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Development of a Microscopic Moiré Interferometry System

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DEVELOPMENT OF A MICROSCOPIC MOIRÉ INTERFEROMETRY SYSTEM

A Thesis
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Master of Science
Mechanical Engineering

by
Jeremy Adam Brougher
August 2007

Accepted by:
Dr. Paul F. Joseph, Committee Chair
Dr. Lonny L. Thompson
Dr. Gang Li
The human tooth is an amazing structure that is worthy of detailed research and analysis. For over half a century, various tests have been conducted to acquire more information about the structural and material properties of dentin, enamel, and the dentin-enamel junction. Unfortunately, variations still exist in the experimental results, even in the most recently collected data. The primary focus of the majority of material property testing on human teeth has been hardness testing utilizing indentation and nanoindentation analysis. However, several limitations still exist with the indentation technique. Thus, the moiré fringe analysis method, specifically microscopic moiré interferometry, has been introduced as a viable alternative testing method for the analysis of biological materials.

Microscopic moiré interferometry is a real-time, full-field deformation analysis technique that allows for in-depth investigation of the mechanics and structures of materials at a microscopic level. In addition, the use of a compact, four beam immersion interferometer allows for near simultaneous analysis of a specimen in both the $U$ and $V$ displacement fields. A modified microscopic moiré interferometry system has been developed which expands on the concepts of both moiré interferometry and standard microscopic moiré interferometry. This modified system was developed specifically for the specialized minute deformation analysis of biological materials. A sensitivity of 4.8 fringes/µm of displacement has been attained using the modified system while increasing the spatial resolution with the capability of analyzing fringe patterns as small
as 22 µm in width. This significant increase is a result of the in-depth analysis, modification, and enhancement of nearly every component in the microscopic moiré interferometry system.

Finally, a system validation test was conducted in which two of the elastic constants of human dentin were experimentally determined, specifically the elastic modulus and Poisson’s ratio. The results obtained in the test procedure were within the acceptable limits of the previously published data. Therefore, it has been demonstrated that the modified microscopic moiré interferometry system possesses the capability of directly determining the mechanical properties of microscopic biological specimens in a timely and accurate manner.
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I thank each of the professors in the Mechanical Engineering Department who have not only taught me how to solve an engineering problem, but have also instilled in me the practices and principles required for a successful engineering career.

Finally, I thank those people who are closest to me. I thank my parents Tom and Shirley Brougher for their loving support and prayers since the day I was born. To my wife Stephanie, thank you for your continual encouragement, devotion, and love. I couldn’t have finished this thesis without you.
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CHAPTER 1
SURVEY OF CURRENT EXPERIMENTAL METHODS FOR DETERMINING
MECHANICAL PROPERTIES OF BIOMATERIALS

1.1 Introduction to Biomimicry

Biomimicry is a design discipline that studies nature’s optimal ideas and then attempts to imitate the designs and processes to solve human problems. The word “biomimicry” is derived from “bios,” meaning life, and “mimesis,” meaning to imitate [1]. The core idea is that many of the problems and limitations which engineers, designers, and researchers struggle with today have already been solved by nature. Biomimicry is not a novel area with many past innovators, such as Leonardo DiVinci and the Wright Brothers, researching and borrowing greatly from the solutions of nature. The primary focus of biomimetic research involves investigation of the structure and function of biological composite materials in an attempt to provide the knowledge necessary for their manipulation and ultimately optimization based on specific applications.

One of the fastest growing biomimetic research areas in the field of engineering is biomimetic material design [1]. Such efforts have focused primarily on new concepts for the design of advanced composites with optimized mechanical properties to weight ratios. Examples of such efforts include the study of abalone shells to develop higher strength ceramics and lightweight building materials, the study of mollusk proteins to develop a higher strength waterproof epoxy, and the study of scales on aquatic animals to develop a
lighter-weight and higher-performance body armor system. Through both observation of the natural world around us and detailed research in biomimetic material design, it is very evident that the natural system seamlessly balances the necessary functional requirements with the anatomical optimizations it ultimately achieves. However, the full extent and detail of functional adaptation has yet to be realized in many materials, specifically small biological materials, due to the difficulty of proper analysis. With the increasing number of biomimetic efforts in a wide variety of scientific fields, the necessity for proper mechanical examination of such biological materials holds great significance.

The motivation and objective of this research project focused on the development of a functional mechanical examination apparatus capable of quantifying material behavior due to mechanical loading at a microscopic level, specifically the development of a modified microscopic moiré interferometer. Through development of the microscopic moiré interferometer, it was desired to include the capability for proper examination of biological materials for biomimetic purposes. Emphasis for this research project focused on the examination of human tooth specimens. The following sections provide a brief background on the structure of the human tooth, a literature review of the current testing methods, and the necessity for development of the modified microscopic moiré interferometer.

1.2 Design of the Human Tooth

Teeth are amazing structures with a design that is seen nowhere else in nature. In a lifetime, a person is given just two sets of teeth: one temporary set which lasts just a
few years, and a permanent set which must endure the remainder of a lifetime. This permanent set of teeth must be capable of handling incredible stresses while dealing with repeated cyclic loading (approximately $10^6$ cycles per year) [2]. This, of course, requires that teeth must be both tough and resistant to wear. Unfortunately, the combination of these material properties does not typically perform well. Materials that are hard and resistant to wear tend to be brittle and crack easily. On the other hand, materials that are tough and can absorb great stresses don’t hold up under continual wear [2]. The human tooth has an amazing design with the capability of handling both of these conditions.

Upon initial inspection, the tooth looks like a relatively simple design with an outer layer of enamel and an inner zone of dentin. The junction of these two materials is called the Dentin-Enamel Junction, or DEJ. Figure 1-1 illustrates the cross-section of a typical tooth, with a close-up of the DEJ. In recent years, an increasing number of studies have been performed to uncover more information about the microscopic material properties and structure of enamel, dentin, and the DEJ.
Figure 1-1. Diagram of a tooth showing the scalloped structure along the dentin-enamel junction.

Enamel is known to be the hardest tissue in the human body. This is due to the fact that enamel is about 85 vol% mineralized [3]. Enamel is a ceramic-like material that is composed of parallel rods that start at the DEJ and run toward the outside of the tooth. Thus, enamel should be considered an anisotropic material. It provides a hard, brittle protective coating that is excellent for harsh wear, but also diverts any load through its depth to the under-lying dentin [2]. Experimental values for the material properties of enamel vary. However, the most recent measurements of the elastic modulus are approximately 87.5 GPa and 72.7 GPa in directions parallel to and perpendicular to the enamel rods, respectively [4]. This makes the outer shell of enamel approximately five times harder than the dentin substrate.
Dentin is a softer, bone-like material with considerably lower stiffness than enamel. Dentin is composed of approximately 50 vol% mineral, 30 vol% organic components, and 20 vol% fluids [5]. The structure of dentin is characterized by tubules, which serve as the pathway for the necessary fluids and minerals during the formation of the tooth [6]. The tubules run from the DEJ toward the pulp chamber of the tooth and are typically 1 to 2 µm in diameter [7]. The material properties of the dentin also vary with respect to the tubules. Peritubular dentin, the material immediately surrounding the tubules, has been reported to have an elastic modulus of approximately 30 GPa. Whereas, intertubular dentin, the material found between the tubules, has been reported to have an elastic modulus of approximately 15 GPa [8]. Figure 1-2 shows a scanning electron microscopy image of human dentin with the location of the tubules, peritubular dentin, and intertubular dentin [obtained from reference 3]. A larger amount of data is available for the material properties of dentin than for enamel. However, there is also much inconsistency within those reported values. Nevertheless, it is accepted that dentin appears to be approximately four times tougher than enamel.
The Dentin-Enamel Junction, or DEJ, is the complex and rather crudely defined zone where the hard enamel layer unites with the tough dentin layer. Much study and testing has been done to better understand the function of this juncture. The structure of the DEJ includes several levels of scallops with the convexities directed toward the dentin and concavities directed toward the enamel [9]. The DEJ is believed to play an important role in the biomechanical integrity of the entire tooth structure [10]. It is critical to the prevention of crack transmission from the enamel to dentin and is considered to play a key role in reducing the stress concentration between the two dissimilar materials [11]. Most experimental work with the DEJ has focused on testing
for the functional width of the zone and the variation of material and chemical properties of the substance around the juncture. Large variations have been reported in the test results, depending mostly on the techniques used for examination. Microhardness tests have determined the functional width of the DEJ to range from 100 to 200 μm, however, nanoscratch testing has determined the width to be as small as 2 μm [11,12,13]. Other studies have also proposed that the DEJ is a region of both functionally graded material properties and structural properties [9,11].

Despite the valuable insight that stands to be gained by acquiring more detailed information about the natural design of the tooth, many large discrepancies are evident in the reported experimental values. Many inconsistencies even exist in experimental values reported using the same testing methods. These discrepancies are believed to be due to the difficulty associated with performing mechanical tests on microscopic and submicroscopic biological specimens. Experimental test results can be significantly affected by variations in specimen moisture content, specimen mineral content, enamel rod orientation, and dentin tubule orientation and density. Therefore, much experimentation remains to be performed and knowledge remains to be gained about the structural and mechanical properties of the intricate elements of the human tooth. Perhaps the value of being able to study such a unique, intelligent design at microscopic scales and finer detail cannot truly be measured at this time.
1.3 General Motivation for Research

In every case where dissimilar materials are bonded together, a stress concentration is developed at the interface. Even two near-similar materials such as brass and steel, when bonded, develop high strain and stress zones at the interface when exposed to thermal variation [14]. Therefore, one would naturally think that the juncture between enamel and dentin, where enamel is nearly five times harder than dentin, would result in an area of huge stress concentration and suffer disastrous consequences when stressed. However, it has been found that the DEJ actually acts as a zone of stress-dissipation, providing a smooth transition of stress from the hard enamel, across the DEJ, to the softer dentin. Unfortunately, despite an increasing amount of work being performed in the recent past, many questions still remain about the tooth’s microstructure and material properties and how that stress and strain is distributed throughout the tooth.

Substantial knowledge still remains to be discovered in order to better understand the process of stress dissipation in teeth. In particular, such knowledge is the next logical step to better predicting the behavior of the dentin-restoration interface [15]. Significant progress has been made in the areas of restorative and preventative dentistry in the past several decades, including new restorative dental procedures that vary from the design of preparations to the choice of bonding methods [5]. However, a key problem remains in the lack of detailed information about the properties of the tooth itself. The more knowledge that is gained about the natural design of teeth, the better we are also able to mimic that design for restorative dental materials.
Restoration of teeth requires the bonding of an artificial material to a treated tooth structure. This process includes the etching and drying of both the dentin and enamel. One primary problem results in the peritubular dentin being etched away, leaving funnel shape openings in the tubules that are not conducive to bonding [5]. Also, if the restorative material is bonded to a partially dried section of dentin, stresses develop when the dentin is restored to its fully hydrated state [16]. A thin layer of cement, and amalgam, is used to hold the filling in place [6]. Traditional materials used for artificial fillings include gold and silver alloys, but newer esthetic restorative materials include resin matrix-ceramic filled composites [6]. Work has been done to form chemical bonds between the filling and the natural substances within the tooth [5]. However, to better understand the restoration-tooth bond, more detailed information about the chemical and material properties of the tooth at the microscopic level must be gained. If longer lasting, stronger bonds can be established, the current dental restoration process could be completely altered. Preventative measures need to become simpler and more reliable, while restorative procedures need to become more conservative and durable [5].

Advanced knowledge about the properties and structure of teeth also has application beyond the dental realm. The excellent biomechanical properties of the human tooth have drawn interest as being a premium biomimetic model for joining dissimilar materials [11]. By gaining greater understanding of the stress trajectories, the resulting strain distribution, and their relation to the structure of the tooth, insight can also be gained into the manner in which teeth function. This information has a wide range of utilization, including new applications for improvements in synthetic materials and
bonds [13]. As more information is learned and understood about natural designs, such as the tooth, we are better able to mimic such principles in new designs and engineering.

1.4 Review of Current Testing Methods

A majority of the work executed in the experimental research and testing of human teeth has occurred in the last half century. Therefore, this region of biomimicry can be considered a relatively new branch of engineering exploration. Also, a majority of the experimental research with teeth has been limited to the investigation of the material properties of dentin. However, the last several decades have seen an increase in the examination of the material and structural properties of the entire tooth. The early testing methods were limited to compression, tension, and bending tests. However, a majority of the experimental research has centered on various indentation testing methods. Microindentation, nanoindentation, and Atomic Force Microscope (AFM) based nanoindentation all employ approximately the same methodology, but with increasing levels of both sensitivity and precision. The most recent attempts at quantifying the material properties of dentin have focused on the use of Resonant Ultrasound Spectroscopy (RUS). Unfortunately, even though the experimental technology and techniques have advanced greatly, the reported experimental values still tend to vary widely. This statement even holds true independent of the testing methods. The following section briefly summarizes each of these procedures and some of the associated experimental results.
1.4.1 Tension and Compression Testing

Tension and compression testing were primarily utilized for early experimentation on the material properties of dentin. Peyton et al. first experimentally determined dentin to have an elastic modulus of 11.6 GPa by compression testing in 1952 [17]. Ten years later, in 1962, Bowen and Rodriguez used tensile testing to experimentally determine dentin to have an elastic modulus of 19.3 GPa [18]. Early tests involved sectioning and attaching slices of dentin to varied designs of compression and tension devices. Strain gauges were typically utilized, either affixed directly to the tooth or to the fixture that was used to apply load to the specimen [19]. These designs were an excellent first attempt at establishing the material properties of dentin and the results obtained were relatively good for the type of testing performed. However, increased knowledge about the microscopic distribution of properties within dentin required more sensitive testing methods to be developed.

1.4.2 Indentation Testing

Indentation, a method where a hardened stylus is brought into contact with a surface, is commonly used to quantify a material’s hardness. Hardness is a measure of a material’s resistance to deformation and is defined as the applied load of the indenter divided by the projected area of indentation. In the last decade, the use of microhardness indentation testing has been applied to mineralized tissues for the analysis of material properties such as elastic modulus [19]. Figure 1-3 shows a sketch of a typical Vickers microhardness indenter with a corresponding picture of an actual indentation into human dentin [obtained from reference 9]. Again, variations existed in the experimental values
for the obtained data. The elastic modulus of enamel ranged from 80 GPa to 94 GPa, depending on the specific tooth tested and the orientation of the enamel [9]. Dentin was measured to have an elastic modulus of 20 GPa [9]. Testing was also performed in the zone of the DEJ and a functional width of 100 μm to 200 μm was proposed [12,13]. However, this value seemed to be much wider than the optical appearance of the DEJ. Therefore, because of the microscopic variance of properties within the materials being tested, higher sensitivity methods with the ability to test at a finer scale were required.

**Figure 1-3.** Sketch of Vickers microhardness indenter with corresponding picture of actual indentation into human dentin.

Nanoindentation has the distinct advantage of using a much smaller indenting stylus in order to test materials with smaller applied loads and at finer spatial resolutions [5]. When utilized for the experimentation of dentin, this provided a significant improvement by enabling more precise testing when dealing with tubule orientation and surface morphology [5]. In 1993, van Meerbeek et al. first utilized nanoindentation and reported the elastic modulus of dentin to be 19.6 GPa. Since then,
nanoindentation has become a common technique for the determination of local mechanical properties of structural features in biological hard tissue [19].

The most recent development in the area of indentation testing came in 1998, when Balooch et al. modified an Atomic Force Microscope (AFM) in order to obtain a high resolution image while simultaneously measuring both hardness and stiffness [20]. The conventional head assembly of an AFM was replaced with a transducer-indentor assembly called a Triboscope [20]. This permitted the positioning of the indentation to be accurate within a few tens of nanometers and allowed measurement of hardness values with indentation depths of less than 20 nm (approximately 5-10 times shallower than the nanoindenter) [5]. The minimum applied load could be less than 1 μN and it could measure a displacement up to 35 μm [20]. The indenter stylus could also be made from a variety of materials and in a variety of shapes and sizes [20]. Figure 1-4 shows an image from an AFM based nanoindentation test across the DEJ of a human tooth [obtained from reference 9]. The key benefit of utilizing the AFM based nanoindenter for experimentation with teeth is the liquid cell that is used to keep the specimen fully hydrated [5]. Thus, the AFM based nanoindenter has the ability to be a force-generating and depth-sensing instrument capable of providing load-displacement curves at specified locations all while the specimen is contained in an ambient, liquid environment. The most recent experimental work performed with the AFM based nanoindenter reported the measurement of elastic modulus values of 30 GPa and 15 GPa for peritubular and intertubular dentin, respectively [8]. The elastic modulus for enamel was also reported to be 87.5 GPa and 72.7 GPa for directions parallel and perpendicular to the enamel rods,
respectively. In addition, a functional width for the DEJ of 12 μm to 20 μm was proposed [9,11]. The AFM based nanoindenter has been utilized to obtain the most precise, experimentally measured data for the material properties of biological materials using the indentation testing technique.

**Figure 1-4.** Image from AFM based nanoindentation test across the DEJ of a tooth.

### 1.4.3 Resonant Ultrasound Spectroscopy Testing

The most recently developed tool for quantifying the material properties of dentin was introduced by Kinney _et al._ in 2003, when Resonant Ultrasound Spectroscopy (RUS) was used to determine the elastic modulus and Poisson’s ratio of human dentin [21]. RUS utilizes Newton’s second law and Hooke’s law to predict the resonant modes of mechanical vibration of a specimen of known shape [22]. The elastic modulus and
Poisson’s ratio of a material can then be directly determined by comparing the frequency spectrum produced by the resulting eigenvalue problem for the test at hand with the measured resonant frequencies of the specimen [22]. The RUS experimentation and analysis conducted on hydrated dentin specimens determined an elastic modulus of 23.3 GPa and 25.0 GPa in directions parallel to and perpendicular to the tubule axis, respectively [21]. Poisson’s ratio was assessed to be 0.45 and 0.29, in directions parallel to and perpendicular to the dentin tubule axis, respectively. On the other hand, the tests on dry dentin specimens displayed an increase in elastic modulus of approximately 4 GPa in both directions, while the anisotropy previously observed in the Poisson’s ratio disappeared [21]. Therefore, while a relatively small amount of experimentation has been performed, the RUS technique looks to be a promising new non-destructive testing method for the experimentation and analysis of biological materials.

1.5 Analysis of Current Testing Methods

Despite significant advances in both the theoretical understanding of the mechanics of composite structures and vastly improved testing methods, discrepancies still remain in the experimental data. Even some of the most recently published material shows evidence of this variance. This provides a true and accurate reflection of the difficulties encountered in performing mechanical testing on such small, biological specimens. Therefore, improvements are still required in the preparation, set-up, and analysis used for the current testing methods. The following paragraphs briefly outline
some of the key constraints in the utilization and interpretation of the past and current testing methods.

The tension and compression tests first performed nearly a half-century ago were an excellent first attempt at gaining information about the structural and mechanical properties of teeth. However, since the human tooth is a very detailed structure with microscopic and sub-microscopic properties, the testing methods for examining these properties must also be microscopic in nature. A majority of these tests made use of strain gauges applied either directly to the specimen or to the loading fixture. While strain gauges are a superb tool developed for testing the macroscopic properties of materials, increased sensitivity was required for more accurate measurements and conclusions. Also, the measurement of material properties such as the elastic modulus, especially in biological materials, is particularly sensitive to specimen preparation, experimental set-up and design, and stress relaxation [19]. Therefore, while the testing and results were a good first attempt, more detailed and sensitive testing methods were required for analysis of the material properties of teeth.

Microindentation and nanoindentation utilize primarily the same methodology in testing. The main disadvantage of microindentation was the large indenter stylus size and, therefore, the decreased level of sensitivity. The size of the microindentation with respect to the microscopic structure of the tooth resulted in merely a composite average for the structure and property differences [5]. For example, the changes and effects of the variations in tubule density at different locations could not be distinguished [5]. The smaller indenter stylus size of the nanoindenter gave it the ability to be more of a site-
specific instrument with a greater level of sensitivity. However, the main disadvantage for the nanoindenter was the inability to position the location of the indentation within less than a few micrometers [5]. Therefore, alignment of the indenter tip with the feature of interest was quite difficult. In addition, perhaps the most significant hindrance to using the microindentation and nanoindentation techniques was the inability to indent in water [19]. This became a serious issue when attempting to accurately measure the material properties of mineralized or biological tissues, where keeping the specimen fully hydrated was a necessity. These significant drawbacks restrained the capabilities of the microindenter and nanoindenter from being a fully useful and accurate measurement tool.

The modified AFM based nanoindentation technique and the RUS technique were both developed in an attempt to overcome the problems associated with the microindentation and nanoindentation testing techniques. The microscopic size of the indenter combined with the powerful depth-sensing indentation equipment and improved positioning capability of the atomic force microscope produced the capability of higher sensitivity measurements while obtaining a high-resolution image of a fully hydrated sample [5]. Also, the RUS technique looks to be a promising new non-destructive testing method for directly determining the elastic constants of biological materials [21]. However, several limitations still remain with the use of both techniques. These limitations produce significant problems when experimenting with biological specimens such as the human tooth. Some of the constraints in the utilization and interpretation of results obtained from both techniques are summarized briefly below.
The primary limitation placed on any indentation technique, as well as the RUS technique, is that assumptions must be made about the structural and material properties of the sample being tested. First of all, while the AFM based nanoindentation experimentation is performed on just a very thin surface layer of the material (<1μm), the results obtained are assumed to be representative of the remainder of the bulk material [7]. Therefore, any slight change on the surface layer of the material can result in disastrous interpretation of test data. Storage conditions, such as solution and time, become important issues since any reactions, such as etching or dissolution, have the ability to affect the surface layer [7]. On the other hand, the RUS technique allows only for the assessment of bulk properties and relies on the assumption that the density of the sample is uniform [22]. Each of these circumstances plays an important role in the determination of the material properties of the specimen.

A second important assumption is that the surface layer displays the same homogeneous characteristics as the bulk material. This becomes critical in the inspection of the three-dimensional, crudely defined structure of the DEJ. The multilevel scalloped sub-structure must be taken into careful consideration when performing any testing around the DEJ. The zone of the DEJ is known to be a highly irregular and non-planar juncture [11]. Figure 1-5 shows an illustration adapted from reference 11 which portrays the capability of sampling varying mixtures of materials on both sides of the junction with the same indentation. Although the two indentations shown are observed to be on the dentin side of the junction, the differing indentation depths give differing results. This appears to give the result of a change in the material properties of dentin
approaching the DEJ, but in actuality, both indentations are sampling differing levels of both dentin and enamel. This conclusion is supported by the trend that decreased indentation depth results in a decreased measured width of the DEJ [11]. Microindentation testing results in a functional width result for the DEJ as wide as 200 μm and nanoscratching as low as 2 μm [11,13]. Therefore, the deeper the indentation, the more of a problem sampling of unwanted materials or areas becomes, thus increasing the likelihood for unwanted interactions.

Figure 1-5. Model of varied indentation depths due to irregular nature of DEJ.

Another important assumption generally followed in the analysis of indentation testing is that the material reacts consistently with a changing load throughout the depth of its structure. Differing opinions still exist on the reaction of the material properties of dentin to depth and the direction of loading. The decreasing tubule density moving away from the DEJ is believed to play a role in altering the bulk material properties of dentin.
Also, enamel has highly anisotropic stiffness characteristics due to the structure of the rods [2]. In a recent finite element model simulating the loading of enamel, it was concluded that the inclusion of anisotropic properties in the structure had a profoundly different stress distribution under load than when compared with similar isotropic models [2]. Therefore, careful attention must be made to the orientation of the specimen and the direction of the loading in the preparation for indentation testing. However, the results could also be misinterpreted if the material properties change under the application of the load itself. Assumptions are made that the hardness (and elastic modulus) does not change with the amount of force applied. In other words, it is assumed that the material properties do not act as a function of the applied force. As the indenter is lowered into the material, it is assumed that the material resists the indenter with a consistent amount of pressure, thus assuming a constant hardness (and elastic modulus). However, if the material displays a non-linear elastic modulus, and resists the pointer with an increasing amount of pressure as it is lowered, a higher value for the hardness will be recorded. Figure 1-6 shows an example of two differing types of specimen deformation under compression. Case A illustrates what is assumed in indentation testing. A linear amount of deformation exists for the increasing load that is applied. Case B shows the possibility of a nonlinear amount of deformation for the same applied load. Thus, the apparent hardness (and elastic modulus), of the material would change according to the depth of the indentation. In each case the same load is applied to the specimens, but different end results are recorded. When performing testing on
anisotropic material such as biological specimens, at this time it is impossible to know if
the assumptions being made are truly valid for the type of testing being performed.

Case A: *(Assumed)*

![Diagram](Figure 1-6)

**Modulus Remains Consistent as Indentation Load Increases**

Case B: *(Possible Alternative)*

![Diagram](Figure 1-6)

**Modulus Changes as Indentation Load Increases**

**Figure 1-6.** Illustration of possible non-linear response to increasing indentation load on
experimental specimens.

The next important limitation to be considered is the effect of the indenter on the
material. As the indenter is compressed into the specimen, there is an undefined area
surrounding the indenter that is affected. Figure 1-7 shows two pictures of photo-elastic
material being compressed by a sharpened pointer. As seen in photograph (A), there is a
wide area of material surrounding the pointer that is affected by the indent. (Note, the diameter of the indenter shown in the picture is approximately 1 mm.) Therefore, it is necessary that sufficient spacing be kept between the indentations.

![Image](image.png)

**Figure 1-7.** Effect of indentation into photo-elastic material.

(A) Observed effect in middle of specimen, (B) Observed effect near edge of specimen.

It has been reported that a spacing of approximately four times the indentation size must be maintained in order to ensure appropriate properties determination [11]. If this determination is accurate, when performing tests over a 12 μm zone (width of DEJ found from AFM based nanoindentation testing), the evaluation can only be based on a very limited number of indentations [16]. Thus, substructure variations may exist in the material or the DEJ zone that may not be detected by the existing instrumentation [16]. When testing a homogeneous and isotropic section of material, the results may give
accurate measurements of the properties in the bulk surrounding material. However, the possibility exists that these bulk properties may not be sufficient if the material properties of both the dentin and enamel vary throughout the tooth, especially near the DEJ [16]. In addition, as illustrated in photograph (B) of Figure 1-7, as the indenter approaches the end of the sample, the stress pattern surrounding the indenter is altered. This becomes significant in the zone surrounding the DEJ. Since the difference in the material properties between the dentin and enamel is sufficiently large, it is reasonable to conclude that there is a considerable effect in the indentations approaching the interface between the two. The plots in Figure 1-8 and Figure 1-9 were constructed to further illustrate this possibility.

Figure 1-8 shows two numerical models of averaged modulus values. In the plot, the two averaged modulus lines were obtained by beginning with a perfect “DEJ” interface at \((x=0)\) and using a value of 20 GPa for the “dentin” on the left \((x<0)\) and 67 GPa for the “enamel” on the right \((x>0)\). The modulus value for the purple line, indicated as “Average Modulus Value of ±3 μm” was obtained by taking an average value of ±3 (y) values at each whole number data point. For example, at \((x=-2)\), the averaged modulus value \((y=33.4)\) was obtained by averaging the following values: \([20, 20, 20, 20, 20, 67, 67]\). The orange line, indicated as “Average Modulus of ±4 μm” was obtained using a similar process and averaging 9 data points. Similar lines with increments up to ±5 μm were completed for the values on each side of the “DEJ” interface. This was done in an attempt to simulate the effect that the indenter would have on the material surrounding the indentation.
Figure 1-8. Plot showing averaged modulus lines around abrupt DEJ interface.

Figure 1-9 shows an overlay of the experimental data values for elastic modulus with the two numerical models of averaged modulus values. The blue line in the plot displays the experimental data points obtained using the AFM based nanoindentation technique [9]. When the original experimental results and the various averaged modulus value lines were compared, it was found that for the “dentin” side, a ±4 μm average closely fit the experimental model. Also, for the “enamel” side, a ±3 μm average fit the experimental model near completely. This combination seems reasonable since the softer dentin would allow for a larger composite average of material than the harder enamel.
The purple and orange line in Figure 1-9 shows the combination of these two averaged modulus value lines. As illustrated, a simple numerical model of averaged values along an abrupt interface is nearly capable of completely mimicking the data obtained experimentally using the indentation technique. Therefore, the affected material surrounding the indentation may have significant effects on the interpretation of the experimental results. Unfortunately, this also exposes a decrease in the level of sensitivity around the area that most requires examination.

**Figure 1-9.** Plot showing AFM based nanoindentation experimentally determined elastic modulus values with combined averaged modulus values.
The last primary limitation on the indentation technique is that a direct measurement cannot be made of the desired material properties such as the elastic modulus and Poisson’s ratio. Indentation provides an excellent evaluation of directly measured hardness values. However, the elastic modulus is typically calculated according to the method developed by Oliver and Pharr [23]. In this method, power-law curves are fitted to a specified percentage of the final unloading curves from the indentation measurement. The contact stiffness and contact depth are then obtained by differentiating and extrapolating these curves. The elastic modulus of the specimen \( (E_s) \) can then be calculated using equation 1-1,

\[
E_s = \frac{\sqrt{\pi}}{2\sqrt{a}} S
\]  

(1-1)

where \( S \) is the calculated contact stiffness and \( a \) is the projected area while the load was applied [20]. The corrected, reduced elastic modulus \( (E_r) \) can then be calculated using equation 1-2,

\[
\frac{1}{E_r} = \left(1 - \nu_s^2\right) + \left(1 - \nu_i^2\right) \frac{E_s}{E_i}
\]  

(1-2)

where \( E_i \) is the elastic modulus of the indenter, and \( \nu_s \) and \( \nu_i \) is the Poisson’s ratio of the specimen and indenter, respectively [4]. The equation for the reduced elastic modulus requires an assumed, isotropic value for the Poisson’s ratio of the specimen. Therefore, the calculation of one material property for a desired specimen requires curve-fitting, extrapolating, and the assumption of a material property (isotropic Poisson’s ratio) that may not be valid. The derivation of other material properties then adds additional
assumptions and uncertainties to the conclusions. Therefore, the ability to conclude directly any material properties other than hardness values is not possible.

The intent of the examination was to provide a true and accurate reflection of the difficulties encountered in performing mechanical examinations on small, biological specimens. The purpose of this examination was not an attempt to prove that any of the testing methods discussed cannot be considered valid for the experimental determination of all material properties. The majority of biological specimens, including the human tooth, are neither homogeneous nor isotropic. While AFM based nanoindentation and RUS analysis provide a closer inspection of the properties of microscopic materials, the necessary assumptions significantly limit the effectiveness of these powerful tools. Therefore, innovative evaluating methods, in which different or no assumptions need to be made, must be examined to increase the current knowledge of the microscopic nature of biological materials.

1.6 Innovative Methods – Moiré Technique

A possible solution to some of the limitations associated with the experimental testing methods detailed above is the moiré technique. The moiré effect occurs any time two similar arrays of equally spaced lines or dots are arranged in such a way that one array can be viewed through the other [24]. The broad dark and light bands which result from this overlap, or interference, are known as moiré fringes [25]. This ideology has been applied to experimentation for a variety of deformation and displacement analysis procedures. In the experimentation, one pattern, or grating, is applied to the sample that
is subject to testing and interfered with a second, undeformed reference grating. The resulting moiré fringe pattern can then be analyzed to directly determine the displacements in the sample under varying levels of stress. An example of the moiré effect is illustrated in Figure 1-10. A more detailed discussion of the moiré technique and moiré interferometry is provided in Chapter 2.

Figure 1-10. Example of the moiré effect.

Wang and Weiner were the first to apply the moiré fringe technique to the testing of a human tooth by directly measuring the in-plane strain distribution on slices of human teeth [13]. On a polished section of tooth, they were able to apply a 200 lines/mm grating (cross-line grating with alternating black and white lines) using a commercial 3s “Super Glue.” The experimental set-up was then performed by overlaying a reference grating with only parallel lines of the same frequency and then applying a horizontal load to the tooth. The interference between the deformed specimen and reference grating produced moiré fringes, generating the two in-plane displacement fields. This was an excellent
initial test in the area of stress-strain analysis of a biological material and showed excellent insight to the mapping of strain on a human tooth. They were able to measure an approximate 200 μm thick zone of dentin, adjacent to the DEJ, which undergoes a larger amount of strain when compressed, which they believed was important to minimizing the stress across the DEJ [13]. However, one limitation they encountered was the sensitivity of their system. With a reference grating frequency of 200 lines/mm, their system was capable of a sensitivity of 0.2 fringes/μm of displacement on the specimen or just 5 μm/fringe order. However, increased knowledge about the microscopic distribution of properties within dentin necessitated the development of more sensitive testing methods. (A detailed description of the sensitivity of a moiré system is included in Chapter 2.)

Wood et al. were the first to apply the moiré interferometry technique to the study of human teeth [16]. In moiré interferometry, a reflective cross-line diffraction grating is utilized with the interference of coherent, collimated laser light to achieve the desired fringe pattern. The cross-line diffraction grating is an array of ridges and furrows in orthogonal directions. A special replication technique was developed which enabled the ability to replicate a reflective grating mold onto the surface of the tooth while maintaining an adequate moisture level in the specimen. A complete description of this technique is given in reference 16. As a result of their testing, Wood et al. were able to develop complete displacement field maps across the tooth surface in both the vertical and horizontal directions showing the effects of moisture level changes through the tooth’s crown [16]. Figure 1-11 shows an example of the fringe patterns obtained using
the moiré interferometry technique on the human tooth samples [16]. They were also able to achieve a higher level of sensitivity. Using their system, they were able to obtain a 2400 lines/mm reference grating frequency. Thus a sensitivity of 2.4 fringes/μm of displacement or 0.417 μm/fringe order could be achieved. This was an excellent advancement in the ability to inspect the details of the stress-flow across the surface of a tooth. However, the amount of detail and accuracy for testing around desired zones such as the DEJ was still not achieved. Therefore, higher sensitivity methods with the ability to test at a finer scale were required.

The microscopic moiré interferometry technique was first introduced by Han and Post [26]. The microscopic technique was based on the same principles as traditional moiré interferometry, but allowed for in-depth investigation and microscopic analysis on the mechanics and structures of materials. This was achieved by utilizing a refractive
medium for the formation of the reference grating, rather than air. In this set-up, the collimated laser beam was directed into a piece of optical material used for the interferometer. The refractive medium allowed for a greater diffraction angle to be utilized, which created a reference grating with twice the frequency of traditional moiré interferometry. The addition of an immersion fluid was necessary to avoid complete internal reflection of the laser beam inside the interferometer. A microscope objective above the interferometer was then used to collect the resulting fringe picture. Using the microscopic moiré interferometry technique, Han and Post were able to acquire a 4800 lines/mm reference grating frequency. This produces a sensitivity of 4.8 fringes/μm of displacement, or a contour interval of 0.208 μm per fringe order [26]. With the addition of a microscope objective, the spatial resolution is restricted only by the capability of the optics being used.

In the current study, the previous concept of microscopic moiré interferometry has been both expanded and modified for use in the realm of experimentation with biological materials, specifically the human tooth. Previous designs by Han and Post allowed for an image scale, horizontal width across image, of approximately 0.4 mm. However, for the meticulous inspection of microscopic and sub-microscopic structures such as the DEJ, the desired scale was nearly an order of magnitude smaller. Therefore, the entire interferometer design necessitated modification to permit the use of high magnification optics including long working distance objectives and ultra-zoom assemblies. Through these modifications, the modified microscopic moiré assembly currently has the capability of specimen analysis down to a scale of approximately 22 μm. Thus, the
current limits of microscopic material property and structural investigation have been both met and stretched.

1.7 Advantages of the Microscopic Moiré Interferometry Technique

The prime advantage of the microscopic moiré interferometer over previous moiré testing methods is the enhanced detail of viewing area and increased sensitivity in measurement. The viewing area is currently at a smaller scale than previously ever recorded for similar optical testing techniques. Also, the sensitivity of the microscopic moiré interferometer has increased nearly 25 times beyond the original tests performed by Wang and Weiner. Therefore, the modified microscopic moiré technique can observe key areas of interest such as the scalloped region of the DEJ and the zones surrounding the dentin tubules. This enhanced ability will be advantageous to the study of both healthy teeth and replacement components for damaged teeth. Examination of healthy teeth will provide valuable insight to the seemingly mysterious mechanical and structural properties that allow the teeth to work so flawlessly. On the other hand, inspection of the hybrid layer between damaged teeth and replacement fillings will offer innovative understanding about the mechanical integrity and breakdown of current artificial bonds.

A second advantage of the modified microscopic moiré technique is the ability to test the specimen in its natural environment. The immersion fluid necessary for the interferometer, typically either water or oil, allows the specimen to remain moist throughout the experimentation process. When coupled with the grating application process previously developed by Wood et al., this allows for experimentation with out
concern of altering the natural properties of the surface layer of the tooth [16]. Therefore, the complete cycle of trial preparation, set-up, execution, and analysis can be completed without affecting the natural state of the tooth.

A valuable inclusion in the modified microscopic moiré interferometer design was the addition of a loading device. The interferometer set-up was designed for a load fixture equipped with a sub-miniature load cell that provides the capability of testing specimens in both tension and compression. Therefore, the modified microscopic moiré interferometer assembly supplies the ability to simultaneously mechanically load a specimen while observing the results. No other previously recorded experimentation methods have proven the ability to test at such a fine scale with an applied mechanical load. Additional detail about the loading device will be included in Chapter 3.

Perhaps the key advantage of the modified microscopic moiré interferometry technique over all other similar testing methods is the ability to perform full-field deformation analysis. The moiré technique is an excellent high-sensitivity tool for measuring in-plane displacements [25]. The displacement at every point can be measured precisely, from which strain can be directly determined. Therefore, the strain across a sample can be found without making any assumptions about material properties. Equations 1-3 through 1-5 show the equations required for the direct determination of strain in two orthogonal directions across the surface of a specimen,
\[
\varepsilon_x = \left( \frac{1}{f} \right) \frac{\partial N_x}{\partial x} \approx \left( \frac{1}{f} \right) \frac{\Delta N_x}{\Delta x} \tag{1-3}
\]

\[
\varepsilon_y = \left( \frac{1}{f} \right) \frac{\partial N_y}{\partial y} \approx \left( \frac{1}{f} \right) \frac{\Delta N_y}{\Delta y} \tag{1-4}
\]

\[
\gamma_{xy} = \left( \frac{1}{f} \right) \left( \frac{\partial N_y}{\partial x} + \frac{\partial N_x}{\partial y} \right) \approx \left( \frac{1}{f} \right) \left( \frac{\Delta N_x}{\Delta y} + \frac{\Delta N_y}{\Delta x} \right) \tag{1-5}
\]

where \(\varepsilon_x\) and \(\varepsilon_y\) are the normal strain values in the respective directions, \(\gamma_{xy}\) is the value of the shear strain across the respective direction, \(f\) is the frequency of the reference grating, and \(N_x\) and \(N_y\) are the fringe orders in the respective directions. Additional detail about the derivation and utilization of these equations with the moiré technique is provided in Chapters 2 and 3.

The microscopic moiré technique provides full-field deformation analysis in two perpendicular fields of interest. Therefore, full-field contour maps in two perpendicular displacement directions can be near simultaneously observed and recorded [16]. Also, the fringe patterns are produced by interference between the known reference and the deformed specimen. Therefore, the deformation can be determined between any two points at any time after the grating has been applied. This is particularly beneficial for long-term studies where there is no need for concern of zero-drift or other unwanted changing test conditions. Therefore, the entire surface of the tooth can be monitored in two separate perpendicular directions for possible long-term studies without concern for unwanted variation in specimen testing conditions.
1.8 Summary

The human tooth is an amazing structure that is worthy of detailed research and analysis. The microscopic properties and structures of the tooth make it difficult to perform effective experimentation. For over half a century, various tests have been conducted to acquire more information about the structural and material properties of dentin, enamel, and the DEJ. However, variation still exists in the experimental results, even in the most recently collected data. The primary focus for the majority of material property testing on the human tooth has been hardness testing through indentation and nanoindentation analysis. However, several limitations still exist with the indentation technique. Key assumptions still need to be made when analyzing the test results to obtain any material property data. The moiré fringe technique was previously utilized to directly measure the strain distribution on a slice of a human tooth [16]. A modified microscopic moiré interferometry technique was developed to obtain the same strain data at a much finer scale. Modified microscopic moiré interferometry has been shown to be a viable technique for the direct determination of strain through full-field deformation analysis on human teeth. This technique will enable valuable insight to be gained about the mysterious mechanical and structural properties that allow teeth to function so flawlessly.
2.1 Introduction to the Moiré Technique

The word “moiré” is a French name for a fabric known as watered silk, which exhibits patterns of light and dark bands [24]. The moiré effect commonly occurs whenever two similar but not quite identical arrays of equally spaced dots or lines are arranged such that one array can be viewed through the other. The broad dark and light bands which result from this overlap, or interference, are known as moiré fringes. As shown in Figure 2-1, perhaps the most common occurrences of the moiré effect can be witnessed in the field of photography. Picture (A) of Figure 2-1 shows two different sets of moiré fringes, one set on the window blinds and a second set on Dr. Judy Wood’s jacket. In this picture, the moiré effect is caused by the interference of the uniform pattern of the blinds (and Dr. Wood’s jacket) with the fine regular pattern of the pixel array in the digital camera. Picture (B) shows a similar, but different, example of the moiré effect. In this case, the fringes are caused by the interference of the jacket design with the dot-matrix layout of a printed newspaper picture.
Figure 2-1. Two common examples of the moiré effect in photography.

Considerable insight into the moiré effect can be gained by studying the relationships that exist between the spacing and inclination of the moiré fringes in conjunction with the geometry of the two interfering arrays that produce the pattern [24]. These relationships are the basis for the displacement and strain analysis techniques utilized in this research project. For example, an analysis of the moiré fringes on Dr. Wood’s jacket in Figure 2-1 in conjunction with the known geometries of the jacket design and camera pixel array could lead to a detailed plot of the surface contour of the jacket. The following section will outline the definitions and relationships for the moiré fringe analysis techniques utilized in this research project.

The arrays used to produce moiré fringes may be a series of straight parallel lines, a series of radial lines emanating from a point, a series of concentric circles, or a pattern of dots. In stress analysis work, two arrays (specimen and reference) consisting of
straight parallel lines are the most commonly used. The following list provides a brief summary of the terminology typically used in moiré stress analysis:

- **Grating**: A parallel line array suitable for moiré stress analysis.
- **Cross-line grating**: A grating that has the same repeating arrangement of parallel lines in two orthogonal directions. Used to measure displacement in both directions.
- **Specimen grating**: A grating applied and adhered directly to a specimen, typically when the specimen is in the undeformed state. Additional detail about the grating application process will be provided in Chapter 3.
- **Reference grating**: A master grating, undeformed, used to measure the change in deformation of the specimen grating.
- **Fringe pattern**: The field of dark and light bands which results from the overlap, or interference, of the specimen and reference gratings.
- **Null field**: A field devoid of moiré fringes. Typically seen when the specimen and reference gratings are essentially identical, or matched, when the specimen is in the undeformed state. The objective is for the specimen and reference gratings to be matched prior to experimentation (deformation) of the specimen.
- **Frequency of the grating**: The number of lines per unit length of grating (1200 lines/mm). Also referred to as the density of the grating. Symbolically denoted as $f$. 
• Pitch of grating: Center-to-center distance between the reference grating lines. Also calculated as reciprocal of the frequency of the grating. Symbolically denoted as $p$.

Perhaps the most valuable feature of the moiré technique is that the relationships between the moiré fringes and grating lines can be determined simply by geometry. This geometrical approach to the interpretation of the moiré technique was first published by Tollenaar in 1945. Later, in 1960, Morse et al. presented a complete study of the geometry of moiré fringes in strain analysis [27,28]. Image analysis techniques are normally employed to measure changes in spacing between the intersecting points of the two overlapping arrays before and after loading. From analysis of these data, the displacements and strains observed in the specimen can be determined using relatively simple analytical procedures. In its simplest form, engineering strain, $\varepsilon$, can be defined according to equation 2-1,

$$\varepsilon = \frac{\Delta l}{l_o}$$

(2-1)

where $l_o$ is the original length of a specimen measured in a given direction and $\Delta l$ is the change in length measured in the same direction after loading.

Applying this principle to the moiré technique, the engineering strain, $\varepsilon$, from a given fringe pattern can be generalized by two expressions for either tensile strains (A) or compressive strains (B) over an arbitrary gage length, as shown in equation 2-2,
\[
\varepsilon = \begin{cases} 
\frac{\Delta l}{l_o} = +\frac{np}{l_g - np} & \text{(A)} \\
-\frac{\Delta l}{l_o} = -\frac{np}{l_g + np} & \text{(B)}
\end{cases}
\]  

(2-2)

where \( p \) is the pitch of the undeformed reference grating, \( l_g \) is the gage length, and \( n \) is the number of moiré fringes in the gage length. Analysis equation 2-2, shows that a moiré fringe is formed within the given gage length in a uniformly deformed specimen each time the specimen grating is extended (or compressed) by an amount equal to the pitch of the master grating. This principle is illustrated in Figure 2-2. Careful inspection of the two arrays shows that the bottom array was elongated such that approximately 8 lines extend beyond the upper array. Therefore, this elongation then produces approximately 8 moiré fringes in the area of interference between the two arrays.

\textbf{Figure 2-2.} Moiré fringes produced by pure, uniform elongation.
The discussion and equations presented above are valid for cases involving uniform elongation (A) or contraction (B). The moiré fringes in Figure 2-2 were formed by elongation of the “specimen” perpendicular to the direction of the lines in the reference grating. However, elongation of a “specimen” parallel to the direction of the lines in the reference grating have no effect on the moiré fringe pattern. Therefore, the need for measurement of displacement in two orthogonal directions and for the use of a cross-line grating on a sample is raised. Additional discussion about the need for near simultaneous measurement of the displacements in two orthogonal directions will be presented later. In addition, simple experimentation with a pair of identical gratings indicate that moiré fringes can also be formed by pure rotation (no elongation or contraction) as shown in Figure 2-3.

![Figure 2-3. Moiré fringes produced by pure rotation.](image-url)
In Figure 2-3, note that the moiré fringes have formed in a direction that bisects the obtuse angle between the lines of the two gratings [24]. The relationship between the angle of rotation of the array, $\theta$, and angle of inclination of the moiré fringes, $\phi$, both measured in the same direction and with respect to the lines of the reference grating is shown in equation 2-3.

$$\phi = \frac{\pi}{2} + \frac{\theta}{2}$$

Equation 2-3

The equations have been provided for cases of pure rotation and pure elongation or contraction of the specimen grating with respect to the reference grating. However, in most cases at any given point on a stressed specimen, a combination of these two effects occurs simultaneously to produce a fringe pattern. The displacement of a specimen is typically caused by deformation of the body, rigid-body movement, or a combination of both. Therefore, in 1948 Weller and Shepard presented an analysis technique that relates a moiré fringe pattern to a displacement field [29]. Using this technique, the fringe pattern can be visualized as a displacement surface where the height of a point on the surface above a plane of reference represents the displacement of the point in a direction perpendicular to the lines of the reference grating. This technique can be likened to a topographical map, where the lines of equal elevation can be likened to lines of equal displacement across the surface of a specimen. This technique also enables the displacements to be measured simultaneously over the whole field of view. To further illustrate this principle, Figure 2-4 shows a sketch of a moiré fringe pattern at an arbitrary point on a stressed specimen [adapted from reference 24].
Figure 2-4. Sketch of a moiré fringe pattern at an arbitrary point on a stressed specimen.

In Figure 2-4, the lines of a deformed specimen grating are superimposed over the lines of a reference grating. Both gratings are assumed to have the same initial pitch, \( p \). The interference, or intersection, of the reference grating and the deformed specimen grating produces the broad, dark moiré fringes. The dashed lines in each grating are assumed to have coincided when the specimen was in the undeformed state. Therefore, the intersection of these two lines has been used to locate the zero order fringe. The zero order fringe indicates the point(s) on the specimen that did not displace. According to the displacement field approach, the moiré fringe located immediately above the zero order fringe is labeled as fringe order 1. All points on this fringe line on the specimen have moved a distance equal to \( p \) in the vertical direction from its original position. Similarly, any point lying on the fringe order of 4 has moved a positive distance of \( 4p \) in the vertical direction from its original position. Conversely, any point lying on the fringe order of -2
has moved a distance of $2p$ in the opposite, or negative, direction from its original position. Using this technique, the relationship between the defined fringe orders and the relative displacement of any point across the surface of a specimen is defined according to equations 2-4 and 2-5.

$$U(x, y) = \frac{1}{f} N_x(x, y)$$  \hspace{1cm} (2-4)

$$V(x, y) = \frac{1}{f} N_y(x, y)$$  \hspace{1cm} (2-5)

Typically, two moiré fringe patterns, or fields, are obtained with orientations perpendicular to the $x$ and $y$ axes. $U$ and $V$ are considered to be magnitudes of the corresponding vector components that point in the $x$ and $y$ directions of displacement, respectively. Therefore, $U$ is defined as the absolute displacement of any point in the $x$ direction. Furthermore, the components of the full-field, in-plane strain profiles can be computed from the derivatives of the displacements (slopes of the displacement surfaces) according to the relationships shown in equations 2-6 through 2-8.

$$\varepsilon_x = \frac{\partial U}{\partial x} = \frac{1}{f} \left[ \frac{\partial N_x}{\partial x} \right]$$ \hspace{1cm} (2-6)

$$\varepsilon_y = \frac{\partial V}{\partial y} = \frac{1}{f} \left[ \frac{\partial N_y}{\partial y} \right]$$ \hspace{1cm} (2-7)

$$\gamma_{xy} = \frac{\partial U}{\partial y} + \frac{\partial V}{\partial x} = \frac{1}{f} \left[ \frac{\partial N_x}{\partial y} + \frac{\partial N_y}{\partial x} \right]$$ \hspace{1cm} (2-8)
Similarly, the in-plane rotation, $\psi$, of any element in the field can be obtained using equation 2-9 [30]:

$$
\psi = \frac{\partial U}{\partial y} - \frac{\partial V}{\partial x} = \frac{1}{f} \left[ \frac{\partial N_x}{\partial y} - \frac{\partial N_y}{\partial x} \right]
$$

(2-9)

Since the direct strain calculations require differentiation of experimental displacement data, a loss in accuracy is unavoidable. Post et al. suggest that a reasonable estimate is if displacements are known to 99% accuracy, the derivatives of displacement can be established to 90% accuracy [25]. Nevertheless, even with this approximate 10% loss in accuracy, because of the high sensitivity and abundance of displacement data from moiré interferometry, reliable strains can be extracted from the displacement fields.

While higher sensitivity strain measurement techniques (moiré interferometry and microscopic moiré interferometry) are also discussed in this study, the equations presented that relate the analysis of moiré fringes to in-plane displacements are identical for each of the techniques. The moiré fringe analysis techniques discussed in the previous pages have focused on the determination of in-plane displacement and strain. However, it should also be noted that different moiré techniques can be utilized to measure out-of-plane displacements. The out-of-plane component, $W$, is typically perpendicular to the original plane of the specimen surface. The $W$ component can be measured independently by classical interferometry methods (e.g., Twyman-Green interferometry), holographic interferometry, shadow moiré, or projection moiré [24]. This research project focused solely on in-plane displacement and strain analysis;
therefore, no further discussion will be presented for the out-of-plane measurement techniques.

2.2 Introduction to Moiré Interferometry

The concept of optical interferometry is based primarily upon the principles of constructive and destructive interference of light. According to Young’s famous double slit experiment, light that is traveling in waves can constructively and destructively interfere with itself in a manner which produces alternating light and dark bands [31]. A combination of the concepts and methods of optical interference with the previously discussed moiré technique provides the basis for moiré interferometry. High-sensitivity moiré interferometry was first introduced by Guild in 1956 as a powerful experimental method for measuring deformation of solid bodies [32]. In moiré interferometry, a reflective diffraction grating, or holographic grating, applied directly to the specimen is utilized in conjunction with a virtual reference grating created by the interference of coherent laser light. Each of these concepts will be discussed in further detail below. The moiré fringe pattern which results from the overlap of these two gratings can then be analyzed using the same methods utilized for the previously described moiré technique.

First of all, moiré interferometry utilizes a diffraction grating, typically a cross-line reflective diffraction grating, for the specimen grating. A diffraction grating divides every incident beam of light into a multiplicity of beams of smaller intensities [31]. An illustration of the effect caused by a reflective diffraction grating is shown in Figure 2-5.
Figure 2-5. Division of a beam of light incident to a reflective diffraction grating.

As shown in the figure, when a beam of light is incident to the diffraction grating (illustrated by the red arrow) at a given angle, $\alpha$, the grating divides it into a series of beams which emerge at preferred angles (illustrated by the black arrows). The divided beams are referred to as diffraction orders and are numbered in sequence beginning with the zero order, not to be confused with the zero order fringe of a moiré pattern. The zero order is always considered to be the mirror reflection of the incident beam for reflective diffraction gratings. The specific angles of the various diffraction orders can then be determined by utilizing equation 2-10,

$$\sin \beta_m = \sin \alpha + m \lambda f$$

(2-10)

where $m$ is the desired diffraction order, $\lambda$ is the wavelength of the incident light, $f$ is the grating frequency, $\alpha$ is the angle of incidence, and $\beta_m$ is the resulting angle of the $m^{th}$ diffraction order.
For stress analysis work, the cross-line reflective diffraction grating is required to be replicated directly on the specimen. The replication process typically utilizes a special mold, which is a cross-line grating itself, and a liquid adhesive. A description of the specialized process required for replication of the grating onto biological materials, such as a human tooth, is included in Chapter 3. Several attributes must exist in a replicated specimen grating in order to ensure accurate experimental test results [25]. First, the grating must be adhered firmly to the specimen in order ensure the surface deformation will be transferred to the grating with high fidelity. Second, the grating must be thin enough to minimize any shear lag between the specimen and grating surface. The resultant grating thickness of the replication process utilized for the biological specimens in this study has been reduced to approximately 1 micrometer. Last, the grating utilized should have high efficiency in the diffraction orders being utilized. Low diffraction orders \((m=\pm1)\) are desired in order to minimize noise and maximize contrast of the resultant fringe pattern. This point will be discussed in greater detail later in this chapter.

The second requirement for moiré interferometry is the use of a virtual reference grating created by the interference of coherent light. A virtual reference grating can be created under a special condition of symmetry where two beams of coherent light illuminate the specimen grating obliquely at angles of \(+\alpha\) and \(-\alpha\). In this condition, the two beams have a common direction of polarization and generate walls of constructive and destructive interference, otherwise known as a virtual reference grating, in their zone of intersection [24]. This interference appears as closely spaced light and dark bars on
the specimen, likened to the reference grating in the traditional moiré technique. The
can be calculated using the following equation:

\[ f_r = \frac{2}{\lambda} \sin \alpha \]  

(2-11)

where \( \alpha \) is the angle of incidence and \( \lambda \) is the wavelength of coherent light.

A typical arrangement representative of moiré interferometry is provided in
Figure 2-6 [adapted from reference 25]. As shown, the two beams of coherent light, \( B_1 \)
and \( B_2 \), illuminate the reflective diffraction grating adhered to the specimen. The
symmetric incident angles, \( +\alpha \) and \( -\alpha \), of the two beams are determined by the diffraction
order relationship provided in equation 2-10. The intersection of the two beams of light
creates the virtual reference grating immediately in front of the specimen surface. In
most applications, the angles of illumination of the incoming beams, \( +\alpha \) and \( -\alpha \), are
initially adjusted so that the diffracted beams emerge normal to the specimen grating. If
the system is properly tuned this initial interference fringe pattern is a null-field.
Therefore, when a load is applied, the specimen deforms and the diffraction grating
replicated on the surface deforms with it. When a specimen is being examined, the
created virtual reference grating interferes with the diffraction orders normal to the
deformed specimen grating. The resulting moiré fringe pattern can then be captured with
a camera.
Generally, near simultaneous pictures of both the $U$ and $V$ displacement fields for a specimen are desired. Together they fully define the in-plane deformation across the entire surface of a specimen. The optical arrangement for the two field moiré interferometer utilized in this research project is shown in Figure 2-7 [obtained from reference 25]. This three-mirror, four-beam system introduced by Czarnek in 1983 provides a compact and easy-to-use system for measurements of displacements in two orthogonal directions almost simultaneously [33]. A large coherent, collimated beam of laser light illuminates the specimen and the three mirrors ($A$, $B$, and $C$). The beam is divided into four parts. Parts $C'$ and $D'$ provide the two symmetric beams of light in the

Figure 2-6. Simplified diagram of a moiré interferometer.
horizontal plane in the same manner as the two beam interferometer discussed earlier. Their diffraction orders interfere, producing a fringe pattern that represents the horizontal component of displacement, $U$. Parts $A'$ and $B'$ provide the two symmetric beams of light in the vertical plane after reflection from mirrors $A$ and $B$, respectively. Thus, the fringe pattern that represents the vertical component of displacement, $V$, is also created. The two patterns can then be recorded by a single camera. The two perpendicular displacement fields can be separated by blocking Parts $A'$ and $B'$ of the collimated beam for the $U$ pattern and $C'$ and $D'$ for the $V$ pattern.

Figure 2-7. Illustration of a three-mirror, four-beam moiré interferometry system.
The sensitivity of experimental displacement and strain measurements using moiré interferometry is strictly controlled by the frequency of the reference grating. The frequency of the reference grating has been defined as a function of the wavelength of coherent light and the angle of incidence (equation 2-11). However, the angle of incidence is also determined by the frequency of the specimen grating (equation 2-10). Therefore, the overall relationship between the frequency of the reference grating \(f_r\), frequency of the specimen grating \(f_s\), angle of incidence \(\alpha\), and wavelength of light \(\lambda\) is provided in equation 2-12 [25]:

\[
f_r = \frac{2}{\lambda} \sin \alpha = 2mf_s
\]

(2-12)

It can also be said that the sensitivity of the moiré technique is determined by the number of fringes generated, \(N_x\), per unit of displacement, \(U\) [25]. This assertion is confirmed by rearranging the displacement equation, \(f = N_x/U\), provided in equation 2-4. Therefore, the sensitivity of the system increases with the frequency of the reference grating, \(f_r\). The reference grating frequency, \(f_r\), typically achieved for a high sensitivity moiré interferometry system is: \(f_r=2400\) lines/mm. Thus, the sensitivity of the system is 2.4 fringes per micrometer of displacement. On the other hand, the contour interval can be calculated as the inverse of sensitivity, \(1/f_r\). The contour interval is defined as the displacement per fringe order of a given system. When \(f_r=2400\) lines/mm, the contour interval for a typical moiré interferometry system is 0.417 \(\mu\)m/fringe order.

While moiré interferometry provides high sensitivity, there still exists an inherent limit to the technique. Inspection of equation 2-12 shows that as the angle of
incidence, $\alpha$, approaches 90°, the frequency of the virtual reference grating is maximized. Therefore, the theoretical limit for the virtual reference grating frequency is dependent solely on the wavelength of the incident light, $f_r = 2/\lambda$. For example, the limit for a reference grating frequency created with coherent green argon-ion laser light of $\lambda=514$ nm is $f_r=3890$ lines/mm. However, this impasse can be resolved by utilizing an alternate refractive medium (other than air).

2.3 Introduction to Microscopic Moiré Interferometry

The microscopic moiré interferometer, also known as an immersion interferometer was developed to increase the basic sensitivity of a moiré interferometry system beyond the previously discussed theoretical limit. The concept of a microscopic moiré interferometer was first introduced by Han and Post in 1992 [26]. This concept is based on the traditional principles and techniques utilized for moiré interferometry, however it allows for significantly increased sensitivity and spatial resolution through the utilization of microscopic analysis of the deformation of stressed materials. The increased sensitivity is achieved through the use of a refractive medium other than air for the creation of the virtual reference grating. A brief discussion of the basic principles and techniques utilized in microscopic moiré interferometry follows.
2.3.1 Increased Sensitivity of Microscopic Moiré Interferometry

The velocity of light in a vacuum, $c$, is provided in equation 2-13,

$$c = \omega \lambda$$  \hspace{1cm} (2-13)

where $\omega$ and $\lambda$ are the frequency and wavelength, respectively, of the given light source in a vacuum [34]. In a refractive medium the velocity of light decreases according to equation 2-14,

$$c_m = \frac{c}{n} = \frac{\omega \lambda}{n}$$  \hspace{1cm} (2-14)

where $c_m$ is the velocity of light in the medium and $n$ is the refractive index of the medium [34]. In addition, since the frequency of a coherent light source is invariant in an electromagnetic spectrum, the wavelength of light must decrease by the same ratio as the velocity [34]. Therefore, the wavelength of light in the refractive medium, $\lambda_m$, can be expressed as shown in equation 2-15.

$$\lambda_m = \frac{\lambda}{n}$$  \hspace{1cm} (2-15)

Using the relationship previously provided in equation 2-12, the frequency of a virtual reference grating generated in a refractive medium, $f_m$, can then be expressed according to equation 2-16.

$$f_m = \frac{2 \sin \alpha}{\lambda_m} = \frac{2n \sin \alpha}{\lambda}$$  \hspace{1cm} (2-16)

Therefore, for a given, coherent light source in a vacuum with a constant wavelength of light, the frequency of the virtual reference grating in a refractive medium is increased by a factor of $n$. The theoretical limit of the sensitivity of the system is also increased by the
same factor. As shown earlier, the theoretical limit for the frequency of a reference grating created with coherent green argon-ion laser light, typically used in microscopic moiré interferometry, of $\lambda=514 \text{ nm}$ is $f_r=3890 \text{ lines/mm}$. However, the wavelength of the laser light is reduced to $\lambda=338 \text{ nm}$ in optical glass with a refractive index of 1.52. Thus, the theoretical limit for the virtual reference grating frequency, $f_r$, is increased to 5910 lines/mm in the optical glass.

2.3.2 Optical Configuration of the Microscopic Moiré Interferometer

Figure 2-8 shows the configuration of a basic microscopic moiré interferometer [26]. The immersion interferometer is typically a prism fabricated from optical material. The collimated, coherent laser beam enters the interferometer from the inclined plane on the right side of the glass. The lower half of the beam is aimed directly at the surface of the specimen, while the upper half of the beam is aimed at the mirrorized surface on the left side of the glass. The reflected upper portion of the beam is then incident to the specimen at an angle symmetrical to the lower half of the beam; this is likened to the conditions required for traditional moiré interferometry. The interference of the two halves of the beam then creates the virtual reference grating inside the refractive medium of the immersion interferometer.
Figure 2-8. Basic configuration of a microscopic moiré interferometer.

The gap between the immersion interferometer and the specimen is filled with an immersion fluid, typically water. The immersion fluid is necessary to avoid complete internal reflection of the laser beam inside the interferometer. When the index of refraction of the immersion fluid is different than that of the immersion interferometer, the angle of the incident beam changes slightly, as shown in Figure 2-9. A reasonable match of the index of refraction between the immersion interferometer and immersion fluid is desired. However, Snell’s law, given in equation 2-17, shows that the frequency of the virtual reference grating is not altered due to this slight change in the angle of incidence [31].

\[ n \sin \alpha = n' \sin \alpha' \]  

(2-17)
Figure 2-9. Effect of Snell’s law between immersion interferometer and immersion fluid.

The complete optical and mechanical arrangement of the microscopic moiré interferometry system proposed by Han and Post is shown in Figure 2-10 [obtained from reference 25]. As shown in the illustration, the incident light for the specimen is provided by a laser light source and is conducted to the specimen via two optical fibers. Two small collimating lenses are then mounted above the immersion interferometer at the appropriate angle of incidence. The compact, four-beam moiré immersion interferometer allows for creation of virtual reference gratings in two orthogonal directions, which in turn allows for analysis of the specimen in both the $U$ and $V$ displacement fields. The interferometer, illuminators, and microscope are all connected to a heavy-duty $x$, $y$ transverse to position the system over the desired portion of the specimen. The imaging system used to collect the fringe pattern is composed of a microscope objective lens and a CCD video camera. The specimen is affixed to a loading frame, which in turn is...
mounted on a rotary table for fine adjustments of angular orientation [26]. Ancillary equipment includes a personal computer with a frame grabber board and TV monitors for microscopic inspection of the specimen.

![Diagram of microscopic moiré interferometry system proposed by Han and Post.]

**Figure 2-10.** Illustration of the arrangement of the microscopic moiré interferometry system proposed by Han and Post.

The previously published work that was conducted using the microscopic moiré interferometry system was performed with coherent laser light of wavelength, $\lambda=514$ nm in air, or $\lambda_m=338$ nm in the optical glass [26]. With an incident angle of $\alpha=54^\circ$, a virtual reference grating was produced with a frequency of $f_r=4800$ lines/mm [26,35]. The corresponding sensitivity of the system is 4.8 fringes/$\mu$m of displacement, and the
contour interval is 0.208 µm/fringe order. Thus, the sensitivity is effectively doubled over that of traditional moiré interferometry. This also exceeds the previously conceived theoretical limit for traditional moiré interferometry [35]. It should also be noted that using this system, Han and Post achieved a spatial resolution with the capability of analyzing zones nearing 400 µm [26]. However, due to the microscopic nature of the biological materials being evaluated in this research study, a system with the capability of even greater spatial resolution was desired.

2.4 Development of the Modified Microscopic Moiré Interferometry System

Two primary advantages of a microscopic moiré interferometry system have been shown that provide significant improvement over traditional moiré analysis methods: the considerable increase in displacement measurement sensitivity and the improved detail of its viewing area through the use of a microscope objective. However, due to the microscopic nature of many biological materials, including the human tooth, the objective of this research project was to modify and enhance the capabilities of the previously described system. As shown in Chapter 1, the features of the human tooth which require examination are truly microscopic, with the width of the DEJ zone being measured as small as 2 µm by nanoscratch testing and the diameter of a dentin tubule ranging from 5 to 15 µm [3,11]. Therefore, the zone of deformation in the stressed specimen also has the potential of being microscopic (2–3 µm). The sensitivity of the previously described microscopic moiré interferometry system (4.8 fringes/µm of displacement) was considered to be sufficient for the purposes of this research project.
However, the spatial resolution capability of the system required an increase of greater than an order of magnitude above that of the previously published work.

The addition of a microscope objective in the microscopic moiré interferometry system allows the limit on the size and detail of the viewing area to be restricted only by the capability of the optics employed. Therefore, the initial objective of this research study seemed relatively simple; modify the current system to allow for the utilization of a higher magnification microscope objective. The higher magnification objective should then relate directly to an increase in the spatial resolution capability of the overall system. However, this seemingly simple modification led to an eventual in-depth analysis and enhancement of nearly every component in the previously described microscopic moiré interferometry system.

2.4.1 Overall Design of the Modified Microscopic Moiré Interferometry System

A final schematic of the modified microscopic moiré interferometry system developed through this research project is presented in Figure 2-11. An argon-ion laser is used to provide green laser light, $\lambda=514$ nm, for creation of the virtual reference grating and illumination of the specimen. A spatial filter assembly consisting of an inverted microscope objective and a pinhole aperture is used to obtain a brighter, cleaner expanding beam. The beam is then collimated and directed by way of several mirrors into the interferometer at the desired angle of incidence for proper reflection and interference. The translating mirror is used to switch the beam between the $U$ and $V$ displacement fields. This provides the capability of near simultaneous inspection of both
orthogonal directions on the surface of the specimen without causing any change in the specimen orientation or rotation.

**Figure 2-11.** Schematic of the modified microscopic moiré interferometry system.

Throughout the course of analysis and enhancement of the microscopic moiré interferometry system, several somewhat reoccurring challenges were encountered. Rather than document the chronological series of modifications to the system or a sequential description of each of the modified components, the following section summarizes the primary obstacles encountered along with the corresponding required modifications. While several additional areas required attention and enhancement that are not included in this research summary, the four key obstacles that necessitated the most consideration were the modification of the immersion interferometer design to
allow for the utilization of a higher magnification microscope objective, the increase in intensity of light throughout the entire system, the vibration isolation of the overall system, and the proper alignment of the optical system.

2.4.2 Design of the Modified Microscopic Moiré Immersion Interferometer

The primary limitation to the previous immersion interferometer designs published by Han and Post were their incompatibility with a high-magnification microscope objective. Even long working distance microscope objectives experience a drastic decrease in working distance as the magnification capability is increased. To clarify, the working distance of a microscope is defined as the space between the objective and specimen under investigation required for proper focus to be achieved. For instance, the Mitutoyo 100x microscope objective utilized in this research project had a working distance of 6 mm, as opposed to the Mitutoyo 20x objective which had a working distance of 20 mm. Previously, the experimental results published by Han and Post using microscopic moiré interferometry were obtained using a 10x microscope objective lens [26].

The two immersion interferometer designs utilized by the Clemson Photomechanics Laboratory prior to the commencement of this research project are shown in Figure 2-12. For each interferometer configuration, a diagram is also included which shows the required path of the coherent laser light, the area in which the virtual reference grating is created, the surfaces which are required to be mirrorized, and the approximate thickness of each of the interferometer designs. As shown in the figure,
both available interferometers had thicknesses greater than the working distance required for the 100x microscope objective. Configuration #2 could be used with objective magnifications of 20x or lower, while Configuration #1 could only be used with objective magnifications of 10x or lower. Therefore, the redesign of a new, modified microscopic moiré immersion interferometer was necessitated.

![Diagram](image)

(A) Configuration #1

![Diagram](image)

(B) Configuration #2

**Figure 2-12.** Two previous designs of microscopic moiré immersion interferometers.

Figure 2-13 illustrates the modified design of the microscopic moiré immersion interferometer. To accommodate the 6 mm working distance of the 100x microscope
objective, the modified immersion interferometer design consists of a fused-silica prism attached to a long, shallow rectangular base of fused-silica optical glass. The primary conceptual difference from the previously utilized designs is that the incident beam does not follow a direct path to the specimen surface. The incident beam takes two extra reflections with the assistance of the two mirrorized surfaces on the top and bottom surfaces of the base of the interferometer. This modification allows the high-magnification objective to be lowered below the height of the prism and get extremely close to the surface of the base of the interferometer. The objective can then focus clearly on the specimen while still allowing for a small amount of lateral and vertical movement. The design methodology utilized in the development of the interferometer follows.

**Figure 2-13.** Illustration of modified microscopic moiré immersion interferometer.
Optimization of the design of the modified interferometer was conducted utilizing a program developed with Microsoft Excel and the assistance of Dr. Wood. The required inputs for the program included the thickness and refractive index of the interferometer base, size and refractive index of the prisms, placement of the prism on the interferometer base, and the width of the incoming collimated laser beam. Two of the output plots from the program are shown Figure 2-14. The program had the capability of computing the desired parameters, which included the trajectories of the reflected beams (Plot A), the projected viewing area of the microscope (green square in bottom corner of Plot B), and the arrangement of the prisms on the base of the interferometer (Plot B).
Figure 2-14. Outputs of Microsoft Excel program showing optimized design of the modified immersion interferometer.

The primary objective in the development of the optimization program was to minimize the effect of back reflections in the zone of interference of the modified immersion interferometer. The bright pink beam in Plot (A) of Figure 2-14 shows the zero order diffraction, or reflection, of the orange incident beam. The plot shows that a portion of the beam reflects back as the beam exits the prism. Similarly, the dark green
beam shows the back reflection of the light green beam. When a beam of light passes through an interface of two differing refractive indexes, a portion of the beam is transmitted, $T$, and the remainder of the beam is reflected, $R$, such that $T + R = 1$. The fraction of the beam that is reflected, $R$, is given in equation 2-18,

$$R = \left( \frac{n_r - n_i}{n_r + n_i} \right)^2$$

(2-18)

where $n_r$ is the refractive index of the material through which the beam is traveling and $n_i$ is the incident material at the interface [31]. Thus, at the interface between the fused silica prism and air, approximately 3.5% of the outgoing beam would be reflected back into the interferometer. Initially this percentage does not seem high, but with the combination of the high intensity laser light and the highly sensitive CCD camera any stray beams could significantly affect the quality of a captured image.

Therefore, the design of the modified immersion interferometer was optimized to ensure that any such back reflections were contained either in the prism or in the area of the base immediately below the prism. This design required that one edge of the base, as shown in Figure 2-13, be polished (with no beveling) and mirrorized to ensure adequate reflection for creation of the virtual reference grating. The opposite edge of the base was then kept in the “ground” state, also shown in Figure 2-13. The ground glass edge helped to dissimilate the coherent beam of light and prevent any further back reflections.

Picture (A) of Figure 2-15 shows a photograph of the modified immersion interferometer. The very compact, four-beam immersion interferometer allows for creation of virtual reference gratings in two orthogonal directions, which in turn allows
for analysis of the specimen in both the $U$ and $V$ displacement fields. Picture (B) shows how closely the interferometer and the 100x microscope objective fit together. The base of the interferometer was fabricated using a fused silica optical window, $n=1.458$, and was polished on the top and bottom faces (as well as the two edges noted earlier) to a surface finish of $\lambda/2$. The dimensions of the base were $31.0\pm0.1\text{mm} \times 31.0\pm0.1\text{mm} \times 4.0\pm0.05\text{mm}$. The two right angle prisms were also fabricated using fused silica optical glass. The prism size was 12.5 mm, measured along the short dimension of the right angle. The two prisms were bonded to the base of the interferometer using Type SK-9 Lens Bond (Summers Optical, Fort Washington, PA). Lens Bond is a very low viscosity adhesive which practically eliminated any air bubble problems. The refractive index of the Lens Bond was $n=1.49$. The difference in refractive indexes between the prism / base material and Lens Bond was deemed not to have any detrimental effects to the performance of the interferometer. The holder was attached to the base of the interferometer using Norland Optical Adhesive 81 (Norland Products Inc, Cranberry, NJ). This adhesive cures to a hard film but will not become brittle. The small amount of resiliency in the adhesive also provided strain relief from any vibrations. The toughness of the adhesive insured the required long term performance of the bond. The mirrorized surfaces (shown in Figure 2-13 and Figure 2-15) were produced by vacuum deposition in the Clemson Photomechanics Laboratory. A clear, thin layer of the optical cement was then coated over the mirrorized surfaces to prevent scratching during contact of the interferometer with the specimen during experimental testing.
It should also be noted at this time that total internal reflection inside a refractive medium is obtained when incident angles are greater than the critical angle. The critical angle, $\theta_c$, can be derived from the law of refraction as shown equation 2-19,

$$\theta_c = \sin^{-1}\left(\frac{1}{n}\right)$$  \hspace{1cm} (2-19)

where $n$ is the refractive index of the medium [31]. Using this equation, the critical angle for the fused silica optical glass used in this study is approximately 43°. Therefore, the top and bottom faces of the base of the interferometer did not need to be mirrorized. However, the vacuum deposition was performed to ensure maximum internal reflectivity of the beam and to avoid potential problems should any external substances (dust or immersion fluid) spread outside the viewing area of the interferometer and disturb the reflective areas.

**Figure 2-15.** Modified immersion interferometer with 100x microscope objective.
The modified microscopic moiré immersion interferometer was designed to create a virtual reference grating with a frequency of \( f_r = 4800 \) lines/mm. According to the definition for the frequency of a virtual reference grating in a refractive medium given in equation 2-16, the required parameters for proper use of the modified immersion interferometer are: refractive index of interferometer equal to \( n = 1.458 \), wavelength of coherent laser light equal to \( \lambda = 514 \) nm, and incident angle into the interferometer equal to \( \alpha = 58^\circ \). Enough flexibility was factored into the design of the interferometer to allow for the incident angle to be adjusted between 52º and 64º. This flexibility enabled a ±10% variation in the frequency of the virtual reference grating. Therefore, carrier patterns could be used to subtract off uniform strain fields in either direction of displacement to extend the dynamic range of measurement to ±10% strain. A carrier pattern is defined as a uniform array of fringes which can be added to a specimen prior to loading. These initial fringes are changed by the deformation of a specimen under loading and are said to “carry” the displacement information. This technique is especially useful in cases of small deformations [25].

2.4.3 Increase of Light Intensity throughout the Entire System

The next significant modification to the microscopic moiré interferometry system was the necessary increase of light intensity throughout the entire system. With the utilization of higher magnification microscope objectives, such as the 100x objective, the intensity of light required to capture a quality fringe pattern significantly increases. In the microscopic moiré interferometry system proposed by Han and Post, optical fibers
were utilized for the transmission of laser light from the source to the specimen [26]. An illustration of how the optical fibers and individual collimating lenses are used to illuminate each displacement field of the immersion interferometer is shown in Figure 2-10.

An optical fiber is fabricated from three primary components: the core, a thin glass center where the light travels; the cladding, an optical material surrounding the core; and the buffer, a protective coating around the outside [36]. The light travels through the core by constantly reflecting off the cladding. While the cladding does not absorb any light from the core, the light signal can degrade within the fiber, mostly due to impurities in the glass [36]. The extent of degradation depends upon the purity of the glass core and wavelength of light. Therefore, maximizing the amount of light efficiency into and out of the optical fibers was the original focus for increasing the intensity of light. To maximize efficiency, the ends needed to be sliced exactly perpendicular to the length of the optical fibers. In addition, the input end of the fiber required precise alignment with the laser light source. After the completion of both efforts, the intensity of the light improved, but still was not good enough to capture a high quality fringe pattern.

Therefore, the decision was made to disregard the optical fibers and to transmit the light from the laser source to the immersion interferometer through a series of reflections. This modification required the addition of three necessary elements: a spatial filter, a single collimating lens, and a series of mirrors. A spatial filter is a relatively simple assembly consisting of an inverted microscope objective and a pinhole aperture.
By aligning the aperture precisely at the focal point of the inverted microscope objective, a brighter, cleaner expanding beam can be obtained. It was found that a 2 µm diameter aperture provided the highest quality results. Figure 2-16 shows a picture of the spatial filter assembly used in this research study.

Figure 2-16. Spatial filter assembly and collimating lens.

Several iterations of collimating lenses of varying diameters and focal lengths were evaluated. After experimentation was performed with each of the lenses available in the Clemson Photomechanics Laboratory, it was concluded that the maximum intensity of light resulted from the smallest diameter lens with the longest focal length. The smaller diameter lens directed the maximum amount of light to the interference zone of the interferometer, thus, no extra light was “wasted” illuminating zones outside the
viewing area. Therefore, in an attempt to maximize the efficiency of the system, a special 6.35 mm diameter / 25.4 mm effective focal length precision achromatic doublet lens was ordered and employed in the system (Newport Corporation, Irvine, CA). A picture of the collimating lens (circled) is included in Figure 2-16.

Finally, a series of mirrors were required to direct the collimated laser beam to the immersion interferometer. A considerable effort was made to ensure that the path of the beam traveled parallel to the surface of the table and that only perpendicular changes of direction were made where necessary. This caution was essential in order to conserve equal polarization of the collimated laser light in each direction. Equal polarization of light is required for consistent constructive and destructive interference of light in the virtual reference grating [25]. Thus, without equal polarization, the resultant fringe pattern will have a higher contrast in one direction than the other. A translating mirror is utilized to switch the beam between the $U$ and $V$ displacement fields. A picture of how the mirrors are utilized to direct the collimated laser beam along the appropriate path and into the interferometer is shown in Figure 2-17. The path of the laser beam has been drawn for clarity purposes.
2.4.4 Vibration Isolation of the Overall System

Excellent vibration isolation is required to maintain stability of the optical elements and to ensure that fringe patterns of high quality are obtained. Microscopic moiré interferometry measures minute displacements, therefore, any inadvertent vibrations can cause fringes to dance at the vibration frequency. An optical table with pneumatic vibration dampers was utilized to provide a base for mounting the equipment of the microscopic moiré interferometry system. However, as spatial resolution of the system was increased through the use of the 100x microscope objective and the modified immersion interferometer, the subtle vibrations which previously could not be seen were now intensified and required to be dampened.

The initial cause of significant vibration was determined to be the laser source. The argon-ion laser source employed an external fan for cooling the laser heads while in
operation. A heavy-duty plastic ventilation tube was used to connect the two parts. While the fan was kept off the table, it was determined that the heavy-duty ventilation tube was transmitting the vibration of the fan through the housing for the laser and to the table. A simple move of the fan to an elevated height on the wall and replacement of the heavy-duty plastic tube with a lighter-weight ventilation hose provided a somewhat easy solution.

The second, and primary, source of vibration in the system was the base and mounting fixtures for the interferometer and optical system. This source of vibration confirmed that the newly discovered vibrations were a result of the increased spatial resolution capability of the system since the existing base and fixtures were adequate for lower spatial resolution experimentation. It was determined that both the interferometer holder and the shelf used to hold the optics system experienced a “diving board effect.” The moment caused by the relatively large load of the optics system and the long arm of the existing interferometer holder, respectively, caused a prolonged vibration in the system any time the optical table was bumped. This vibration would eventually cease if the entire system remained motionless. To correct this problem, heavy-duty reinforcement supports were affixed to the base of the system. For the optical system, a heavy-duty fixture was fabricated which included a counter-balance of the same moment as the system. For the interferometer, a heavy-duty fixture was constructed which minimized the moment arm for the interferometer holder. In addition, two additional degrees of freedom were designed into each of the fixtures to assist with the alignment issues which will be discussed in further detail in the following section.
One additional source of movement in the system was determined to come from the immersion fluid between the interferometer and the specimen. Tap water was originally used as the immersion fluid for all experimentation. However, as the previous vibrations in the system were isolated and dampened it was discovered that when experimentation was performed on biological specimens for extended periods of time, small particles and active microscopic organisms were being drawn out of the specimen and moving across the specimen surface. Several trials were run using various index matching oils for the immersion fluids with varying levels of success. However, due the inherent difficulty of working with oils in a clean room environment and the difficulty of cleaning the interferometer after each round of experimentation, it was determined that the use of distilled water as the immersion fluid which could be flushed somewhat frequently over the course of an experimental run provided satisfactory results.

2.4.5 Proper Alignment of the Optical System

Unfortunately, after the design and fabrication of the modified immersion interferometer was completed and the light intensity and vibration concerns were resolved, several additional issues arose which resulted either directly or indirectly from the short working distance of the 100x microscope objective. Figure 2-18 shows an example of an early fringe picture which was taken utilizing the 100x objective in conjunction with the modified immersion interferometer. As shown in the figure, an overwhelming amount of background patterns and extraneous grid lines now made it difficult locate the either desired fringe pattern or features on the specimen surface. As
shown in the picture, at least two different levels of undesired noise made the fringe pattern nearly impossible to interpret. Therefore, several rounds of analysis were conducted through the entire microscopic moiré interferometry system in order to increase the clarity and contrast of the fringe patterns.

![Fringe pattern image](image)

**Figure 2-18.** Example of early fringe picture captured using 100x objective and modified immersion interferometer design.

The first, and perhaps most beneficial, enhancement dealt with the specimen grating frequency. With a virtual reference grating frequency of 4800 lines/mm, as is achieved with a microscopic moiré interferometry system, the ideal specimen grating frequency is 2400 lines/mm. Such a grating would function in its $m=\pm1$ diffraction orders. However, only cross-line diffraction gratings with a frequency of 1200 lines/mm
were readily available in the Clemson Photomechanics Laboratory for experimental use. Initially this did not pose a problem since such gratings are satisfactory to use in the $m=\pm 2$ diffraction orders. Although, due to its shorter working distance the 100x microscope objective actually collected the two extra diffraction orders of the 1200 lines/mm specimen grating. Figure 2-19 presents a simplified illustration which displays the root of this problem. The two extra diffraction orders collected by the microscope objective were then interfering inside the optical system which appeared as overwhelming grid lines in the background of the fringe pictures. Therefore, a cross-line diffraction grating with a frequency of $f_s=2400$ lines/mm was purchased and replicated for experimental use. The harsh grid lines shown in Figure 2-18 immediately disappeared. As an added bonus, doubling the specimen grating frequency also increased the overall intensity of the resultant fringe picture. This intensity increase is a direct result of the lack of formation of the extra two diffraction orders. Due to its optimal diffraction efficiency and minimal creation of noise, the addition of the 2400 lines/mm specimen grating is considered one of the most beneficial additions to the modified microscopic moiré interferometry system.
Even after the addition of the 2400 lines/mm specimen grating, several less obvious background patterns and grid lines remained. Through many iterations of analysis and experimentation, it was determined that each of the extraneous patterns were a result of various forms of misalignment in the system. Figure 2-20 shows two of the most common patterns which developed as a result of misalignment. As can be seen, the overwhelming pattern caused by the 1200 lines/mm specimen grating is no longer evident. However, as outlined on each of the pictures, a near vertical pattern is evident in picture (A), and a circular pattern is evident in picture (B).
Figure 2-20. Examples of background patterns resulting from misalignment in the system.

The vertical pattern in picture (A) was determined to be a product of misalignment of the modified immersion interferometer. The optimization program developed in the design stage for the interferometer excellently predicted the path of the back reflections in a perfectly aligned system. However, if the interferometer was even slightly rotated along the \( x \) or \( y \) axis of the system, the path of the back reflections would alter and eventually escape through the viewing area. Furthermore, the existing mounting fixture for the interferometer only possessed the capability for two degrees of freedom (translation along the \( x \) and \( y \) axis). Therefore, a new mounting fixture was designed that was both vibration resistant (as described earlier) and included all six degrees of freedom. Two pictures of the modified mounting fixture are included in Figure 2-21. This modification ensured the capability of the interferometer to be aligned and calibrated.
concurrently with the incoming beam of light, the specimen grating, and the microscope objective.

**Figure 2-21.** Modified mounting fixture for the immersion interferometer.

The circular background patterns shown in picture (B) of Figure 2-20 were determined to result from the misalignment of the optical system. Initially, the optical system had the capability of just one degree of freedom (translation along the $z$ axis), which enabled the system to focus on the specimen surface. Therefore, the most beneficial modification for the optical system was the addition of two degrees of freedom to the mounting fixture (translation along the $x$ and $y$ axis), as noted earlier. In addition, several upgrades were made to the individual components of the optical system. The complete system utilized by the Clemson Photomechanics Laboratory prior to the commencement of this research project consisted of the microscope objective, zoom
assembly, adaptor tube, and CCD camera. The first significant upgrade was the purchase of an ultra-zoom assembly with a built-in adjustable iris (Navitar, Inc., Rochester, NY). The ultra-zoom assembly featured an analog adjustment between zoom levels of 1x and 5x, which corresponded to a spatial resolution capability of analyzing zones from 110 µm to 22 µm when coupled with the 100x microscope objective. The built-in adjustable iris had the ability of closing down at the focal point of the collected picture in order to block any unwanted beams or reflections. Thus, the iris worked similarly to the pinhole aperture in a spatial filter assembly. In addition, a new longer adapter tube was purchased which increased the resolution of the collected picture. A picture of the upgraded optical system is included in picture (A) of Figure 2-22. Together, the closed-arrangement consisting of the microscope objective, ultra-zoom assembly with adjustable iris, adaptor tube, and CCD camera worked well when the system was properly aligned. However, proper alignment seemed to be an ongoing struggle. The slightest misalignment of any part of the entire modified microscopic moiré interferometry system, including the spatial filter, incoming angle, specimen and interferometer orientation, and finally the objective and camera placement, could cause detrimental end results. In addition, the built-in adjustable iris worked as described only at the 3x zoom level. If the zoom was set to a higher or lower level, the focal point in the optical system would shift and closing the iris would cause the diameter of the collected picture to get smaller and eventually disappear.
Figure 2-22. Examples of the closed (A) and open (B) arrangements of the optical system.

Therefore, additional experimentation was performed with the optical system in an open-arrangement, as shown in picture (B) of Figure 2-22. In this modified arrangement, the adapter tube was removed from the system and an additional lens was placed immediately above the ultra-zoom assembly. The built-in adjustable iris was then left open and not utilized. At the highest zoom level (5x), the lens assisted with the formation of a focal point of the collected picture approximately 8 inches above the zoom.
assembly. An additional manual adjustable iris could then be placed at the newly formed focal point of the collected picture to effectively block out any unwanted beams or reflections. A similar arrangement was also developed when the zoom was set to the lowest level (1x). Since the additional lens, additional iris, and CCD camera were all independently adjustable, this open-arrangement was much more forgiving to misalignments in the rest of the modified microscopic moiré interferometry system. This optical set-up worked very well and some of the clearest fringe pictures were obtained using the open-arrangement. However, the additional weight and height of the fixturing that was required to hold the supplementary lens and iris, as well as the CCD camera, caused minor vibrations in the system, especially at higher zoom levels. Also, since the CCD camera was not used in a closed system, it was very sensitive to ambient light which could diminish the contrast in many of the fringe pictures. Therefore, while the open-arrangement ensured the highest quality fringe pictures, it was determined that the ease and consistency of the closed-arrangement provided the preferred optical system set-up for experimentation purposes.

The last source of extraneous background patterns was determined to be the actual specimen grating. On many of the specimens, small spots were observed with a pattern of concentric circles radiating outward. These spots were found to result from imperfections in the grating or the grating replication procedure. Excessive care must be used in the grating replication process to produce gratings of equal thickness and minimal flaws. With the moiré technique the deformation of the specimen surface is sought, however the deformation on the outside surface of the grating is actually observed and
measured. In this research project, the procedure developed by Dr. Wood for replication of a specimen grating onto biological materials was utilized to reduce the specimen grating thickness. However, perfecting this procedure was found to be extremely difficult. More detail about this replication procedure will be provided in Chapter 3.

2.4.6 Resultant Fringe Pictures of Completed System

Figure 2-23 through Figure 2-26 shows several examples of fringe patterns captured with the modified microscopic moiré interferometry system. These pictures demonstrate the increased spatial resolution capability of the overall system, with the scale of the image zones ranging from 500 µm to 22 µm in width. Each set of pictures also demonstrate the capability of the system to capture near simultaneous fringe patterns in both the $U$ and $V$ displacement fields. While the pictures were taken under a no-load condition, carrier patterns were added to show the improved clarity and contrast of the fringes. It is evident that through proper alignment of the entire system, the extraneous grid lines and background patterns have been removed from the fringe patterns. However, it should also be noted that several pictures have spots and minor patterns resulting from imperfections in the specimen grating. Nevertheless, the following pictures and fringe patterns were captured during experimentation on the zone surrounding the DEJ of a human tooth. The specific patterns have been selected for inclusion in this research summary because of the clear definition of the DEJ zone and the obvious differences in surface quality of the dentin and enamel substrates.
The three pictures shown in Figure 2-23 were captured using the 20x microscope objective with the modified immersion interferometer. The 500 µm scale represents the approximate spatial resolution capability of the microscopic moiré interferometry system proposed by Han and Post [25]. Pictures (A) and (B) show the $U$ and $V$ displacement fields, respectively. Picture (C) shows the same area of the specimen surface illuminated with white light. It is evident from the pictures that the enamel has a smoother cast across the surface while the dentin has a more porous appearance where the tubules emerge to the surface. The DEJ has been highlighted on the two fringe patterns for clarity purposes.
Figure 2-23. Fringe patterns across the DEJ of a human tooth at 500 µm scale.

Pictures (A) and (B) of Figure 2-24 show the $U$ and $V$ displacement field fringe patterns, respectively, at the 110 µm scale. The corresponding pictures were captured using the 100x microscope objective at the 1x zoom level. Once again, the DEJ in the picture has been highlighted on the two fringe patterns for clarity purposes. Also, while less obvious, the differences in surface quality between the dentin and enamel substrates can still be observed.
Figure 2-24. Fringe patterns across DEJ of a human tooth at 110 µm scale.

Pictures (A) and (B) of Figure 2-25 show the $U$ and $V$ displacement field fringe patterns, respectively, at the 50 µm scale. The corresponding pictures were captured using the 100x microscope objective at a 3x zoom level. As was noted earlier, this is the only zoom level where the built-in adjustable iris on the ultra-zoom assembly works as desired. The increased contrast and picture clarity from the partially closed iris helps to clearly display the surface features of the dentin substrate.
Figure 2-25. Fringe patterns across dentin in a human tooth at 50 µm scale.

Finally, pictures (A) and (B) of Figure 2-26 show the $U$ and $V$ displacement field fringe patterns, respectively, at the 22 µm scale. The corresponding pictures were captured using the 100x microscope objective at a 5x zoom level. This combination of objective and zoom level represents the maximum spatial resolution capability of the modified microscopic moiré interferometry system. The two fringe patterns were captured using the closed-arrangement of the optical system. Close inspection of both fields shows that a faint orange peel-like pattern is apparent across the specimen. This pattern is believed to be caused by multiple internal reflections between the elements contained in the ultra-zoom assembly. Typically, the broadband antireflection coating on the lens elements is not adequate to eliminate all reflectance in high intensity systems. Such patterns are only visible at the highest magnification levels and in lower intensity fringe patterns. However, this faint pattern does not disrupt the quality of the image and
is not considered detrimental to the experimental results. Therefore, while the picture clarity may not be as high as if the open-arrangement optical set-up were utilized, the surface features of the underlying dentin substrate are still visible and the contrast of the fringe pattern is still fine enough for quality experimentation purposes.

Figure 2-26. Fringe patterns across dentin in a human tooth at 22 µm scale (maximum spatial resolution capability).

2.5 Summary

A modified microscopic moiré interferometry system has been developed for specialized minute deformation analysis of biological materials. This modified system expands the spatial resolution capabilities beyond the microscopic moiré interferometry system developed by Han and Post. The previously developed microscopic moiré interferometry system achieved a spatial resolution with a capability of analyzing zones
nearing 400 µm [26]. However, utilizing the modified system, a displacement sensitivity of 4.8 fringes/µm of displacement has been attained while increasing the spatial resolution with the ability of analyzing zones as small as 22 µm in width. This significant increase is a result of the in-depth analysis, modification, and enhancement of nearly every component in the microscopic moiré interferometry system. The modified system allows for real-time full-field in-plane displacement and strain analysis of microscopic specimens. In addition, the use of a compact, four beam modified immersion interferometer allows for the near simultaneous analysis of the specimen in both the $U$ and $V$ displacement fields. The next chapter will outline the system validation test in which the Poisson’s ratio of human dentin was experimentally determined.
CHAPTER 3

SYSTEM VALIDATION – EXPERIMENTAL DETERMINATION OF
POISSON’S RATIO AND THE ELASTIC MODULUS OF HUMAN DENTIN

3.1 Material Testing at the Microscopic Scale

One of the most important tasks in a material testing and analysis laboratory is the experimental determination of the mechanical properties of materials, such as the elastic constants. For constants such as Poisson’s ratio (negative transverse strain divided by axial strain) and the elastic modulus (axial stress divided by axial strain) precise determination of stress and strain is essential. In the case of metals, plastics, and other isotropic, homogeneous materials where the stress and strain distribution are predictable and uniform, strain gauges and extensometers provide sufficient information about the deformation of a specimen and allow easy measurement of the elastic constants. However, biological materials such as the dentin substrate of a human tooth are anything but isotropic or homogeneous. In addition since many of the structures and properties of biological materials are microscopic in nature, traditional testing methods provide only composite averages of the properties being evaluated. Chapter One of this thesis outlined the intrinsic difficulties and limitations involved with the use of strain gauges and other traditional material property testing methods on biological materials. The necessary assumptions involved with such testing methods can be highly uncertain and can lead to significant errors and variability in experimental data.
Czarnek reported that laboratory tests conducted on three specimens of non-isotropic, non-homogeneous composite samples using moiré interferometry gave better quality information about the elastic modulus and Poisson’s ratio than tens of specimens tested with conventional instrumentation [37]. Since the moiré technique provides full-field information about the distribution of displacements across the surface of a specimen, the full-field distribution of strains can then be obtained through simple differentiation. From this distribution, one can either calculate the average value of strain or closer inspect the non-uniformities across the evaluation area. Therefore, any errant data can be discarded rather than statistically averaged. The addition of a microscope objective in the microscopic moiré interferometry system allows the limit on the size and detail of the evaluation area to be restricted only by the capability of the optical system employed.

Initially, a series of validation experiments were performed on material with known mechanical properties. In this chapter, an additional system validation experiment is developed where two of the elastic constants of human dentin are experimentally determined. The primary purpose of this trial was to exhibit the application of the modified microscopic moiré interferometry system to experimentation with biological materials. Therefore, the focus of this chapter will be the enhanced system capabilities and the procedure used to demonstrate that the modified system possesses the ability to directly determine the mechanical properties of microscopic biological specimens in a quick and accurate manner. Each required step for the preparation and set-up of the experiment as well as the collection and reduction of data will be discussed.
3.2 Experimental Preparation

In addition to the preparation and alignment of the modified microscopic moiré interferometry system which was discussed in Chapter 2, two key system enhancements were required for experimentation with biological specimens. First of all, a micromechanical test fixture was designed and fabricated for compressive loading of the specimen. Second, an improved process was utilized for replication of the diffraction grating onto the specimen. In each case the modifications and enhancements were governed by the naturally miniature size of the specimen, the necessity for proper hydration levels in the specimen through all test phases, and trouble free implementation into the modified system. This section illustrates the steps employed in the development of the micromechanical test fixture and discusses the general procedure used for proper specimen grating replication.

3.2.1 Micromechanical Test Fixture

The micromechanical test fixture was developed specifically for use with the modified microscopic moiré interferometry system to examine biological specimens ranging from several centimeters to just a few millimeters in size. Since dental samples generally function under a combination of compressive and shear loading conditions, the development of a compressive loading and unloading test fixture was deemed adequate. Due to the limited size of the dental specimens, tensile examination was not considered to be practical. In addition, the standard uni-axial examination of materials utilizes “dog-bone” shaped specimens. However, due to the size and nature of the dental specimens it
was impractical to obtain and examine specimens in such a manner. Therefore, the test fixture was designed for strictly rectangular specimen profiles.

The micromechanical test fixture is shown in Figure 3-1. As illustrated, two fine threaded screws, 100 threads per inch, were used for loading and unloading the specimen. The loading rate applied to the specimen was further reduced by the introduction of a wedge configuration, which simply translated in the provided groove and transferred the vertical loading force of the fine threaded screw to the horizontal force incident to the specimen. This reduction of loading rate was necessary due to the typically miniature size of the specimen and the high sensitivity to displacement measurements inherent to the modified system. Any component which translated relative to a different component during the loading process was fabricated from bead blasted stainless steel to reduce the resultant coefficient of friction. A sub-miniature load cell (Entran, Model ELFS-T4) with the capability of measuring loads in both compression and tension was then mounted directly in-line with the specimen. Overall, the developed configuration provided a relatively wide compressive loading range of approximately 2 to 120 lbs which allowed for quasi-static loading assessments. Finally, the entire test fixture was mounted on a solid six degree of freedom stage which allowed for proper alignment of the specimen relative to the optical system. The micromechanical test fixture and specimen then fit compactly underneath the modified immersion interferometer for experimental testing.
As shown in Figure 3-1, the test fixture was originally designed with opposing specimen holders which contained machined shelves where the specimen was placed during the loading process. This configuration was found to be suitable for trials in which the specimen size was greater than 10 mm × 10 mm and when magnification levels of 20x or lower were acceptable. However, for smaller specimens or higher magnification levels a modified specimen holder was required. The increase in spatial resolution of the higher magnification levels made even the slightest amount of out-of-plane motion or rotation of the specimen intolerable. Therefore, the modified specimen holders were designed to minimize such movements. Picture (A) of Figure 3-2, shows the basic design of the modified specimen holders. High precision wire electrical discharge machining (EDM) was utilized to obtain the near perfect corners and required
dimensional tolerances. The slot on the outside face of the modified holders was designed to mate with the original holders. On the inside face a ledge for the specimen was formed by the addition of a precision cut piece of plastic. The inclusion of a movable plastic base to the ledge was necessary for experimentation with greater thickness specimens. The addition of an angle bracket to each end of the holders, as shown in Picture (B) of Figure 3-2, prevented the undesired out-of-plane motion and rotation. A slot which aligned precisely with the attachment screw holes was machined into the angle bracket using wire EDM thus allowing for only lateral translation between the two holders. Therefore, a restriction to only one degree of freedom was introduced. Furthermore, the addition of a wet sponge wedged between the inside faces of the two modified holders allowed for proper specimen hydration during the experiment.

Figure 3-2. Design of the modified specimen holders.
3.2.2 Specimen Grating Replication Technique

All experimentation on biological materials in this research study utilized the improved specimen grating replication technique introduced by Wood et al. to ensure high moisture content in the specimen and a replicated specimen grating of minimal thickness [38]. A detailed description of this specimen preparation and grating replication method has been presented by Du [39]. For traditional moiré interferometry experimentation, the thickness of a typical specimen grating is approximately 25 µm [25]. The thickness of the specimen grating is very important, whereas, when the deformation measurement of the specimen surface is sought, the deformation on the outside surface of the grating is actually observed and measured. This difference is negligible for most experimentation utilizing traditional moiré interferometry. However, in micromechanics studies, where the strain gradients can be relatively large, this difference could be significant. Shear lag through the thickness of a specimen grating can mask the true displacement behavior at discontinuities or in zones of abrupt changes, such as at the DEJ and around dentin tubules. The replication technique utilized in this research study effectively reduces the specimen grating thickness by an order of magnitude, to as little as 1 µm. Therefore, the specimen grating thickness is slightly smaller than the spatial resolution of the imaging system employed. Accordingly, the width of the shear lag region is approximately the same as the spatial resolution and, thus, the obtained grating thickness is considered to be acceptable for the following experimentation.
All human tooth samples were obtained with proper permission from the Medical College of Georgia (Dr. David Pashley, Department of Oral Biology, School of Dentistry). The preparation of samples for use with the microscopic moiré interferometry system required a strict methodology to minimize flaws in the grating and underlying material surface. Each of the samples was kept refrigerated and stored in distilled water. A low-speed diamond blade wet saw was used to obtain the desired dentin samples. Once sectioned, the samples were polished to a 1200 grit finish under a continuous supply of fresh water. After polishing, the samples were immersed in distilled water for at least one hour to ensure complete hydration.

A schematic of the technique used for the replication of the reflective diffraction grating onto the polished specimen surface is shown in Figure 3-3. A 2400 lines/mm diffraction grating was used for all experimentation. In the initial step, an epoxy grating was coated with a thin aluminum layer via vacuum deposition. Second, the adhesive was applied to the specimen surface. The preferred adhesive for use with biological materials is cyanoacrylate, specifically M-Bond 200 (Vishay, Raleigh, NC). This adhesive allows for a very fast cure time, which is further accelerated by water. In order to ensure a uniform cure across the surface of the specimen, pressurized air was used to remove any water droplets from the polished surface after the sample was removed from the distilled water. The tip of a paper clip was then used to place a tiny drop of the cyanoacrylate on the corner of the specimen surface. At this point the 2400 lines/mm epoxy grating mold was positioned on the surface of the specimen and held with pressure for several seconds. The cyanoacrylate cures almost instantaneously in the shape of the grating mold, while
the pressure exerted on the specimen provides for a uniform layer of glue. When the grating mold was removed, the aluminum layer remained bonded to the cyanoacrylate which was also adhered to the specimen surface. Thus, an aluminum coated reflective diffraction grating is replicated on the surface of the specimen. The highly viscous cyanoacrylate allows for very thin grating layers, as thin as approximately 1 µm. If successful the outlined procedure lasted less than one minute. The specimens were then immersed in water for at least two hours prior to testing to allow for any necessary re-hydration.

**Figure 3-3.** Schematic of the specimen grating replication technique.
3.3 Validation Experiment Design

Each of the dentin specimens utilized in the system validation experiment were obtained from pre-molar tooth samples. Illustration (A) of Figure 3-4 shows the approximate location of the dentin specimens with respect to the parent slices of the sample teeth. After sectioning a small sliver from each of the parent slices, the enamel was carefully separated from the dentin at the DEJ. The objective in this validation experiment was to evaluate the mechanical properties of the dentin substrate under a compressive load. However, previously published reports have postulated that dentin possesses functionally graded material and structural properties, especially in the area leading up to the DEJ [9,11]. Therefore, it was decided to investigate two adjacent dentin specimens in the configuration shown in illustration (B) of Figure 3-4. As shown, the two dentin samples were arranged in the loading fixture end-to-end, in opposing orientations. This configuration prevented any in-plane rotation of the loading fixture due to the uneven length of the specimen edges or the possibility that the properties of the dentin were graded across the length of the specimen. Thus, care was taken to ensure that the two dentin specimens were obtained from two adjacent parent slices of teeth and were cut to precisely the same dimensions. For each sample, an appropriate specimen size was acquired in order to avoid any edge effect interference with the experimental results.
3.4 Test Procedure A – Examination for Functionally Graded Properties

The initial test procedure in the validation experiment was a general examination for the possibility of functionally graded properties across the surface of both dentin specimens. As noted in Chapter 1, previously published reports have postulated that a smooth transition occurs between the harder enamel and the softer dentin [9,11]. This smooth transition zone, also described as the functional width of the DEJ, is thought to occur on the dentin side of the DEJ. However, significant variations have been reported in the experimental test results for the determination of the functional width of the DEJ. Table 3-1 shows a brief summary of the testing methods and corresponding published experimental results.

Figure 3-4. Location of the dentin specimens and general configuration of compression test.
Table 3-1. Comparison of Experimental Results for Functional Width of DEJ.

<table>
<thead>
<tr>
<th>Test Method</th>
<th>Experimental Functional Width of DEJ</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microhardness Indentation</td>
<td>100 to 200 µm</td>
<td>12,13</td>
</tr>
<tr>
<td>Fracture Toughness</td>
<td>50 to 100 µm</td>
<td>10</td>
</tr>
<tr>
<td>AFM Based Nanoindentation</td>
<td>12 to 20 µm</td>
<td>9,11,40</td>
</tr>
<tr>
<td>Nanoscratching</td>
<td>1 to 3 µm</td>
<td>11</td>
</tr>
</tbody>
</table>

In addition, the observations of a smooth transitional zone between enamel and dentin are in contrast with data acquired using high-resolution imaging at the DEJ. This imaging data shows that only a narrow interfacial zone between the enamel and dentin is present [41,42]. Thus, the DEJ is more typified by an abrupt structural demarcation, comparable to the presence of a distinct interface between the enamel and dentin.

Therefore, to closer investigate the zone leading up to the DEJ using the moiré technique, a series of regions across the length of each specimen were observed under varied compressive loads and magnification levels. Initially, the 100x microscope objective was utilized at the 1x zoom level (corresponding to an image scale of 110 µm in width). With the two dentin specimens under a zero load condition, a series of fringe pictures were captured while translating the camera across the center of the specimen and toward the DEJ zone. This progression was then repeated at two increased load conditions, corresponding to the mid-range and maximum load capabilities of the subminiature load cell. The specimens were then immersed in water for at least two hours.
After proper re-hydration of the specimens, the entire procedure was repeated at two additional magnification levels: first utilizing the 100x objective at the 3x zoom level (corresponding to an image scale of 50 μm in width), and second utilizing the 20x objective at the 3x zoom level (corresponding to an image scale of 250 μm in width).

Overall it was determined that at the lower magnification levels (image scales of 250 μm and 110 μm) virtually no variation was observed in the full-field deformation patterns obtained across the surface of the specimen leading up to the DEJ. At the highest magnification level (image scale of 50 μm) some relatively minor non-uniformities were noted. However, these indications were concluded to result from variations in the surface structure of either the specimen grating or the dentin substrate and were not indicative of the bulk properties of the dentin. The observations noted above were consistent regardless of the respective load conditions of the specimens.

Therefore, the results of the initial test procedure of the validation experiment indicate that human dentin does not possess functionally graded material properties in the zone leading up to the DEJ. In other words, the bulk mechanical properties of dentin are consistent regardless of the location of examination or the magnification level used. Thus, experimentation with the modified microscopic moiré interferometry system concurs with the high-resolution imaging data which shows a distinct interface between the enamel and dentin. However, this initial procedure does not rule out the possibility of dentin having a non-linear response with respect to increasing load. Since each of the sets of captured fringe patterns were compared at the same compressive load (and at the same magnification), the possibility still exists that dentin may not react in a linear
manner under increasing load levels. This possibility will be closer evaluated in the next test procedure.

3.5 Test Procedure B – Determination of Elastic Constants

The objective of the second test procedure in the validation experiment was to obtain the necessary data for determination of the elastic constants of human dentin, specifically Poisson’s ratio and the elastic modulus. Since the first test procedure determined that the bulk material properties of dentin were consistent across the surface of the sample, an investigation area near the center of Specimen A was selected. Figure 3-5 illustrates the approximate area of investigation on the specimen, along with the dimensions of the specimen and the corresponding scale of the fringe patterns. The patterns were captured utilizing the 100x microscope objective at a 1x zoom level. The resultant fringe pattern had a scale of 180 µm in width. This scale value is slightly different than the previous values obtained with the 100x objective at a 1x zoom level due to the utilization of the open optical system arrangement. This spatial resolution level and optical arrangement was selected for the second test procedure to illustrate the quality of fringe patterns and displacement measurements that could be obtained using the 100x microscope objective.
3.5.1 Data Collection

Figure 3-6 through Figure 3-9 shows the four sets of fringe patterns obtained during the second test procedure of the validation experiment. For each set of patterns, the $U$ and $V$ displacement fields are shown. The resultant patterns from four compressive load levels between the minimum and maximum capabilities of the load cell were selected. However, as illustrated in Figure 3-4, two near-identical specimens were compressed simultaneously in the test fixture. Since only Specimen A was being evaluated, the compressive load values provided in each figure caption correspond to half the total load output by the load cell. Figure 3-6 shows the near-null field condition for a compressive load of 1 lb. An initial load of 1 lb was selected, rather than no load, to ensure the modified specimen holders and dentin specimens were in direct contact.
Carrier patterns were added to both the $U$ and $V$ displacement fields shown in Figure 3-7 through Figure 3-9. As described in Chapter 2, the addition of carrier fringes helps to highlight the displacement information of a given field, which is especially useful in cases of small deformations. Inspection of each set of fringe patterns shows that the deformation is greater in the $U$ displacement field than in the $V$ displacement field. This difference is expected since the specimen was compressed in the $x$ direction.

**Figure 3-6.** Fringe patterns captured at a compressive load of 1 lb.
Figure 3-7. Fringe patterns captured at a compressive load of 10 lbs.

Figure 3-8. Fringe patterns captured at a compressive load of 24 lbs.
3.5.2 Data Reduction

Moiré interferometry provides an enormous amount of data in the form of full-field contour maps of displacement components. In some cases this form is convenient. For example, such patterns can be compared with computer generated contour maps resulting from finite element analysis techniques. However, in most cases material testing and analysis laboratories are interested in the stress and strain distributions of the specimen. It has been proven possible to produce derivatives of displacement fields optically, but the amount of effort involved and the limited resolution makes these methods quite unattractive [37]. The most promising methods are computer-assisted data reduction and processing techniques. The technique used to analyze the fringe patterns obtained during the second test procedure of the validation experiment is discussed below.
Data acquisition for the modified microscopic moiré interferometry system was based on the use of the CCD camera attached to the optical system in conjunction with a personal computer equipped with a frame grabber board. A television monitor attached to the CCD camera was also utilized for real-time inspection of the behavior of the specimen. The CCD camera utilized in this research study (Pulnix, TM-72EX) provided an array of $640 \times 480$ pixels and operated at 30 frames-per-second. The frame grabber board (Bitflow, R64-CL) also operated at a rate of 30 frames-per-second.

Once the desired fringe picture was captured, the $\Delta N_x$ and $\Delta N_y$ values could be simply calculated for any line across either the $U$ or $V$ displacement fields. Since the bulk material properties of the dentin substrate were desired, the lines selected for analysis corresponded to the exact center of the fringe picture in the direction of the desired displacement. Thus, to determine the appropriate $\Delta N_x$ value, a horizontal line was selected across the $U$ displacement field and the number of fringes that crossed the line was counted. Similarly, a vertical line was selected for determination of the $\Delta N_y$ value in the $V$ displacement field. In addition, due to the small displacements in the near-null field and the $V$ displacement field fringe patterns where a limited number of fringes were created, a LabVIEW program developed in the Clemson Photomechanics Laboratory was utilized which had the capability of calculating and outputting the $\Delta N_x$ and $\Delta N_y$ values for any line across either the $U$ or $V$ displacement fields down to a fraction of a fringe order.
Table 3-2 shows a summary of the mechanical property data for the dentin specimen evaluated in the second test procedure. The output data from the load cell and LabVIEW program, calculated values, and additional required measured values are each identified in the table. As can be noted, the desired elastic constant values for Poisson’s ratio and the elastic modulus were easily calculated using the output data and the measured values. The following list provides a brief description of each of the equations utilized in the calculation of the elastic constant values.

- Load (N) – Conversion of output load from SI to metric units. Required for compressive stress calculation.

- Compressive stress, $\sigma_{xx}$ – Load (N) divided by cross-sectional area of the specimen, $A_s$.

$$\sigma_{xx} = \frac{\text{Load}}{A_s}$$  \hspace{1cm} (3-1)

- Compressive strain, $\varepsilon_{xx}$ – Output of number of fringes in $x$ direction, $\Delta N_x$, divided by the product of the horizontal length of the fringe pattern, $\Delta x$, and the reference grating frequency.

$$\varepsilon_{xx} = \frac{1}{f_r} \left( \frac{\Delta N_x}{\Delta x} \right)$$  \hspace{1cm} (3-2)

- Transverse strain, $\varepsilon_{yy}$ – Output of number of fringes in $y$ direction, $\Delta N_y$, divided by the product of the vertical length of fringe pattern, $\Delta y$, and the reference grating frequency.

\[ \varepsilon_{yy} = \frac{1}{f_r} \left( \frac{\Delta N_y}{\Delta y} \right) \]  

(3-3)

- Poisson’s ratio, \( \nu \) – Transverse strain, \( \varepsilon_{xx} \), divided by compressive strain, \( \varepsilon_{yy} \).

\[ \nu = \frac{\varepsilon_{yy}}{\varepsilon_{xx}} \]  

(3-4)

- Elastic modulus, \( E \) – Compressive stress, \( \sigma_{xx} \), divided by compressive strain, \( \varepsilon_{yy} \).

\[ E = \frac{\sigma_{xx}}{\varepsilon_{xx}} \]  

(3-5)

<table>
<thead>
<tr>
<th>Output Data</th>
<th>Calculated Values</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Load (lbs)</strong></td>
<td>**( \Delta N_x )</td>
</tr>
<tr>
<td>1</td>
<td>0.086</td>
</tr>
<tr>
<td>10</td>
<td>0.617</td>
</tr>
<tr>
<td>24</td>
<td>1.338</td>
</tr>
<tr>
<td>52</td>
<td>2.402</td>
</tr>
</tbody>
</table>

**Additional Measured Values:**
- Thickness of Specimen (\( t_s \)) = 0.84 mm
- Average Length of Specimen = 5.1 mm
- Cross-Sectional Area of Specimen (\( A_s \)) = 4.28 mm²
- Horizontal Length of Fringe Pattern (\( \Delta x \)) = 180 μm
- Vertical Length of Fringe Pattern (\( \Delta y \)) = 135 μm
- Reference Grating Frequency (\( f_r \)) = 2400 lines/mm
Figure 3-10 and Figure 3-11 show two plots of the mechanical properties which were calculated for the dentin specimen evaluated in the second test procedure. The plot of compressive stress versus compressive strain shown in Figure 3-10 is beneficial for the graphical analysis of the elastic modulus. Likewise, the plot of transverse strain versus compressive strain shown in Figure 3-11 is beneficial for analysis of Poisson’s ratio. A brief discussion of the validation experiment results is included in the next section.

![Graph](image)

**Figure 3-10.** Plot of compressive stress versus compressive strain.
3.5.3 Test Discussion

A relatively large amount of data has been published for the experimental determination of the elastic modulus of human dentin. However, despite its importance there is great inconsistency with the reported values of the elastic modulus of dentin. Measurements span from approximately 10 to 20 GPa and appear to be independent of the testing method. Furthermore, it is interesting that despite marked advances in both the theoretical understanding of the mechanics of composite structures and improved testing methods, the large discrepancies in measured values persist even in the most recently published data [9,21]. On the other hand, a relatively small amount of data
exists for the experimental determination of Poisson’s ratio of human dentin. The range of reported values for Poisson’s ratio varies greatly, from approximately 0.01 to 0.45, depending primarily upon the specimen orientation and testing method utilized. Table 3-3 shows a brief summary of the testing methods and corresponding published experimental results for the elastic modulus and Poisson’s ratio of human dentin.

**Table 3-3.** Comparison of Experimental Results for the Elastic Constants of Dentin.

<table>
<thead>
<tr>
<th>Test Method</th>
<th>Experimental Elastic Modulus (GPa)</th>
<th>Experimental Poisson’s Ratio</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compression</td>
<td>11.0 to 18.3</td>
<td>–</td>
<td>17,43,44</td>
</tr>
<tr>
<td>Tension</td>
<td>11.0 to 19.3</td>
<td>–</td>
<td>18, 45</td>
</tr>
<tr>
<td>Four-Point Bend</td>
<td>12.3</td>
<td>–</td>
<td>46</td>
</tr>
<tr>
<td>Three-Point Bend</td>
<td>8.6 to 15.8</td>
<td>–</td>
<td>47</td>
</tr>
<tr>
<td>Cantilever Bend</td>
<td>11.1 to 19.3</td>
<td>–</td>
<td>48</td>
</tr>
<tr>
<td>Microhardness Indentation</td>
<td>8.7 to 20.3</td>
<td>–</td>
<td>49,50</td>
</tr>
<tr>
<td>AFM Based Nanoindentation</td>
<td>28.6 to 31.0 (peritubular)</td>
<td>–</td>
<td>8,9</td>
</tr>
<tr>
<td></td>
<td>15.0 to 16.3 (intertubular)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acoustic Impedance</td>
<td>–</td>
<td>0.20 to 0.30</td>
<td>51,52</td>
</tr>
<tr>
<td>Quasi-Static Compression</td>
<td>–</td>
<td>0.014</td>
<td>53</td>
</tr>
<tr>
<td>Resonant Ultrasound Spectroscopy</td>
<td>23.2 to 25.0</td>
<td>0.29 to 0.45</td>
<td>19,21</td>
</tr>
</tbody>
</table>
Initial inspection of the two plots given in Figure 3-10 and Figure 3-11 indicate a slightly non-linear trend for the elastic modulus and Poisson’s ratio values. Originally, this observation was rather surprising since most engineering materials display a linear relationship between stress and strain within the elastic region. However, a logical explanation exists for the observed non-linear phenomenon. Recall the illustration provided in Figure 1-6, which presented the assumption required for microhardness testing that the material reacts linearly with the increasing load of an indenter. The possibility also exists that in a micromechanical compression test the dentin could display a non-linear response with respect to increasing load. If the dentin material displayed such non-linear properties, the material would increase stiffness as the compressive force was increased. This reaction would also be consistent with that of a porous constructed of two phases, such as the peritubular dentin and intertubular dentin as shown in Figure 1-2. In addition, this conclusion agrees with the published results by Wood and Pashley which state that the elastic modulus increases linearly when plotted as a function of compressive stress [54].

Another typical explanation for a non-linear trend in elastic modulus values is mechanical hysteresis. Mechanical hysteresis is defined as the energy absorbed in a complete cycle of loading and unloading within the elastic limit and is represented by a closed loop on a stress-strain curve [55]. Mechanical hysteresis becomes an important consideration when conducting fine measurements in a loading curve, such as the high-resolution measurements obtained with microscopic moiré interferometry. However,
mechanical hysteresis would a result in a trend of the opposite direction and is not considered to play a role in the observed results from this validation experiment.

The obtained values for both the elastic modulus and Poisson’s ratio of human dentin fell well within the respective previously published data ranges. The averaged elastic modulus value was 15.22 Gpa, whereas the averaged Poisson’s ratio value was 0.202. Since the objective of this research study was the development and exhibition of a modified microscopic moiré interferometry system for experimentation and analysis of biological materials, no further testing was required at this time. However, additional experimental data obtained utilizing the modified system will be referenced in the Future Work section. Therefore, it has been demonstrated and validated that the modified microscopic moiré interferometry system possesses the capability of directly determining the mechanical properties of microscopic biological specimens in a quick and accurate manner.

3.6 Future Work

One additional enhancement was included in the development of the modified microscopic moiré interferometry system. The incorporation of a piezoelectric transducer to the design of the modified interferometer holder discussed in Chapter 2 provided the increased capability of fringe shifting to the modified system. Figure 3-12 shows a picture of the modified interferometer holder with the incorporated piezoelectric transducer.
Figure 3-12. Modified interferometer holder with piezoelectric transducer.

While not an objective for this research study, fringe shifting is another method which can be used to increase the number of data points in a fringe pattern. Fringe shifting is accomplished by shifting or moving the reference grating relative to the specimen grating [25]. Thus, the configuration shown in Figure 3-12 utilizes the piezoelectric transducer to deflect the interferometer holder, which in turn displaces the attached interferometer. The displacement of the interferometer then results in the slight translation of the virtual reference grating. Alone, this technique does not substantially increase the resolution of displacement measurements. However, a large improvement can be achieved if coupled with a supplementary fringe sharpening technique such as optical / digital fringe multiplication or phase shifting.

Since a significant amount of time lapsed between the completion of the development and experimentation of this research study and the completion of this thesis, a somewhat unique situation exists where the future work for this project has already
been completed. Sobolewski was able to utilize the developed modified microscopic moiré interferometry system and expand the system capability to include phase shifting data analysis. Using the phase shifting method, the phase of a captured fringe pattern is determined directly through either Fourier transform analysis or a phase-shifting technique. The key benefit of this method is that partial fringe orders for the acquired fringe pattern are determined utilizing the fringe shifting technique described above. This enhancement featured simultaneous phase shifting in two in-plane fields to obtain a basic contour interval of 0.052 µm/fringe order. Thus, the use of phase shifting enabled an additional 400% increase to the sensitivity of the modified system (the inherent sensitivity of the modified microscopic moiré interferometry system was 0.208 µm/fringe order). A complete description of the phase shifting technique is presented in the thesis by Sobolewski [56].

In addition, Sobolewski was able to utilize the modified microscopic moiré interferometry system with the enhanced phase shifting technique to obtain additional data on several dental specimens. One such trial was conducted using dental specimens in the same orientation as the validation experiment detailed above. However, the experimental data was acquired using a fringe pattern scale of 650 µm in width, corresponding to a zone of analysis nearly 4 times larger than that used in the validation experiment. Sobolweski’s experimental results provided a value for the elastic modulus of 16 GPa (±1) and a value for the Poisson’s ratio of roughly 0.26 [56]. Thus, the values acquired with Sobolewski’s enhanced system are relatively close to the initial results obtained in this validation experiment. In addition, even with the decreased spatial
resolution of the fringe patterns utilized in the analysis, a slight hysteretic behavior was still noted in the dentin samples. Thus, the initial observations made during this system validation experiment were also confirmed by later experimental analysis performed by Sobolewski using the same modified system. A complete description of the additional examinations performed on biological specimens using the modified moiré interferometry system and the enhanced phase shifting technique is presented in the thesis by Sobolewski [56].
The human tooth is an amazing structure that is worthy of detailed research and analysis. For over half a century, various tests have been conducted to acquire more information about the structural and material properties of dentin, enamel, and the DEJ. Unfortunately, variations still exist in the experimental results, even in the most recently collected data. The primary focus of the majority of material property testing on human teeth has been hardness testing through indentation and nanoindentation analysis. However, several limitations still exist with the indentation technique. Thus, the moiré fringe analysis method, specifically moiré interferometry, has been introduced as a viable alternative testing method for the analysis of biological materials.

Moiré interferometry is a real-time, full-field deformation analysis technique that allows for in-depth investigation and analysis of the mechanics and structures of materials. The moiré technique is characterized by several beneficial qualities. First, it is highly sensitive to in-plane displacement measurements with virtually no sensitivity to out of plane measurements. Second, moiré interferometry has a high signal-to-noise ratio; therefore the displacement field fringe patterns have the capability of high contrast and excellent visibility. Last, the moiré technique is compatible with a large, dynamic range of displacements.

A modified microscopic moiré interferometry system has been developed which expands on the concepts of both moiré interferometry and standard microscopic moiré
interferometry. This modified system was developed specifically for the specialized minute deformation analysis of biological materials. A displacement sensitivity inherent to the system of 4.8 fringes/µm has been attained while increasing the spatial resolution with the capability of analyzing fringe patterns as small as 22 µm in width. This significant increase is a result of the in-depth analysis, modification, and enhancement of nearly every component in the microscopic moiré interferometry system. The modified system allows for real-time full-field in-plane displacement and strain analysis of microscopic biological specimens. In addition, the use of a compact, four beam modified immersion interferometer allows for near simultaneous analysis of the specimen in both the $U$ and $V$ displacement fields.

Finally, a system validation test was conducted in which two of the elastic constants of human dentin were experimentally determined. The primary purpose of this trial was to exhibit the application of the modified microscopic moiré interferometry system to experimentation with biological materials. The obtained values for both the elastic modulus and Poisson’s ratio of human dentin fell well within the respective previously published data ranges. (The elastic modulus was experimentally determined to be 15.22 Gpa, while a value of 0.202 was obtained for the Poisson’s ratio.) Thus, it has been demonstrated and validated that the modified microscopic moiré interferometry system possesses the capability of directly determining the mechanical properties of microscopic biological specimens in a quick and accurate manner.
APPENDICES
Appendix A

Effects of Drying and Freezing on Bovine Bone

Presented at the 2002 SEM Annual Conference on Experimental Mechanics,

Milwaukee, WI, June 10-12, 2002.
EFFECTS OF DRYING AND FREEZING ON BOVINE BONE

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ABSTRACT

Moiré interferometry was used to observe the effects of drying and freezing on bovine bone. By using changes in temperature and moisture content, we were able to observe the induced strain in the bone. After initial studies, points of interest such as osteon cross-sections and the inner Haversian canals were noted and observed closely for small deformations. Fresh bone was obtained and sections were prepared and polished using fine grit wet abrasive papers. An aluminum-coated diffraction grating was then applied to the moist specimens. Great care was taken to maintain the moisture content in the samples during the preparation process. The specimens were then observed in a moiré interferometer before and after freezing and thawing. The observed fringe patterns were captured using both film and a digital CCD camera for later analysis.

INTRODUCTION

Current research involving bones uses samples of both fresh and frozen bone without any distinction being made between the two. Assumptions must be drawn that no distinct changes occur in the material properties or structure of bone in the freezing and thawing process. However, bone is a vascularized material that has many cavities and canals which contain various fluids and blood vessels. Portions of bone such as Haversian systems have been found to be integral in supplying nutrients necessary for maintaining and remodeling new bone [1]. These fluid-filled compartments would rupture causing internal stresses in the bone when frozen.

Moiré interferometry can be used for full-field deformation analysis of a sample. By applying it to cross-sections of bone, we are able to compare the structure and strain fields in a bone both before and after it is frozen. Moiré interferometry is especially useful in comparing how one part of the bone deforms relative to another. Thus, precise observations can be taken to analyze any changes which may take place in the freezing and thawing process.

MATERIALS AND METHODS

Specimen and Specimen Preparation

The specimens were cross-sections of bovine rib bone. Pictures of the specimen orientation and geometry are shown in Figure 1. The fresh bone was sectioned using a low-speed sectioning saw (Isomet, Buehler, Lake Bluff, IL). They were then polished using fine grit wet abrasive papers. Only the finer 600 and 1000/3000 Silicon Carbide papers were used in order to keep the maximum amount of blood in the cavities of the bone.

![Figure 1: Lateral (A) and horizontal (B) bovine bone sections with applied gratings](image)

The specimen grating was formed by replicating a grating mold onto the surface of the specimen. In order to ensure that the moisture content of the bone was maintained throughout the preparation, a special replication process using cyanocrylate was utilized. A diagram of the replication process is shown in Figure 2. A 1200 lines/mm grating mold was first coated with a thin film of aluminum in a vacuum deposition chamber. The aluminum is necessary to enhance the diffraction efficiency of the final grating. After lightly wiping and blowing any excess water off the surface of the bone, a thin layer of cyanocrylate (M-BOND 200, Measurements Group) was spread across the surface using the drag method. It is necessary that this layer of glue be as thin as possible. The goal is for the layer thickness to be approximately 1 μm. After the cyanocrylate was spread,
the grating mold was immediately applied. The curing period used was approximately 30 seconds for the cyanoacrylate to cure in the shape of the mold. When the mold was removed, the cured cyanoacrylate remained bonded to the bone section. If any depth of the layer was thicker than desired, the glue would not completely cure in that section, thus we ensured that any area of obtained grating was of the desired thickness. The samples of bone were stored at all times in a sealed container with several pieces of a wet sponge. This technique was used to keep the bone moist without excess water washing the blood out of the bone's cavities.

![Image of bone replication process with grating](image)

**Figure 2: Replication of specimen grating using cyanoacrylate onto bovine bone specimen**

Moiré interferometry

A moiré interferometer was used to view the sections of bone. A collimated laser beam was divided into two beams that interfere with each other to create a virtual reference grating. This can be done in both the horizontal ($u$) and vertical ($v$) fields, with respect to the bone section. The reference grating frequency can be obtained by $f_{r} = (2/\lambda) \sin \alpha$, where $\lambda$ is the wavelength of the incident coherent light and $\alpha$ is the angle of incidence of the two beams. An argon-ion laser ($\lambda=514$ nm) was used, giving a reference grating frequency of $f_{r} = 2400$ lines/mm. This gives a sensitivity of 0.417 μm/fringe order in the displacement fields. A detailed description of this method is given in Ref. [2]. The moiré pattern is produced by the interaction of the specimen grating and the virtual reference grating. The resulting images were recorded for later analysis.

For small strains, the rate of change of fringe order, i.e., the fringe gradient, is a measure of the local strain by:

$$\varepsilon = \frac{\partial U}{\partial x} = \frac{1}{f} \left[ \frac{\partial N_u}{\partial x} \right], \quad \varepsilon = \frac{\partial V}{\partial y} = \frac{1}{f} \left[ \frac{\partial N_v}{\partial y} \right]$$

$$\gamma = \frac{\partial U}{\partial y} + \frac{\partial V}{\partial x} = \frac{1}{f} \left[ \frac{\partial N_u}{\partial y} + \frac{\partial N_v}{\partial x} \right]$$

where $\varepsilon$ and $\gamma$ are normal and shear strains respectively, and $N_u$ and $N_v$ are the number of fringe orders in the $U$ and $V$ displacement fields, respectively.

**Experimental Design**

A portion of bovine rib bone was obtained from the local Winn-Dixie grocery store. The sample had been previously refrigerated, but never frozen. One horizontal cross-section and one longitudinal section were cut from the bone and polished. Special attention was taken to ensure that the bone remained moist at all times during the preparation process. The four-beam moiré interferometry system was initially set to a null-field in both the $u$ and $v$ fields using the aluminum-coated grating mold. The grating was applied to the specimens at room temperature (70°F). Immediately after applying the grating to each specimen, the specimen was aligned in the interferometer and several images were recorded for later comparison. Special care was taken to observe the initial strain distribution contours around points of interest such as osteons and Haversian canals.

After the initial fringe pictures were taken, the bone specimens were frozen for 40 hours at a temperature of 20°F. The samples were then aligned in the interferometer, observing the same points that were noted earlier. The continuous change in temperature of the specimen and the corresponding times of observation were noted. Wet blankets were also placed around the frozen bone specimens after the initial observations to check for strain induced from drying during the testing process. Several series of pictures at varying levels of zoom on the bone were taken both before and after the bone was frozen. The pictures were then analyzed for any small deformations that occurred in the process of freezing. The pictures were purposely over exposed in order to more clearly examine the surface structure of the bone along with the interference fringes.
RESULTS

Freezing

The fringe pictures in Figure 3 show the horizontal section of the bone specimen after freezing and re-hydration. A complete null field across the entire section of bone in both directional fields (u and v) is shown. Two almost completely matching null fields were also recorded previous to the bone being frozen. The similarity of the null fields, before and after freezing, shows that no permanent deformations were caused in the bone due to freezing.

Drying

The fringe pictures in Figure 4 show the same section bone as above after approximately 15 minutes of spontaneous drying. Figure 5 then shows the bone after approximately 2 hours of drying. Some rotation was added to the specimen previous to drying in order to get a better picture of the full-field deformation across the surface of the bone. When the bone was fully moist, the fringes were almost completely horizontal in the u direction and vertical in the v direction. As the specimen dried, the fringes in the u field began to turn down on the outer left edge of the bone. The fringes in the v field also turned toward the outer part of the bone. These changes were determined to be a positive gradient on the outer, dry part of the bone.

DISCUSSION

Before the bone was frozen, a uniform fringe pattern, in both the u and v fields, was obtained and recorded. Any local deformations in the pre-frozen bone were noted for later reference. When the specimens were taken out of the freezer and observed, it was evident that changes were taking place in the contour of the fringes. As time progressed and the specimen warmed to room temperature definite deformations occurred in both specimens. Initially these were thought to be strains induced from the change of temperature. However, wet blankets were then placed around the specimen in order to account for any loss of moisture that may have taken place during the testing of the specimen. The newly formed fringes steadily began to disappear. The fringe pattern of the specimen returned to almost the exact same null field as the pre-frozen sample of bone.

The uniformity of the null field of both specimens, after freezing and re-hydration, shows that no permanent deformations occurred during the freezing and thawing process. Areas of the bone such as osteons and Haversian canals initially showed localized stress concentrations, but they remained unchanged throughout the freezing and thawing process. The overall full-field deformation analysis, after the freezing, thawing, and re-hydration of both bone sections, showed no relative deformations on the surface caused by the freeze-thaw cycle.

However, definite changes in the bone due to a loss of moisture were noted as time progressed in the experiment. First along the outer edge, then further toward the inner part of the bone sections, it was evident that a deformation due to drying was taking place. A negative gradient was initially observed in the moist specimens. However, this turned to a positive gradient around the outside of the bone as the drying progressed. Thus, it was found that the outer portion of the bone was actually expanding as it dried. This observation was found to be consistent along the outside of both bone sections. The bones were then re-hydrated and frozen and the testing was repeated with the same end results.

CONCLUSION

The freezing and thawing cycle was shown not to have a permanent effect on the material properties or structure of bovine bone. However, as the bone lost its moisture, it was found to display an interesting principle. As the bone dried, its outer edges expanded, rather than contracted. This was found to be true repeated times and for both a horizontal and lateral cross section of bone. The full-field deformation analysis technique of moiré interferometry was used for the analysis.

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Figure 3: Null field of bovine bone after freezing, thawing and re-hydration

Figure 4: Specimen with added rotation after 15 minutes of drying

Figure 5: Specimen with added rotation after 2 hours of drying
Appendix B

Measurement of Strain Distribution in an Elastomer Using Moiré Interferometry

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MEASUREMENT OF STRAIN DISTRIBUTION IN AN ELASTOMER USING MOIRE INTERFEROMETRY

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ABSTRACT
This analysis focused on measuring the strain gradient of an elastomer (rubber), using moire interferometry. Performance of rubber applications can be improved by analyzing the rubber’s specific deformation characteristics under applied loads. Acoustic characteristics such as vibration of car tires can be reduced, by controlling the rubber’s response, using smart piezoelectric materials. Moire interferometry was used in the preliminary analysis of the rubber to measure the strain resulting from deformation induced by piezoelectric ceramic. Moire interferometry is a high sensitivity measuring method that requires precise specimen fabrication. Moire also requires a smooth, planer surface to obtain accurate results. Due to the drastic difference in the material properties of the components being merged, a significant amount of precision was required when preparing the test specimen. Various techniques were developed to prepare the rubber and ceramic in order to obtain a polished surface of high optical quality. This analysis yielded a strain distribution in which strain is a function of position relative to the point of the applied load.

INTRODUCTION
Moire interferometry was used due to its high levels of magnification and non-contact technique. This method, compared to more traditional methods, allowed us to observe deformations in small areas without changing the structure. Moire utilizes the interference between gratings to produce fringes that clearly stand out and creates a pattern, which indicates the amount of strain experienced by the specimen [1]. These fringes are illuminated and magnified by a laser beam so that any conventional photographic device may record the pattern. Features that are generally difficult to detect using conventional strain gauges could be clearly seen and analyzed using moire interferometry.

Figure 1: Sub-components of test specimen (front)

Figure 2: Assembled Specimen and General Area of Strain gradient analysis
Moire interferometry was utilized in order to measure the viscoelastic effects between the rubber and the piezoelectric ceramic piece. The $x$ and $y$ strain gradients for the rubber specimen were analyzed at the rubber/piezoelectric interface. Electrified piezoelectric ceramic was used to deform the rubber. Moire interferometry was used to measure the deformation. In order to employ moire, the rubber specimen needed a highly polished surface. This was necessary to adhere a diffraction grating to the location were the strain data was desired (see Figure 2). An aluminum-coated diffraction grating with a frequency of 1200 lines/mm was used. A continuous surface with no free edges or gaps was achieved by arranging the rubber and piezoelectric components (as seen in Figure 1) into the assembled specimen (seen in Figure 2). A 2-D strain gradient was produced by the deformation in the material caused by electrified piezoelectric material. Time dependent distortion effects of the rubber ( creep) were then observed as a variation in the strain gradient due to time.

MATERIALS AND METHODS

Each component (Rubber and piezoelectric) needed to be prepared separately since the same polishing technique could not polish both materials simultaneously. This was because of the differing properties of the two materials. The modulus of rubber is 0.1% of piezoelectric material (64 GPa). Since rubber is a much softer material than piezoelectric material, different polishing techniques had to be used for each.

The rubber used is categorized as an amorphous polymer [2]. Strain analysis for an amorphous polymer is more involved than that of metals or ceramics due to the magnitude of deformation depending on time, temperature, and the rate at which the load is applied.

These variables determine the extent of viscoelastic effects that the specimen will experience. A polymer subjected to high strain rates and low temperatures will behave as a brittle material (little chain slippage). However, low strain rates and high temperatures will result in a viscous liquid behavior (easy chain slippage).

When polishing the rubber, it was extremely important to prevent overheating. The viscoelastic effects resulting from rises in temperature due to friction had the ability to cause damage to the rubber surface. Therefore, the rubber specimens were frozen before polishing in order to minimize these effects.

Freezing increased the rigidity of the specimen, which provided a more planar surface over the length of the rubber component. Freezing also reduced the Poisson effects of the rubber. Poisson effects cause the polishing surface to develop a concave shape as seen in Figure 3. Concavity in the specimen introduces shear lag and is a means of trapping air bubbles in the adhesive. Shear lag results from a thick layer of glue between the grating and the specimen surface. It causes the measured value of strain to be less than the actual value due to deformation of the adhesive. Any of the above factors could result in decreased accuracy of the moire fringe analysis. Reducing the temperature also reduced the coefficient of friction, which allowed a finer abrasion.

![Effect of Freezing](image)

**Figure 3:** Deformation effects of rubber at room temperature, as well as, the corresponding effect of freezing.

![High Stresses](image)

**Figure 4:** The rough fracture that occurs near the end of cutting the piezoelectric layer.
Figure 5: Method of removing and polishing burrs on piezoelectric surface

The frozen rubber was polished using a 1200-grit silicon-carbide sandpaper while immersed in a 1:1 solution of water and dish soap. The soap created a finer abrasion by lowering the coefficient of friction, ultimately eliminating a stick-slip effect. This process was repeated in order for the specimens to remain frozen. The final polishing stage was done with 100% (undiluted) dish soap. The specimen was then rinsed.

A low speed diamond saw (Isomet, Buehler, Lake Bluff, IL) was required to cleanly cut the brittle specimen. While cutting the piezoelectric layer, the last portion developed high stresses due to the decreasing cross sectional area. Figure 4 shows how the fractured just as the saw was finishing the cut. This fracture caused the shims to not be flush with the electrified portion of the piezoelectric layer. It was found that the rough edge could be removed by putting a slight lateral pressure with one finger on the slowly spinning saw blade (see Figure 5). Thus, the 0.25 in. of the diamond-cutting surface could be used to remove the protruding edge or any other burrs that had developed.

The alignment and adhesion of the surfaces required extra attention and care. PC-10 was found to be the optimal adhesive. Figure 6 illustrates the layering of the specimen and the grating application process. A perpendicular guide was used to align the components during adhesion.

The deformation of the specimen was calibrated and an equation of deformation as a function of voltage was found. An AC power source was then used to induce an electric field through the piezoelectric layer. The deformation of the piezoelectric specimen caused a strain distribution in the sections of rubber. When viewed in a moiré interferometer, this created a fringe pattern. A graph of the strain distribution across surface of the specimen could be obtained from the resulting fringes.

Experimental Design

The objective was to obtain the viscoelastic properties of rubber. A supplemental experiment was conducted in order to simulate the viscoelastic effect of a uniform dimensional change imposed by the piezoelectric device. A temperature change was used to create a uniform dimensional change $\Delta T = 10^\circ \text{C}$. An environmental chamber was used to elevate the specimen and grating to the temperature of $34^\circ \text{C}$. An aluminum-coated grating on low coefficient of thermal expansion glass was used for replication. The same grating was then used in aligning the reference for the moiré interferometer. The fringes captured were a result of the difference between the thermal expansion of the grating and mechanical loading from the piezoelectric sample (due to the change in temperature). Since the grating was replicated from low-expansion glass, the thermal expansion of the grating was assumed to be small. Several fringe patterns were captured starting immediately after the specimen was removed from the environmental chamber and continuing until it reached room temperature ($24^\circ \text{C}$).

RESULTS

Figure 7(a) shows the obtained fringe pattern from the specimen immediately after it was removed from the environmental chamber ($t=0$). Figure 7(b) shows the same sample after approximately 110 minutes of cooling ($t=110$). The corresponding pictures show the $V$-field displacement on the surface of the specimen (in the $y$-direction). Rotation was added to gain a better picture of the full-field deformation across the surface of the specimen. Figure 8 shows the graph of the micro-strain (in the $y$-direction) versus the lateral height of the specimen. The data was taken along a line near the center of the specimen (as shown in the figure) from both of the above fringe pictures. The two lines plotted on the graph correspond to the strain gradients of the specimen at $t=0$ and $t=110$ minutes.
DISCUSSION

Strains can be computed from a quantitative analysis of the gradient of the fringes obtained from moire interferometry [1]. The viscoelastic effects can be seen from the changes in these fringe patterns over time. Figure 8 illustrates the strains resulting from these effects. The change in amplitude of the two strain curves show that the piezoelectric piece reduced in size more than the rubber. This accounts for the increase in strain around the rubber/piezoelectric interfaces ($y = 3 & 4$) and the middle of the piezoelectric section ($y = 3.5$). This also displays a successful bond between the two highly dissimilar materials.

CONCLUSION

Successful methods of polishing both rubber and piezoelectric material were developed and implemented. A surface of high optical quality was fabricated allowing the application of a diffraction grating. Moire interferometry was then used to determine the time dependent characteristics of rubber due to piezoelectric ceramic deformation.

REFERENCES


Appendix C

Development of a Mechanical Testing System Utilizing Microscopic Moiré Interferometry

Development of a Mechanical Testing System Utilizing Microscopic Moiré Interferometry

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Abstract

This paper presents the process involved in the development of a mechanical testing system which utilizes microscopic moiré interferometry. The utilization of microscopic moiré interferometry grants us the opportunity to analyze both microscopic specimens and diminutive regions of larger specimens. High spatial resolution and microscopic analysis methods were required to further analyze material and structural behavior in a variety of test specimens. The developed microscopic system has the ability to analyze specimen sections from a few millimeters down to approximately 20 μm. The motivation for this project originates from the need to analyze material behavior at bi-material interfaces, such as the dentin-enamel junction in the human tooth. Knowledge of the mechanical properties of the human tooth is necessary for better understanding how strain is distributed throughout the tooth. This information has a wide range of application, most specifically the ability for advanced design and implementation of tooth reconstruction.

Introduction

Teeth are amazing structures with a design that is seen no where else in nature. In a lifetime, a person is given just two sets of teeth: one temporary set which lasts just a few years, and a permanent set which must endure the remainder of a lifetime. The permanent set of teeth must be able to handle incredible stresses while dealing with repeated cyclic loading. This, of course, requires that teeth must be both tough and resistant to wear. Unfortunately, this combination of material properties does not work well together. Materials that are hard and resistant to wear tend to be brittle and crack easily. On the other hand, materials that are tough and can absorb great stresses don’t hold up under continual wear. The human tooth has an amazing design with the capability of handling both of these conditions.

The excellent biomechanical properties of the human tooth have drawn interest as being a premium model for biomimetic studies. Knowledge of the mechanical properties of the human tooth is necessary for better understanding how strain is distributed throughout a tooth. However, many questions still remain about the tooth’s microstructure and material properties. By gaining a greater understanding of the stress trajectories, the resulting strain distribution, and their relation to the structure of the tooth, insight can also be gained into the manner in which teeth function. This information has a wide range of utilization, most specifically the ability to better model the behavior of the dentin-restoration interface. As more information is learned and understood about natural designs, such as the tooth, we are better able to mimic such principles in new designs and engineering.

Despite the importance of acquiring more detailed information about the natural design of the tooth, many large discrepancies are evident in the reported experimental values. Even the same testing methods have resulted in varying experimental outcomes. Values still differ widely for the elastic modulus and shear modulus, and other material properties and constants have not even been experimentally measured. This is largely due to the difficulty in performing mechanical testing on small, natural specimens. Therefore, there is still much knowledge to be gained about the structural and mechanical properties of enamel, dentin, and the dentin-enamel junction.

Moiré interferometry has been introduced as an excellent tool for providing full-field, high-sensitivity measurements for in-plane displacements [1]. A moiré pattern is produced by interfering two similar light beams on a holographic grating. When the grating is placed on a specimen subject to testing, the resulting pattern can be analyzed to determine the displacements and strains observed in the material. Full-field contour maps of the displacement fields in opposite directions can be observed and recorded. The distinct advantage of the moiré technique is that the entire specimen surface can be studied and the resulting deformations can be analysed at any time during the experiment. The objective of our project was to utilize a microscopic moiré system to enable an accurate determination of the material behavior in diminutive regions of biological specimens such as the human tooth, specifically at bi-material interfaces.
Background

Wang and Wainer were the first to apply the moiré fringe technique to the testing of a human tooth and directly measure the in-plane strain distribution on slices of human teeth [2]. On a polished section of tooth, they were able to apply a 200 lines/mm grating (cross-line grating with alternating black and white lines) using commercial “super glue.” The experimental set-up was then performed by overlaying a reference grating with only parallel lines of the same frequency and then applying a horizontal load to the tooth. The interference between the deformed specimen and reference grating produced moiré fringes, giving the two in-plane displacement fields. This was an excellent initial test in the area of stress-strain analysis of a biological material and showed excellent insight to the mapping of strain on a human tooth. They were able to measure an approximate 200 μm thick zone of dentin, adjacent to the DEJ, which undergoes a larger amount of strain when compressed, which they believed was important to minimizing the stress across the DEJ [2]. However, one limitation they had was in the sensitivity of their system. With a reference grating frequency of 200 lines/mm, their system had the capability of a sensitivity of 0.2 fringes/μm or just 5 μm/fringe order. However, increased knowledge about the microscopic distribution of properties within dentin necessitated the development of more sensitive testing methods.

Wood et al., were the first to apply a moiré interferometry technique to the study of human teeth [3]. In moiré interferometry, a reflective cross-line diffraction grating is utilized with the interference of coherent, collimated laser beams to achieve the desired fringe pattern. The cross-line diffraction grating is an array of ridges and furrows in orthogonal directions. A special replication technique was developed which enabled the ability to replicate a reflective grating mold onto the surface of the tooth while maintaining an adequate moisture level in the specimen. For a complete description of the technique see [3]. As a result of their testing, Wood et al. were able to develop complete displacement fields due to the effects of moisture level changes through the crown of a human tooth [3]. They were also able to achieve a higher level of sensitivity. Using their system, they were able to obtain a 2400 lines/mm reference grating frequency. This gave them an increased sensitivity of 2.4 fringes/μm or 0.417 μm/fringe order. This was an excellent advancement in the ability to inspect the details of the stress-flow across the surface of a tooth. However, the amount of detail and accuracy for testing around desired zones such as the DEJ was not achieved. Therefore, higher sensitivity methods with the ability to test at a finer scale were required.

Microscopic Moiré Interferometry

The development of microscopic moiré interferometry was first introduced by Han and Post [4]. This process is based on the same principles as macroscopic moiré interferometry, but allows for in-depth investigation for microscopic analysis of the mechanics of materials. This is basically achieved by creating the reference grating in a refractive medium instead of air. Figure 1 shows the diagram of a basic microscopic moiré interferometer [4]. A collimated laser beam is directed into a piece of optical material used for the interferometer. Half of the beam is diffracted directly off of the specimen while the second half is first reflected off a mirrorized surface. The overlap of these two beams create the virtual reference grating. The immersion fluid is necessary to avoid complete internal reflection of the laser beam inside the interferometer. A microscope objective above the interferometer can then be used to collect the resulting fringe picture.

![Diagram of a Basic Microscopic Moiré Interferometer](image)

Figure 1: Diagram of a Basic Microscopic Moiré Interferometer
The advantage of the microscopic moiré interferometer is seen in the greater detail of its viewing area and the clear increase in sensitivity. By the addition of microscope objectives, the limit on the size and detail of the viewing area is restricted only by the ability of the optics which are being used. By utilizing the combination of high-magnification objectives and ultra-zoom assemblies, we have currently achieved the ability to analyze specimens down to a scale of approximately 20 μm. Also, the current design of the interferometry system provides a reference grating of 4800 lines/mm. This translates to a sensitivity of 4.8 fringes/μm of displacement, or a contour interval of 0.208 μm per fringe order. Thus, we have been able to stretch the current limits of data analysis.

Optical Set-up

Figure 2 provides a simplified diagram of the optical set-up used in our experimental design. An argon-ion laser is used to provide green laser light (λ = 514nm) for illumination of the specimen. A spatial-filter assembly consisting of an inverted microscope objective and a pinhole is used to obtain a brighter, cleaner expanding beam. The beam is then collimated and directed by way of several mirrors into the interferometer at the desired angle for proper reflection and interference. The translating mirror is used to switch the beam between the u and v fields. This provides us with the capability of inspecting both orthogonal directions on the surface of the specimen without causing any change in the specimen orientation or rotation.

![Figure 2: Optical set-up of microscopic moiré system](image)

New Interferometer Design

One of the first problems which was discovered with the previously described microscopic moiré set-up and design was the inability to use a high-magnification microscope objective. At the time of original design, most available objectives experienced a drastic decrease in working distance as the magnification increased. For instance, the 100x microscope objective which was previously acquired had a working distance of 6mm, as opposed to a 20x objective which had a working distance of 20mm. The problem arose when trying to focus on the specimen through the interferometer. All of the current designs of microscopic moiré interferometers had thicknesses greater than the working distance of the 100x objective. Therefore, to achieve any greater magnification than with a 20x objective, the current interferometer design would have to be modified.

Figure 3 illustrates the renovated design of the microscopic moiré interferometer. To accommodate the shorter working distance of the 100x objective, the interferometer design consisted of a fused-silica prism attached to a longer, shallow square section of fused-silica. The main difference from the previously shown design is that the beam does not have a direct path to the specimen surface. The beam takes one extra reflection within the interferometer. Therefore, the edges of the interferometer had to be polished and mirrorized in the areas shown to allow for the reflection of the beam. This allowed extra distance for the laser beam to come in beside the objective. The interferometer could then allow high-magnification objectives to get extremely close to the surface in order to focus clearly while still allowing for a slight amount of room for movement.
New Problems

Unfortunately, several other problems arose which resulted either directly or indirectly from the shorter working distance of the high-magnification objective. Figure 4 shows an example of an early fringe picture which was taken using our new interferometer design. While the ability to focus was now achieved with the new design, a whole new problem of picture clarity had developed. The overwhelming amount of background patterns and various grid lines made it hard to focus and pick out the location of the desired fringes. A whole new round of inspection and analysis of every detail of the microscopic moiré system had to be run in order to increase the intensity, clarity, and contrast of the fringes.

Figure 4: Example of background patterns and grids in early fringe picture using new design
The first major beneficial change dealt with the specimen grating frequency. Figure 5 is a simplified illustration which displays the root to the problem. Using a 1200 lines/mm grating, two extra diffraction orders were being collected by the microscope objective. These two beams were then interfering inside the camera to cause the overwhelming grid lines in the background of the fringe picture. However, if a 2400 lines/mm grating was used, the two extra diffraction orders were not caused and the resulting background pattern disappeared.

![Figure 5: Diffraction orders resulting from 1200 vs 2400 lines/mm gratings](image)

Intensity of light in the picture was the next major hurdle with which we had to deal. When using higher-magnification objectives, the intensity of light becomes more of a problem. Original designs for the microscopic moiré system included the light being transmitted through fiber-optic cables. Maximizing the amount of light efficiency into and out of the cables became a major task. Ends needed to be sliced precisely and the entire set-up was very fragile. Therefore, a system utilizing mirrors and a single collimating lens was implemented. To maximize the intensity of light from using the lens, it was necessary to use the smallest diameter and focal length lens possible. Therefore, no extra light would be wasted in illuminating zones outside of the desired viewing area. While we have attempted to maximize the potential of light intensity in our system, it will most likely always be a problem as we continue to push the lower limits of image size.

Alignment seems to be the ongoing struggle in dealing with all of our clarity and intensity struggles. Figure 6 shows two of the examples of the patterns that can develop as a result of misalignment. As you can see, the overwhelming background pattern resulting from the 1200 lines/mm grating is no longer evident. However, as I have outlined on the pictures, a circular pattern is evident in the picture on the left and a near vertical pattern is evident in the picture on the right. Explanations of the direct cause of the problems are not called for at this time. However, it is good to note that the slightest misalignment in anything, including the spatial filter, incoming angle, specimen and interferometer orientation, and objective and camera placement, can be the cause of rather detrimental end results.

![Figure 6: Example of background fringes and grids using current design](image)
Completed Set-Up

Figure 7 shows several pictures of the completed set-up of the new interferometer design and loading fixture. It can be seen how closely together the interferometer and the 100x objective fit. In its design, it was necessary to include all six degrees of freedom for the alignment of the interferometer in order to ensure its exact calibration with the specimen and the objective when testing. This can be seen from the adjustment screws attached to the interferometer. This new design of the interferometer proved to be quite a tight fit when everything is assembled, but it does not prove to be a problem when everything is working properly. The loading fixture used to test the specimen is also shown. A wedge is used in the fixture to minimize the amount of compression for each turn of the screw head. The compressive force is measured directly by a sub-miniature load cell placed in line with the specimen. This provides the ability to test specimen of varying sizes and shapes at a wide variety of compressive loads. The load fixture and specimen can then fit easily underneath the interferometer for steady testing.

![Figure 7: Pictures of completed set-up of new design of interferometer and loading fixture](image)

Figure 8 shows several examples of the most recent fringe pictures taken with the new design set-up of the microscopic moiré interferometer. In each picture, it is labeled that the enamel is on the top half of the picture and the dentin is on the bottom half. The DEJ has been highlighted in order to make the difference in the materials more observable. The enamel has a smoother cast on the surface and the dentin has a more porous appearance where the tubules emerge to the surface. The pictures illustrate well the ability to take like pictures in both the u and v displacement fields. Also, the scales of 500 µm and 110 µm were chosen because of their clarity and the ability to clearly see the change in materials across the DEJ. The system has the capability of taking pictures at a scale approximately 5 times smaller, but due to the lower levels of intensity it was decided not to include them in this publication.

Conclusion

A microscopic moiré interferometer with the capability of measuring microscopic full-field deformations at magnifications not previously recorded has been introduced. This system will allow for analysis of microscopic regions of specimens ranging from a few millimeters down to approximately 20 µm. The microscopic moiré interferometer was developed in order to provide high spatial resolution and microscopic analysis methods that can be used to further analyze material and structural behavior in a variety of test specimens. The purpose of this paper was not to show any testing or analysis methods, but rather to illustrate the design methodology which went into the construction of the microscopic moiré interferometry system. Several of the problems that were encountered and were unique to our design were discussed and outlined. It is expected that in the direct future the developed microscopic system will prove to be capable of determining material behavior in a quick and accurate manner. This microscopic moiré interferometer will prove to be especially useful in the analysis of bi-material interfaces, such as the dentin-enamel junction in a human tooth.
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