great majority of all contractile events studied. There are a number of explanations for this effect in addition to the unlikely option of tissue compressibility. It is possible that the vessel length and geometry may distort as it contracts and expands. Physical and optical properties of the vessel may also change during contractions. A hydration or dehydration problem could conceivably affect the measurements over an extended period of time but we expect this effect to be small since here the tissue is maintained in a controlled environment. Another possibility is
that the edge-detection algorithm does not find the edges accurately due to the image integrity being affected by the propagation of light through and scattered by the vessel.

Figure 4: The time-dependent variation of the cross sectional area of the blood vessel wall. The cross sectional area, A, is calculated as \( A = \pi (b^2 - a^2) \), where \( b \) is the external radius and \( a \) is the radius of the lumen.

Figure 5: The modal wall thickness to the total sum of cross section for the vessel calculated along the axis

A further insight into these considerations may be gained by plotting the same information as in Figure 4 with respect to the modal vessel wall thickness (Figure 5), instead of time. The relationship implied by Figure 5 is reflected in other analysed sequences, not shown here. In the next section a semi-empirical model is developed which will enable progress to made in understanding the origin of the effects described by Figures 4,5 and should help in distinguishing any measurement errors.
3. Model

The physical model adopted will reduce to a Beer-Lambert law or one-dimensional diffusion approximation (with an effective scattering and absorption parameter) but will incorporate sufficient extra functional complexity to model the observed phenomena in an empirical way. The transmission coefficient across a blood vessel with a circular geometry where \( a \) represents the radius of the lumen and \( b \) the external radius of the vessel is approximated by

\[
T(x) = T_0 \exp(-OD(x))
\]

\[
OD(x) = \gamma(x)d(x)
\]

\[
\gamma = \gamma_0 + c_1d(x) + c_2d^2(x) + c_3d^3(x)
\]

\[
d(x) = \left[ \sqrt{b^2 - (x - x_o)^2} - \sqrt{a^2 - (x - x_o)^2} \right]
\]

where \( d(x) \) is the pathlength, \( \gamma \) models the absorbance and scattering parameter as well as any higher order effects such as geometric distortions which depend on pathlength, \( x \) labels the pixel value, \( x_o \) is the centre of the vessel image, and \( c_1, c_2, c_3 \) are constants. Estimating typical values of \( \gamma_0 \) for white light illumination of arteries in a saline bath is difficult as there exists little spectroscopic information and there is likely to be some variation for tissue type, physiological loading and for source wavelength. Some work has been performed on frozen aorta samples suggesting a total absorption coefficient of 312 cm\(^{-1}\) at 633nm in the media region [6]. A comparison of the optical properties of a frozen sample to a sample immersed in saline solution gives very little difference in the scattering and total absorption parameter at this wavelength [7].

Assumptions made by the model are that the optical density is not affected by absorption inside the lumen and the effective absorbance \( \gamma_0 \) in the vessel wall is only constant to zero order. It is now further assumed, for the sake a diagnostic analysis, that the vessel wall is incompressible; which is a good approximation for many physiological loadings [5]. For vessels held in a perfusion myograph the lowest order approximation assumes that the length is constant, so the cross sectional area \( A \) of the incompressible wall is also constant, giving a simple relationship between external radius and lumen radius as \( b = \sqrt{A/\pi - a^2} \). The semi-empirical model, for a range of vessel dimensions, is shown in Figure 6 in reduced form and as fitted to a data profile from Figure 1.
Figure 6: The Semi-empirical model (thick line) and the Beer-Lambert or zeroth order diffusion model (thin line) for a range of vessel profiles with constant cross section \( A = 5000 \), \( \gamma_0 = 312 \text{ cm}^{-1} \), for five different lumen radii \( a = 9 \) to \( a = 65 \). A real profile (triangles) is taken from the image in figure 1.

For the reduced model, an edge-detection algorithm would exactly locate the position of the lumen since it coincides with the maximum gradient, although this is not the case for the extended model. However the latter model clearly demonstrates a better fit to the raw data profile and shows the detected edge inside the real edge. Furthermore, a comparison of the modeled lumen edge shows a difference that varies as the vessel contracts (Figure 7).

The extended model reveals an interesting feature in which the scattering effect degrading the image is not a linear function of the effective optical density and tissue geometry. It might be postulated that the diffusion process affects the image integrity non-linearly, although other explanations outlined above may also be
important. The curve of figure 7, if interpreted as a means of calibration, could be very influential on the end results of the cross-sectional sum. Judging roughly, the change would be in the range of 5-20% for the lumen diameter, resulting in a shift of approximately 20-30% to the cross sectional sum. This magnitude is sufficient to describe the deviations seen in figure 4.

4. Discussion

The vessel used in this experiment is a muscular artery, with the media consisting largely of spirally arranged smooth muscle cells. These are disposed in multiple layers, with small amounts of connective tissue, collagen, and elastic tissue between them [8]. Elastin and collagen are made up of long chains of protein molecules and their structure react to temperature and dehydration. In this experiment the tissue is alive and is maintained in an isotonic solution at 37°C for short periods of time. This treatment is expected to reduce any dehydration affects but quantitative details on this phenomenon are not yet available.

The physiological behaviour and characteristics of tissue as it contracts and expands appear to be inconsistent with the widely held view that arterial tissue is incompressible. This paper has highlighted the potential for a range of other explanations of the inconsistency, which indicate that a better understanding of the tissue optical properties in relation to image formation is an important area for future study. The empirical model developed here will be used as a basis for an extended statistical investigation of arterial morphology and this should lead to a better interpretation of the experimental data.

Acknowledgement

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References
