Abstract

Understanding vertebral mechanics is of interest for identifying persons at risk of fracture, whether that is due to everyday loading such as in osteoporotic fracture or as a result of dynamic loading leading to a traumatic fracture. Vertebral fractures negatively impact the quality of life of patients and represent a large financial burden on the healthcare system. A powerful but underutilized tool that can be used to study vertebral loading and fracture is digital image correlation (DIC). DIC is a non-contact optical method for measuring the displacement on the surface of materials, including bone. In this thesis, DIC was used in a laboratory setting to provide a more complete understanding of the response of vertebral bodies to compressive loading.

The first investigation compared measurements from DIC with strain gages, a commonly accepted experimental method for measuring the bone surface response. For porcine vertebral bodies, the agreement was strong between the strain gages and DIC-measured strains indicating that DIC can be successfully used on bone. Based on those findings, experimental studies were performed using DIC to identify fracture of the anterior cortex and to quantify rate-dependency of the vertebral body response. For the fracture study, high DIC strains on the anterior cortex of vertebral bodies corresponded well with the locations of damage identified by observation of the video. For the rate-dependency study, the DIC displacement patterns were similar for the slow and fast rate tests, but the displacements from the slow rate tests had higher magnitudes, as expected for viscoelastic materials such as bone. Finally, specimen-specific finite element (FE) vertebral body models were created and DIC was used to validate the displacement and stiffness response. The FE models were predictive of the experimental stiffnesses measured using DIC on the surface of the vertebrae.

This thesis demonstrates the utility of DIC for experimental vertebral body investigations and for validation of FE models. Through these studies and future work, DIC has advanced and will continue to advance the understanding of vertebral mechanics under everyday loads as well as in simulated osteoporotic and healthy bone trauma.
Preface

A version of Chapter 2 was published previously: Gustafson H., Siegmund G., Cripton P. Comparison of Strain Rosettes and Digital Image Correlation for Measuring Vertebral Body Strain. ASME. J Biomech Eng 2016;138(5):054501-054501-6. The article was republished with permission from ASME. I was responsible for conducting the testing, analyzing the data, and writing the manuscript. The idea for the manuscript was developed jointly with Peter Cripton. Peter Cripton and Gunter Siegmund advised on data analysis and edited the manuscript.

Chapter 3 is in preparation for publication. I performed the testing, analyzed the data, and wrote the manuscript. Angela Melnyk assisted with the testing, advised on data analysis and edited the manuscript. Peter Cripton and Gunter Siegmund advised on data analysis and edited the manuscript.

Chapter 4 is in preparation for publication. I was responsible for performing the tests with assistance from Angela Melnyk, analyzing the data, and writing the manuscript. The initial idea for the investigation was in collaboration with Kohle Merry who also designed the original impactor and performed pilot testing. Angela Melnyk, Peter Cripton, and Gunter Siegmund advised on data analysis and edited the manuscript.

Chapter 5 is in preparation for publication. The specimens in this study were the same specimens as in Chapter 3. The finite element (FE) methodology presented was developed by Benedikt Helgason, Stephen Ferguson, and students they supervised. I scanned the specimens, modified the FE methods, setup the models, performed the segmentations, performed the data analysis, and wrote the manuscript. Benedikt Helgason provided background literature, advised on the data analysis, and edited the manuscript. Stephen Ferguson and Peter Cripton edited the manuscript.

Studies in Chapter 3, Chapter 4, and Chapter 5 were performed with ethical approval from the University of British Columbia Human Ethics Board for use of cadaveric spine segments under certificate H04-70219 -Biomechanics of Spinal Cord Injury: High Speed Experimental Investigations.
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<tr>
<td>aBMD</td>
<td>Areal Bone Mineral Density</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone Mineral Density</td>
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<tr>
<td>CC</td>
<td>Cross-Correlation</td>
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<tr>
<td>CCC</td>
<td>Concordance Correlation Coefficient</td>
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<tr>
<td>CI</td>
<td>Confidence Interval</td>
</tr>
<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>DEXA</td>
<td>Dual-Energy X-ray Absorptiometry</td>
</tr>
<tr>
<td>DIC</td>
<td>Digital Image Correlation</td>
</tr>
<tr>
<td>DLT</td>
<td>Direct Linear Transformation</td>
</tr>
<tr>
<td>DVC</td>
<td>Digital Volume Correlation</td>
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<tr>
<td>FE</td>
<td>Finite Element</td>
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<tr>
<td>FMT</td>
<td>Fiducial Marker Tracking</td>
</tr>
<tr>
<td>FSU</td>
<td>Functional Spinal Unit</td>
</tr>
<tr>
<td>F</td>
<td>Female</td>
</tr>
<tr>
<td>HR-pQCT</td>
<td>High Resolution Peripheral Quantitative Computed Tomography</td>
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<tr>
<td>IVD</td>
<td>Intervertebral Disc</td>
</tr>
<tr>
<td>LVDT</td>
<td>Linear Variable Transducer</td>
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<tr>
<td>M</td>
<td>Male</td>
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<tr>
<td>MDR</td>
<td>Modulus-Density Relationship</td>
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<tr>
<td>MMM</td>
<td>Material Mapping Method</td>
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<tr>
<td>MTS</td>
<td>Materials Testing System</td>
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<tr>
<td>PMMA</td>
<td>Polymethylmethacrylate</td>
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<tr>
<td>QS</td>
<td>Quasi-static</td>
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<tr>
<td>RMS</td>
<td>Root Mean Square</td>
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<tr>
<td>SD</td>
<td>Standard Deviation</td>
</tr>
<tr>
<td>SSD</td>
<td>Sum of Squared Differences</td>
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<tr>
<td>vBMD</td>
<td>Volumetric Bone Mineral Density</td>
</tr>
<tr>
<td>2D</td>
<td>Two-dimensional</td>
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<tr>
<td>3D</td>
<td>Three-dimensional</td>
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<th>Symbol</th>
<th>Definition</th>
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<tr>
<td>$E$</td>
<td>Young’s modulus</td>
</tr>
<tr>
<td>$\varepsilon_y$</td>
<td>Yield strain</td>
</tr>
<tr>
<td>$\varepsilon_u$</td>
<td>Ultimate strain</td>
</tr>
<tr>
<td>$\rho_{\text{app}}$</td>
<td>Apparent bone density</td>
</tr>
<tr>
<td>$\rho_{\text{ash}}$</td>
<td>Ash density</td>
</tr>
<tr>
<td>$\rho_{\text{QCT}}$</td>
<td>Quantitative computed tomography density</td>
</tr>
<tr>
<td>$\varepsilon_{xx}, \varepsilon_{xy}, \varepsilon_{yy}$</td>
<td>Strain tensor components</td>
</tr>
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Chapter 1: Introduction

The biomechanics of the human spine have been investigated in the laboratory using human cadaveric tissue for the purpose of understanding spinal kinematics, quantifying the response of the intervertebral disc or vertebrae to mechanical stimuli, and for comparing healthy and pathological biomechanics [1]. Typically, the response of the bone is quantified with tools such as load cells, displacement transducers, marker tracking to measure the rigid body motion, and strain gages on the surface of the bones. Digital image correlation (DIC) is an additional tool that can be used to measure full-field displacements and strains on bones in the laboratory, but its use has been limited, to date. The goal of this work was to explore the use of DIC to improve understanding of the response of vertebrae to compressive loading.

In this chapter, background will be provided about vertebral fractures, spinal anatomy, experimental testing of vertebrae in the laboratory with a focus on DIC studies, and computational modeling of vertebrae. This establishes the basis for Chapters 2 through 5 in this thesis, which represent experimental work to better quantify the mechanical response of vertebrae loaded in compression and computational work to apply DIC measurements to models of vertebrae. In Chapter 6, the findings of the studies will be synthesized, and contributions and future work will be discussed.

1.1 Anatomy

The human vertebral column consists of individual vertebrae and has five regions: cervical, thoracic, lumbar, sacral, and coccygeal. There are a total of 33 vertebrae in the spine. Humans have seven cervical vertebrae, twelve thoracic vertebrae, and five lumbar vertebrae that articulate. The sacrum consists of five fused vertebrae which are connected to coccyx, consisting of three to five fused vertebrae, by a fibrocartilaginous joint. The cervical and lumbar regions have a lordotic curvature (concave posteriorly) while the thoracic and sacral/coccygeal regions have a kyphotic curvature (concave anteriorly) (Figure 1.1).
Figure 1.1: Sagittal view of the spine showing the natural curvatures. Modified from Atlas and Text-book of Human Anatomy, Saunders, Philadelphia, 1909 (Copyright expired).

With the exception of the Atlas, the most superior vertebra articulating with the skull, each vertebra has a vertebral body which is a mass of bone located anteriorly (Figure 1.2). In general, the widths and heights of the vertebral bodies increase as you move inferiorly [2], [3]. When viewed in the sagittal plane, the anterior edges of the vertebral bodies are generally concave posteriorly.

Figure 1.2: Lateral view of lumbar vertebra. Modified from Gray’s Anatomy, Lea & Febiger, Philadelphia, 1918 (Copyright expired).
Attached to each vertebral body are the posterior elements or vertebral arch (Figure 1.3). The vertebral arch is formed by the pedicles and laminae, and the transverse processes, inferior and superior articular processes and the spinous process are attached. The transverse processes are lateral projections that are the attachment points for muscles and ligaments. The inferior and superior articular processes, known as facets, have articulating surfaces covered with cartilage. The spinous process is a posterior projection and, similar to the transverse processes, is an attachment point for muscles and ligaments. The spinal cord runs through the center of the vertebral arch along the vertebral foramen.

![Figure 1.3: Top view of a vertebra. Modified from Gray’s Anatomy, Lea & Febiger, Philadelphia, 1918 (Copyright expired).](image)

While the vertebrae exhibit generally similar structure, there are regional differences between the vertebrae. The cervical vertebrae have larger vertebral foramen, bifid spinous processes, and a foramen in their transverse processes which contains the vertebral artery and vein. The thoracic vertebrae have articular cartilage joints where the heads of the ribs articulate with the vertebral bodies. The facets in the thoracic spine are generally more vertically oriented than the facets in the cervical spine. The lumbar vertebrae are the largest vertebrae, and the facets in the lumbar region are the most vertically oriented.

The articulated vertebrae move relative to each other with motion allowed at two zygapophyseal joints (facet joints) and the intervertebral disc (IVD). The facet joints, formed at the junction of the facets of adjacent vertebrae, are synovial joints and are surrounded by a joint capsule. The IVD is a cartilaginous joint that connects the vertebral bodies. The IVD consists of
an outer ring of fibrocartilage called the annulus fibrosis, an inner gel-like substance made of water and a collagen network called the nucleus pulposus, and the hyaline cartilage and fibrocartilage layers between the bone and nucleus/annulus called the endplates. (Figure 1.4). The IVD is important for absorbing axial load and has been compared to a shock absorber [4]. With age, there is progressive degeneration of the annulus laminae [5] and the water content of the nucleus pulposus is reduced [6] which influences the mechanical response of the disc [7].

![Figure 1.4: Intervertebral disc (IVD) showing the annulus fibrosis and the nucleus pulposus. Modified from Atlas and Text-book of Human Anatomy, Saunders, Philadelphia, 1909 (Copyright expired).](image)

1.2 Bone

Vertebrae have a cortical shell of dense bone that surrounds a spongy cancellous core (Figure 1.5). The thickness of the cortical shell of a lumbar vertebra is correlated with the strength of the vertebra [8] and has been investigated in previous studies. The average thickness of the cortical shell at L1 has been reported between 0.35 and 0.69 mm [8]–[10], while the average for the L5 vertebra was 0.67 mm [9]. The variation in the cortical shell thickness between these studies may be a result of the varying age ranges used in different studies and where the authors delineated the cortical shell and trabecular bone structures. In general, the average thickness of the anterior shell is thicker than the posterior shell and endplates [9], [10].
Both cortical bone and cancellous bone are porous. In healthy adults, cortical bone has a porosity of between 2 and 3% while cancellous bone porosity ranges from 70 to 80% [11]. The porosity of both types of bone increases with age (Figure 1.6). For experiments on cortical and trabecular bone, it is important to distinguish if the testing was performed on the bone tissue itself or a sample of the bone which would have varying levels of porosity. Some investigations test individual trabeculae or account for the porosity by performing statistical regressions to find the properties for a no porosity condition [12]–[14] while other investigations report the properties of the bone sample and do not account for porosity [15]–[17].
Figure 1.6: Microcomputed tomography reconstructions of (A) trabecular bone from the femoral neck and (B) cortical bone from the femoral diaphysis. The images on the left are from a young male while the images on the right are from an elderly female. Republished from [18]. Licensed under the terms of the Creative Commons 4.0 license, © Boerckel et al., licensee BioMed Central. 2014.

The mechanical properties of cortical bone have been investigated previously using experimental samples of cadaveric bone (Table 1.1). In human femoral cortical bone samples, the Young’s modulus, yield stress and strain, and ultimate strain were independent of sex [12], but ultimate stress and strain decreased significantly with age [17]. Comparing compression with tension, cortical bone samples have higher ultimate strength [12], [16], and cortical tissue has higher yield stress and strain in compression [13]. In the femur, the yield strain in tension was 0.67% while the yield strain in compression was 0.98%; the ultimate strain in tension was 1.9% while the ultimate strain in compression was 1.3% [12]. Experiments on vertebral cortical bone have been limited due to the thinness of the cortical shell in vertebrae.

The cancellous bone consists of interconnected struts called trabeculae. Bone marrow, a soft tissue responsible for blood cell production, is found in the spaces between the struts. Compared to cancellous bone, cortical bone has lower porosity and remodels more slowly [11]. With increasing age, the thickness of the horizontal trabeculae decreases and for the horizontal
and vertical trabeculae, the distance between the trabeculae increases [19]. The combination of thinner and fewer trabeculae results in reduced strength of the trabecular structure.

Similar to cortical bone, the properties of trabecular bone have been investigated using cadaveric bone samples. Like cortical bone, trabecular bone samples have a higher yield strain in compression than in tension. The yield strain for human vertebral trabecular bone samples was 0.78% in tension and 0.84% in compression [15]. For femoral trabecular bone tissue, the ratio of the tensile to compressive yield strength was 0.63 [13]. The ultimate strain for cubes of trabecular bone was 1.6% in tension and 1.4% in compression but these values were not significantly different [15]. At a quasi-static rate, the yield strains for trabecular bone are similar to the yield strains for cortical bone and the yield behavior of trabecular bones can be characterized well with a bilinear material model [20].

Efforts have been made to compare the material properties of cortical and trabecular bone. Since the bones have different porosities, one investigation [13] used both experiments and computational models to isolate the mechanical properties of the bone. The study controlled for porosity by establishing a linear relationship between mechanical properties and the vascular porosity in both the cortical and trabecular bone. The linear relationships were extrapolated to a porosity of zero. The study found that cortical bone was stronger, had higher yield strains, and had a higher Young’s modulus than trabecular bone [13]. Another approach used nano-indentation and acoustic microscopy and determined that for femoral bone the longitudinal Young’s modulus of cortical bone was higher than the Young’s modulus of trabecular bone [21]. These studies show that the bone tissues are different, even when controlling for the bone porosity.
Table 1.1: Young’s moduli, yield strains, and ultimate strains for bone loaded in compression and tension

<table>
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<tr>
<th>Young’s modulus (E) (GPa)</th>
<th>Cortical bone</th>
<th>Trabecular bone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Compression</td>
<td>Tension</td>
</tr>
<tr>
<td>19.9&lt;sup&gt;f&lt;/sup&gt;</td>
<td>18.0&lt;sup&gt;f&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>ε&lt;sub&gt;y&lt;/sub&gt; (%)</td>
<td>0.98&lt;sup&gt;f&lt;/sup&gt;</td>
<td>0.67&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>0.84&lt;sup&gt;v&lt;/sup&gt; or 1.04&lt;sup&gt;v&lt;/sup&gt;</td>
<td>0.76&lt;sup&gt;v&lt;/sup&gt;</td>
</tr>
<tr>
<td>ε&lt;sub&gt;u&lt;/sub&gt; (%)</td>
<td>1.3&lt;sup&gt;f&lt;/sup&gt;</td>
<td>1.9&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>f</sup> = tissue from cadaveric femur [12], [13]
<sup>v</sup> = tissue from cadaveric thoracolumbar vertebrae [15]
* = study controls for bone porosity

Previously, the anisotropy of cortical and trabecular bone has been demonstrated [14], [16], [22]–[24]. Some have characterized bone as orthotropic [22], [24], requiring nine constants to describe the material, while other studies have characterized bone as transversely isotropic [16], [23], requiring five constants. Transversely isotropic materials are a specific subset of orthotropic materials that assume the material response is the same within a plane but different in the normal direction. The variation in Young’s modulus for off-axis loading compared to on-axis loading can be relatively high. For instance in trabecular bone samples from various anatomical locations, the Young’s modulus in the cranial-caudal direction was found to be 63 MPa while in the anterior-posterior and medial-lateral directions, the modulus was 29 and 25 MPa, respectively [14]. For this reason, experimental tests on bone samples should consider the orientation of the bone relative to the loading direction [25].

When load is applied axially to a vertebral body, the force is transmitted through the endplate and is distributed between the cortical shell and trabecular bone [11]. Measuring the proportion of load carried through the cortical shell compared to the trabecular bone is of interest for understanding how healthy bones bear load and for characterizing the effects of age and disease. The load proportion through the cortical shell has been investigated experimentally [26], [27] and computationally [28]–[31]. Experimentally, one study found that in specimens from donors older than 40 years, removal of the cortical bone resulted in a 65% reduction of the maximum load sustained [26] while another study found that removal of the cortical shell reduced the axial load the bone could sustain by 21 to 54% compared to the load carrying ability of the other T12-L5 vertebrae from the same donor [27]. Computationally, one study predicted
that the removal of the cortical shell would result in a 52\% reduction in the load carrying ability with a range of 38\% to 68\% for 13 specimens [28]. The large ranges seen both experimentally and computationally indicate the varying influence of the cortical shell for different specimens.

1.3 Epidemiology of spine fractures

Spinal fractures may occur as the result of everyday activities in fragile osteoporotic bones or as the result of trauma.

Osteoporosis is a medical condition in which bone mass decreases and spaces within the bone increase resulting in porosity and fragility. In a review of industrialized countries, it was estimated that osteoporosis affects 9 to 38\% of women and 1 to 8\% of men over the age of 50 [32]. Osteoporotic vertebral compression fractures represent 27\% of all osteoporotic fractures in the United States [33], and most commonly occur at the T12 or L1 level [34]. The estimated cost of all osteoporotic fractures in the United States in 2005 was $16.9 billion US, and osteoporotic vertebral fractures account for more than $1 billion US [33]. Osteoporotic spinal fractures may lead to kyphosis, back pain, and loss of overall height [35], [36], as well as sleep disorders, anxiety, depression, or loss of self-esteem [36], [37].

Traumatic spinal fractures represent a relatively small proportion (< 1\%) of all traumatic fractures [38]. However, traumatic vertebral fractures can have serious consequences, particularly if the fracture results in injury to the spinal cord through vertebral column misalignment or direct impingement by a bone fracture. Neurological deficits occur in between 4 to 38\% of vertebral fracture cases [39]–[42]. Similar to osteoporotic fractures, these fractures are typically compression or burst type fractures and occur most commonly in the lumbar spine [39], [41], [42]; unlike osteoporotic fractures, traumatic spinal fractures occur most commonly among males and are typically the result of falls or traffic accidents [39], [41], [42].

The consequences and prevalence of spinal fractures motivates research regarding the mechanics of vertebral fracture and methods for identifying persons at risk for fractures. Identification of patients at risk can help target prevention and treatment programs to patients most prone to vertebral fracture.

1.4 Experimental testing of vertebrae

1.4.1 Compressive testing of vertebrae

Many studies have tested isolated vertebral bodies quasi-statically in compression using a materials testing system (MTS) (Table 1.2). The posterior elements were removed for the testing
in all of the studies listed. Load cells were used to measure the maximum force reached before there was a drop in the force due to presumed or directly observed compromise of the bone structure. There is variability as to which lumbar vertebra can sustain the highest fracture load but literature shows that the lumbar fracture tolerance is higher than the cervical and thoracic tolerances [43].

Table 1.2: Summary of studies measuring the fracture loads of lumbar vertebral bodies under compressive loading in materials testing machines

<table>
<thead>
<tr>
<th>Author, year [reference]</th>
<th>Specimens</th>
<th>Donor ages</th>
<th>Loading rate (mm/s)</th>
<th>Potting</th>
<th>Fracture load (N)</th>
</tr>
</thead>
</table>
| Singer, 1995 [44]        | 287 thoracolumbar vertebrae (10 M, 8 F donors) | 29-88 | 0.25 | Bone Cement | L1: 3000  
L2: 2800  
L3: 2200  
L4: 2500  
L5: 2750 |
| McBroom, 1985 [45]       | 20 L1 and L3 vertebrae | 63-99 | 0.1 | Bone Cement | L1: 3160  
L3: 3385 |
| Yoganandan, 1988 [27]    | 63 T12-L5 vertebrae (5 M, 6 F donors) | 56-91 | 2.54 | None | Male  
L1: 4800  
L2: 3400  
L3: 5000  
L4: 4900  
L5: 4600  
Female  
L1: 3500  
L2: 2600  
L3: 3300  
L4: 3500  
L5: 3900 |
| Hansson, 1980 [46]       | 109 lumbar vertebrae (63 F, 46 M vertebrae) | 31-79 | 0.083 | None, 3 mm of disc was left | L1: 3260  
L2: 3760  
L3: 4109  
L4: 4807 |
| Myers, 1994 [47]         | 61 L2-L4 vertebrae (16 M, 6 F donors) | 52-75 | 1.5 | Methacrylate | All Levels: 5565 |
| Ebbesen, 1999 [48]       | 101 L3 vertebrae (50 M, 51 F vertebrae) | 18-96 | 0.083 | Removed endplates | L3: 5800 |

Sex-related differences have been shown for compressive loading of vertebrae from donors. A study of quasi-static (QS) compression of thoracolumbar vertebral bodies found that female donors had lower ultimate force compared to male donors [49]. In a study of lumbar vertebral bodies loaded dynamically (0.2 to 4 m/s), a non-significant trend (p<0.1) was found
that female donors had a lower ultimate force than male donors [50]. Morphologically, a study of L3 vertebral bodies (n = 101) found females had smaller vertebral volumes and smaller cross-sectional areas than males [48]. The study also loaded the vertebral bodies in QS compression and found that while females had lower ultimate compressive force, there were no differences based on sex when normalizing the load sustained by cross-sectional area. This suggests that for compressive loading of vertebrae, quantifying anthropometry may be a better indicator of failure load than reporting the sex of the specimens.

Changes in the compressive tolerance of vertebrae have also been observed with age. For compressive loading of L3 vertebrae (n = 101), the ultimate load, the ultimate load normalized by cross-sectional area and BMD each decreased with increasing age [48]. In a study that tested thoracolumbar vertebral bodies with the endplates removed to create parallel surfaces, the maximum stress (load/area) sustained decreased with increasing age [51]. However, in a study with lumbar vertebrae (n = 77), age was not found to be a significant predictor of strength; this result may be due to elderly donors (average: 85 years, range: 54-97 years) and a relatively small standard deviation (SD) for the ages of all the donors [49]. In experiments that tested trabecular cores from vertebrae, decreases in the maximum stress and ash density were observed with increasing age. However, the changes with age were larger than the changes in ash density [52] indicating that, besides density, there are changes with age such as mineralization or trabecular structure that influence the strength.

To summarize, sex and age are commonly correlated with the ultimate strength. The ultimate strength changes with age and sex are related to the BMD and size of the vertebral body, as well as other factors such as trabecular connectivity and degeneration.

1.4.2 Types of spine specimens

Different types of ex vivo cadaveric specimens can be tested to characterize the spine (Figure 1.7). For instance, trabecular bone cores have been used to establish the relationship between density and bone strength [53], while three-vertebrae segments have been used to investigate tolerance of the spine in lateral bending [54]. In general, smaller specimens provide more detail on the specimen tested and allow for more control over the boundary conditions while larger specimens provide more physiologic conditions. In testing a single vertebra, the bone can be tested intact, with the posterior elements removed, or with the endplates removed. Experimentally, the strength of the vertebrae is typically tested with the posterior elements
removed. This is justified since in healthy individuals the facet joints typically carry less than 20% of the load in neutral posture [55]–[57]. Removal of the endplates can further simplify the boundary conditions and may lead to more consistent testing [58], [59]. However, removal of the endplates and the posterior elements represents a less physiologic condition.

Figure 1.7: Types of ex vivo spine specimens that are used in testing. Republished from [1] with permission of World Scientific Publishing Co.; permission conveyed through Copyright Clearance Center, Inc. Load application

In cadaveric spine and vertebra testing, load is typically applied either with a MTS [27], [44]–[48], [50] or a drop weight [60]–[62]. In general, the loading rates that can be applied are lower in a MTS. However, MTSs have been used in vertebra testing at rates as high as 4.0 m/s [50]. One difference between MTSs and drop weight systems is the energy that they supply. In a MTS, the energy supplied by the actuator to the bone is essentially infinite while in a drop weight system, the amount of energy supplied depends on the height from which the weight was dropped and the mass of the weight. In the drop weight system, the bone acts on the drop weight to slow the loading while in the MTS, the loading is applied at a rate specified by the experimenter. Although the drop weight may be more representative of loading in the real world, use of a MTS provides a consistent loading condition which justifies its continued use.
1.4.4 Potting

For compression of vertebrae, typically the bones are potted in a material to create plano-parallel surfaces for compressing the bone (Figure 1.8). The properties of three common materials used for potting (dental stone, polymethylmethacrylate (PMMA), and a low melting point alloy called Wood’s metal) were compared, and it was found that Wood’s metal had the highest compressive modulus while PMMA had the highest yield stress (Figure 1.9) [63]. In an experiment comparing Wood’s metal with PMMA for potting functional spinal units (FSUs), differences were measured for the disc stiffnesses when potting in the two materials and the authors caution against comparing studies with different potting materials [64]. For the studies in this thesis, PMMA was selected because of its high strength and toughness compared to the other materials.

Figure 1.8: Cross-sectional view of a vertebra potted in a filler material. Modified from [63] with permission of the American Society of Mechanical Engineers (ASME).
1.4.5 Loading rate

Cortical bone was found to be viscoelastic [65]–[69]. In particular, cortical bone exhibits creep, relaxation, a dependency of stiffness on strain rate, and hysteresis indicating dissipation of energy [11]. The viscoelasticity of the cortical bone is due to the collagen content of the bone, interfaces in the bone such as the cement lines between osteons, and fluid flow through the canals in the bone [11]. One study loaded human femoral samples of cortical bone in compression and tension at strain rates representative of what might occur in high-energy real-world injuries (0.14 to 29 s$^{-1}$ for compression and 0.08 to 17 s$^{-1}$ for tension) [70]. The Young’s modulus increased while the yield stress and strain for both tension and compression decreased with increasing strain rate (Figure 1.10).

Figure 1.9: Stress-strain curve for the three potting materials tested. Modified from [63] with permission of ASME.
Figure 1.10: Strain vs. strain rate for compression of cortical bones. The yield strain decreases with strain rate while the failure strain increases to a point and then decreases. Modified from [70] with permission of ASME.

Similarly, cancellous bone is viscoelastic [71]–[73]. In one experiment, cylindrical samples from human cadaveric bone (n = 100) and bovine bone (n = 24) were tested in compression in a MTS at strain rates ranging from 0.001 to 10 s\(^{-1}\) [71]. The Young’s modulus of the bones was larger for higher strain rates. It was suggested that the Young’s modulus is proportional to the strain rate raised to an exponent of 0.06. A similar investigation was performed with a modified method in terms of the cylinder dimensions and bone constraint [72]. In this study, it was suggested that the exponent on the strain rate should be slightly lower (0.047), possibly due to different constraint on the bone during testing. Both of these studies show that strain rate influences the response of cancellous bone samples. Viscoelasticity in the cancellous bone is thought to be a result of fluid flow of the marrow, blood, and other interstitial fluid through the bony trabecular matrix [71], [74].

The role of load rate has been investigated with whole vertebrae and in spinal segments. Rabbit spinal segments were impacted in a drop tower at rates of 1.7, 2.0 and 3.0 m/s [62]. In a study with actuator loading using a material testing system (MTS) of cadaveric lumbar vertebrae, the bone response for loading at 0.01 m/s was compared to loading at 2.5 m/s [75]. Another study investigated thoracic vertebrae loaded with an actuator at loading rates of 0.89, 0.089, and 0.0089 m/s [76]. More recent work has used an actuator and compared the response of lumbar vertebrae compressed at low speeds (average: 0.6 m/s) and high speeds (average: 3 m/s) [50].
Studies testing whole vertebrae in compression at varying impact rates are summarized (Table 1.3). Other work has also used porcine [77], [78] or baboon [79] spinal segments to evaluate the effect of loading at high and low velocities.

**Table 1.3: Summary of studies loading human cadaveric vertebral bodies in compression at varying impact rates using a material testing machine**

<table>
<thead>
<tr>
<th>Author, year, [reference]</th>
<th>Specimens</th>
<th>Donor ages</th>
<th>Loading rates (mm/s)</th>
<th>Potting</th>
<th>Fracture force (N)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kazarian, 1977 [76]</td>
<td>48 thoracic vertebrae (4 M donors)</td>
<td>26-38</td>
<td>0.089, 8.9, 890</td>
<td>Dental Acrylic Resin</td>
<td>Slow: 3900 Medium: 5370 Fast: 6630</td>
</tr>
</tbody>
</table>

Although the loading rates varied across studies, the overall findings of the studies on vertebrae are similar. The ultimate strength and stiffness of the bone increase with increasing loading rates, and the displacement was generally lower at higher loading rates [50], [75]–[79]. More energy is absorbed at failure for high rate tests compared to low rate tests [62], [75]. Furthermore, in one study, a trend was observed where there were more injuries to the endplate at low velocities and more injuries to the vertebral body at high velocities [78]. These studies indicate that rate is an important factor in determining the response of vertebrae.

In some studies of long bones, the viscoelastic trend of bones has not been observed under all loading conditions. For instance, in a four point bending test comparing contralateral limbs, the tibia and femur were not significantly stiffer when loaded at a higher strain rate, while the fibula was stiffer when loaded at a higher strain rate [80]. However, the high rate loading was only ten times faster than the low rate loading and only twelve bones were tested. A study from our laboratory using whole proximal femora (n = 20) loaded the bones once at a non-injurious QS rate in a MTS and then dynamically in a drop tower [81]. The study found that there was no statistically significant difference in the stiffness between the QS and dynamic loading. However, the bones that were stiffer at the QS loading had lower BMD (Figure 1.11). It was postulated that the bones with lower BMD may have had less trabecular structures through which...
the bone marrow moved thus reducing the viscoelastic effects. However, it is also possible that the bones with lower BMD were more likely to fail, perhaps in subtle ways, during the dynamic loading prior to the peak force. This would make the bones appear less stiff in the drop tower based on the method used to calculate the stiffness.

![Image of box plot](image.jpg)

**Figure 1.11: Comparison of BMD for femora.** The femur specimens that were stiffer in the fall simulator (FS, dynamic loading) had higher areal BMD (aBMD) compared to the specimens that were stiffer in the QS loading. Republished from [81] with permission of Pergamon; permission conveyed through Copyright Clearance Center, Inc.

In summary, cortical and trabecular bone are rate dependent and therefore whole bones also generally exhibit rate-dependent responses. In some cases, the influence of viscoelasticity among specimens may not be observed due to low sample sizes, a low range of rates, or anatomical variation between specimens. In vertebrae, viscoelasticity has been shown in compressive loading; these studies have observed an increase in stiffness and ultimate strength for higher loading rates.

### 1.5 Measuring strain on and within bone

Strain can be measured for bones in various ways including with strain gages, with DIC or with digital volume correlation (DVC). In the following sections, the advantages and disadvantages of these methods will be discussed as well as applications for understanding bone behavior.
1.5.1 Strain gages

The most common method to measure bone strain is with strain gages which have been used on both cadaveric specimens and in living animals or humans. Typical strain gages consist of a thin wire attached to a flexible sheet; when the bone is strained, the resistance of the wire changes resulting in a change in voltage in a Wheatstone bridge circuit containing the gage that can be measured to determine the strain. Strain gages can be a single-element gage that measures strain in a single direction or stacked gages (called a rosette) that measure strain in three directions, allowing for calculation of the principal strains. Strain gages have many advantages such as their relative inexpensiveness and low signal noise. However, there are limitations of strain gages including that they only measure the average strain at a discrete location, achieving a quality bond between the bone and the gage can be difficult, application of the gages can be time consuming, and strain gage attachment may reinforce the bone thus changing mechanical behavior [82]. Strain gage readings may also be affected by temperature [83].

Instructions on attachment of strain gages to bone have been published by various groups [83], [84]. In general the methods are similar. First, soft tissue should be removed from the bone and scraped with a scalpel. Next, the bone is degreased using alcohol or acetone; multiple applications of the degreasing fluid may be required. After that, the gage is attached to the bone using cyanoacrylate and pressure should be applied while the glue cures. Once the gage is set, the wires should be secured to provide strain relief and prevent the lead wires becoming detached from the gage. Finally, it is recommended that the gage should be waterproofed using polyurethane.

1.5.1.1 Strain gages on vertebrae

Strain gages have been used on vertebrae to characterize the strain across the bone during loading [85]–[87]. Previously, three-vertebra segments (L3-L5, n = 6) with 17 strain gages on the L4 vertebra were used to evaluate the strain distribution [85]. Strain was highest on the base of the pedicles, and there was higher strain on the superior vertebral rim than on the inferior rim. Another similar study used thoracolumbar spinal segments (n = 21) with 11 strain gages applied (Figure 1.12) [87]. The highest strain was found at the base of the pedicle and higher tensile strains were found on the superior rim than on the inferior rim. More recently, the response of lumbar vertebra segments to different loading configurations was measured using strain gages [86]. The most consistent strain distribution was found for axial compression since all the gages
were applied circumferentially around the center of the vertebral body while the other loading configurations, such as torsion and compression of the bone with a 15° tilt, had more variable strain distributions.

Figure 1.12: Experimental setup for strain gage recordings. Republished from [87] with permission of Wolters Kluwer Health, Inc.; permission conveyed through Copyright Clearance Center, Inc.

1.5.2 Digital image correlation

A more recently developed method of measuring strain on bones is DIC, a non-contact optical technique that uses computer algorithms to measure the full-field displacement and strain on the surface of an object. Two-dimensional DIC was first developed by researchers at the University of South Carolina during the 1980s [88]–[90]. To measure the three-dimensional deformation on a curved surface, DIC based on stereophotogrammetric techniques was later developed using two or more cameras to measure the deformation of the surface [91].

The basic principle of DIC is that the deformation of the surface of an object can be measured by identifying the location of subsets of pixels in the undeformed state and in deformed states. To identify subsets of pixels, random texture is applied to the object. The similarity of the subsets in the initial image (reference image) and the deformed images are evaluated with a similarity metric. Given an undeformed subset of pixel intensities and a deformed subset of pixel intensities, f(x,y) and g(x,y) respectively, similarity metrics can be calculated [92] (Figure 1.13). Typically, either cross-correlation (CC) criteria or sum of squared differences (SSD) formulations are used. While the most basic criteria are CC and the SSD, these criteria only find the best match if the pixel intensities are not changing from the undeformed to the deformed image, which is uncommon in real experimental conditions [93]. Therefore, the
normalized and zero-normalized criteria can be used for pattern matching that accounts for changes in the pixel intensities (Table 1.4).

Table 1.4: Criteria for DIC pixel subset agreement between \( f(x, y) \) and \( g(x, y) \), undeformed and deformed pixel subsets of size \( M \times M \) pixels, respectively. Modified from [92], DOI: 10.1088/0957-0233/20/6/062001. © IOP Publishing. Reproduced with permission. All rights reserved

<table>
<thead>
<tr>
<th>Criteria name</th>
<th>Equation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cross-Correlation (CC)</td>
<td>[ CC = \sum_{i=-M}^{M} \sum_{j=-M}^{M} [f(x_i, y_j)g(x_i', y_j')] ]</td>
</tr>
<tr>
<td>Normalized Cross-Correlation (NCC)</td>
<td>[ NCC = \sum_{i=-M}^{M} \sum_{j=-M}^{M} \frac{f(x_i, y_j)g(x_i', y_j')}{\bar{fg}} ]</td>
</tr>
<tr>
<td>Zero-Normalized Cross-Correlation (ZNCC)</td>
<td>[ ZNCC = \sum_{i=-M}^{M} \sum_{j=-M}^{M} \left[ \frac{f(x_i, y_j) - \bar{f} \times \bar{g}}{\Delta f, \Delta g} \right] ]</td>
</tr>
<tr>
<td>Sum of Squared Differences (SSD)</td>
<td>[ SSD = \sum_{i=-M}^{M} \sum_{j=-M}^{M} [f(x_i, y_j) - g(x_i', y_j')]^2 ]</td>
</tr>
<tr>
<td>Normalized Sum of Squared Differences (NSSD)</td>
<td>[ NSSD = \sum_{i=-M}^{M} \sum_{j=-M}^{M} \left[ \frac{f(x_i, y_j) - \frac{\bar{f}}{\bar{g}} g(x_i', y_j')}{\Delta f, \Delta g} \right]^2 ]</td>
</tr>
<tr>
<td>Zero-Normalized Sum of Squared Differences (ZNSSD)</td>
<td>[ ZNSSD = \sum_{i=-M}^{M} \sum_{j=-M}^{M} \left[ \frac{f(x_i, y_j) - \bar{f} g(x_i', y_j') - \bar{g}}{\Delta f, \Delta g} \right]^2 ]</td>
</tr>
</tbody>
</table>

Values used in the table:

\[
\begin{align*}
f_m &= \frac{1}{(2M + 1)^2} \sum_{i=-M}^{M} \sum_{j=-M}^{M} f(x_i, y_j) \\
g_m &= \frac{1}{(2M + 1)^2} \sum_{i=-M}^{M} \sum_{j=-M}^{M} g(x_i, y_j) \\
\bar{f} &= \sqrt{\sum_{i=-M}^{M} \sum_{j=-M}^{M} [f(x_i, y_j)]^2} \\
\bar{g} &= \sqrt{\sum_{i=-M}^{M} \sum_{j=-M}^{M} [g(x_i, y_j)]^2} \\
\Delta f &= \sqrt{\sum_{i=-M}^{M} \sum_{j=-M}^{M} [f(x_i, y_j) - \bar{f}]^2} \\
\Delta g &= \sqrt{\sum_{i=-M}^{M} \sum_{j=-M}^{M} [g(x_i, y_j) - \bar{g}]^2}
\end{align*}
\]
Figure 1.13: Schematic of DIC showing that the speckle pattern creates a unique intensity pattern in an undeformed image. That same intensity pattern can be identified in the deformed image based on a similarity metric.

For the random texture, high contrast is important. Typically black and white paints are used to create the texture. The object is painted with a thin layer of one color of paint and then a speckle pattern is applied with a spray can or an airbrush in the other color. It has been shown that the size, shape, and distribution of the speckles affects the measurement error [94]. The speckle should be appropriately sized relative to the subset size [95]. If the speckles are small, the uniqueness of the pattern is reduced; however, if the speckles are too large, there are too few speckles per subset and accurate identification of the subset from image to image is difficult [94].

Speckle pattern assessment parameters have been suggested such as speckle size relative to the analysis subset size [96], subset entropy [97], and mean intensity gradient [98]. While these methods are useful for comparing the relative accuracy of different speckle patterns, no standards exist for thresholds for acceptable speckle patterns and quantitative measures of speckle pattern are rarely reported in literature.

To account for the fact that the shape of the subset changes in the deformed image a displacement mapping function is used to map coordinates from the reference image to the deformed image. To do this, it is common to use a first order shape function that accounts for translation, stretch, and shear (Figure 1.14). Therefore, the coordinates of each pixel in the deformed subset can be written based on the original location and the transformation terms:
Where \( x' \) and \( y' \) are the new pixel locations, \( x \) and \( y \) are the original locations, \( \Delta x \) and \( \Delta y \) are the distance of the pixel from the center of the subset, \( u \) and \( v \) are the translations, \( \frac{\partial u}{\partial x} \) and \( \frac{\partial v}{\partial y} \) are the stretch terms, and \( \frac{\partial u}{\partial y} \) and \( \frac{\partial v}{\partial x} \) are the shear terms.

**Figure 1.14:** Visualization of six possible linear transformations for DIC. One transformation or a combination of these transformations can be used to map the location of points in an undeformed image to the points in a deformed image. Modified from [99] with permission from Justin Blaber and Antonia Antoniou.

Although open source DIC code exists [100], DIC is typically performed using commercial software. Common software packages include Strainmaster (LaVision, Inc., Göttingen, Germany), VIC-2D or VIC-3D (Correlated Solutions, Inc., Columbia, SC, USA), and ARAMIS (GOM, mbH, Braunschweig, Germany). In these packages, the user loads the images.
collected during deformation. In two-dimensional (2D) applications, a single length measurement may be used to calibrate the system. For three-dimensional (3D) applications, a calibration object such as a grid or checkerboard is typically used to identify the relative location of the cameras to one another.

For the DIC analysis in the software, the user can select the subset size, as well as the distance between the subset centers, also called step size. For instance the user might select a subset size of 15 x 15 pixels and a distance of 15 pixels between the subset centers. In this case, there would be no overlap between subsets. If instead a spacing distance of 7 pixels was selected, each subset would overlap with each other neighboring subset by approximately 50% of its area. For both displacement and strain calculations, subset size affects the noise and resolution with smaller subsets providing better resolution while larger subsets have less noise [101]. For displacement calculations, the choice of step size affects the spatial resolution but is less influential for the frame-to-frame noise. For strain calculations, the choice of step size has a large influence on both the spatial resolution and the frame-to-frame noise [101]. The same frame of an analysis for a vertebra with different parameters is shown to demonstrate the effect of the spacing between subsets and the subset size (Figure 1.15). The maps showing the minimum principal strain have more spatial resolution when the spacing between subsets is smaller.
Figure 1.15: Strain maps from DIC on a vertebral body. All the maps are from the same frame of the same test but were analyzed using different parameters. The smaller spacing distances and the smaller subsets have a higher resolution but are more variable from frame-to-frame.

The DIC accuracy also depends on the experimental setup parameters including lighting [102], image contrast [102], camera resolution [92], and image distortion caused by the camera lenses [92].

1.5.2.1 DIC on biological tissue

Among its many applications, DIC has been applied to study biological tissue such as vessels [103], [104], intervertebral discs [105], bone samples [106]–[108], and whole bones [109]–[117]. Non-biological composite bone with similar geometries and material properties to human bones have also been investigated [118]–[121]. The purposes of the studies include determining the mechanical properties of the tissue, developing strain-based fracture criterion, and validation of finite element (FE) models. DIC has also been used to investigate joint replacement such as total knee arthroplasty [122], [123] and total hip replacements [118], [124];
these studies measured the change in the strain patterns due to implantation or compared the strain magnitudes and patterns for different implants or implant positions.

1.5.2.2 DIC on vertebrae

DIC has been used in investigation of vertebrae in only two recent studies [115], [116]. One investigation used porcine spines (n = 8) to evaluate cervical spine injury mechanisms due to axial loading using DIC based on images from high-speed cameras [115]. The average peak maximum principal strain on the anterior surface, as a result of first-order buckling reported for the time prior to observable fracture, was 4.6%. However, the DIC reconstruction was poor and there were areas where the DIC could not be tracked. This was perhaps due to the quality of the speckle pattern, bringing into question the reliability of the DIC data in that study. Another study investigated the role of DIC experimental parameters and software settings on the noise of the DIC signal for an unloaded lumbar vertebra [116]. Using flat metal pieces, their optimal parameters were found to maximize the accuracy and precision of the signal in the unloaded state. Using this, they then applied the same settings to a cadaveric L5 vertebra; lower accuracy and precision were measured on the bone as compared to the flat samples. However, the study only focused on noise in the unloaded state and did not investigate the strain on bone during a loading scenario.

1.5.3 Digital volume correlation

Digital volume correlation (DVC) is a technique similar to DIC except that DVC matches volumetric subsets instead of 2D subsets. These volumetric subsets can be from CT scans, magnetic resonance images, or other volumetric imaging modalities. DVC can be used to determine the strain in bones based on the trabecular architecture [125]. The algorithm uses small volumes to identify the motion of the trabecular bone as the bone is deformed. While DVC is a unique technology that allows for measurement of internal strains in bones, it can have high errors and requires a large number of CT images to perform [126]. Furthermore, the types of loading that can be applied are limited since it must fit within a CT scanner, and the loading can only be applied in a stepwise fashion.

1.5.3.1 DVC of bone

To date, DVC of bone has been done with images from CT scanners [125]–[129]. The first application of DVC to bone was with a lumbar vertebra and a proximal tibia [125]. This was compared to FE models; the coefficient of determination ($R^2$) for the relationship between
experimental and FE axial displacement of the vertebra was 0.90 while the R² for the axial strain was 0.33 [130]. Strain measurements may be more sensitive to errors in the predicted measurements since strain is the derivative of displacement. Another study used strains measured by DVC to investigate vertebral failure patterns of L1 human cadaveric vertebrae (n = 30) loaded in compression [126]. They found high compressive strains near the endplates and the highest strain in the axial direction. A related study investigated vertebral failure with and without an IVD [129]. They found that the presence of the disc affected the strain distribution and resulted in lower ultimate forces. More recently, DVC has been used to investigate axial and axial-flexion loading in a three-vertebra segment and quantify displacements (Figure 1.16) [128]. As expected, endplate deflection was associated with vertebral failure in both loading modes. More displacement and failures were seen at the superior endplate than at the inferior endplate. DVC provides the ability to quantify displacements and strains internally in vertebrae, and these measurements are important for understanding vertebrae mechanics and failure.

Figure 1.16: Three-quarters view of the displacement measured using DVC immediately before and after the peak loading in a combined anterior-flexion loading scenario. Modified from [128] with permission of John Wiley and Sons; permission conveyed through Copyright Clearance Center, Inc.

1.6 Finite element analysis

FE modeling is used commonly in engineering to theoretically evaluate structures or bodies. One definition of the FE method is, “A computer aided mathematical technique for obtaining approximate numerical solutions to the abstract equations of calculus that predict the response of physical systems subjected to external influences” [131]. The FE method gets its name from the fact that the structure being analyzed is discretized into numerous small elements. After the structure or body is divided into elements, relationships are developed for the elements
(e.g. between displacement and force). Then the equations of the various elements are assembled and solved as a system of equations to predict the overall response of the structure [132].

To develop accurate FE models, use of the framework of verification, validation, and sensitivity analysis is advocated [133], [134]. Verification is the process of evaluating the model’s numerical accuracy. In particular, evaluating the software if using a custom code and evaluating the effect of the mesh size on the model predictions. Validation is the comparison between model predictions and controlled laboratory experiments. Typically models are validated for a single boundary condition or a small subset of boundary conditions; however, there are many boundary conditions that the model is not validated against. Validation is done to prove that the model can provide reasonable predictions for one or more controlled experiments. However, when extrapolating the model for new boundary conditions, caution should be used.

The final step is sensitivity analysis which is the changing of model parameters to evaluate their influence on the model predictions. By doing so, researchers can focus on accurately measuring parameters with a large influence on the model results. These steps promote quality in models, and reporting this process allows other model users to evaluate the utility of the model.

1.6.1 Finite element analysis and bone testing

There are strong synergies in a research study that uses both experimental testing and FE models (Figure 1.17). The block diagram shows that experimental testing can provide material properties and validation data for the computational models while the computational models can help ensure that sound decisions are made in experiments regarding the loading, boundary conditions, and sensor placement to maximize the value from experiments, which can be resource intensive. There are limitations of both experiments and modeling, and performing both can mitigate these limitations [135].
1.6.2 Specimen-specific FE models of vertebrae

Specimen-specific FE vertebrae models have been developed to predict the stiffness or fracture response of vertebrae. Clinically, such models are relevant because they may allow for better identification of patients at risk for bone fracture as a result of bone loss. Better prediction using FE QCT models as compared to DEXA was shown in previous studies [49], [136] and motivates the further development and improvement of FE models to predict the vertebral response.

1.6.2.1 Assigning heterogeneous properties

To assign the material properties in specimen-specific FE models, experimental relationships between the bone density and the Young’s modulus are used. To develop these relationships, small samples of bone are tested in a MTS. Typically the displacement and force are measured using an extensometer and a load cell which can be used to create a stress-strain
plot and determine experimentally the Young’s modulus. The Young’s modulus can be correlated with the QCT density ($\rho_{QCT}$), the ash density ($\rho_{ash}$) or the apparent density ($\rho_{app}$).

The QCT density is found by scanning the bone in a CT scanner and comparing the bone density with a calibration phantom of hydroxyapatite solution at specific densities. The ash density for the bone is measured by burning off the organic matter from a bone sample leaving the mineral content; the ash density is defined as the ash mass divided by the original volume of the bone. Ash density is not equal to the QCT density due to bone attenuation and the homogenous nature of the phantoms which is different than the mineral distribution in the bone, but the linear correlation is strong ($R^2 = 0.997$) [137]. The ash density can be found from the QCT phantom density using the following relationship:

$$\rho_{ash} = (\rho_{QCT} + 0.09) \cdot 1.14$$

Equation 1.3

The apparent density is the wet mass divided by the total volume of the specimen. The process for finding the apparent density was as follows: wash the bone sample in a bleach ultrasound bath three times, use an air jet to further remove marrow and water and repeat the air jet process until the difference in mass between the cycles is less than 0.5% [25]. The apparent density is related to the ash density by the relationship $\rho_{ash} = 0.6\rho_{app}$ [137]. These studies are summarized in a review article on material mapping [53]. In most cases, the densities are related to the Young’s modulus with power relationships but linear fits have also been used. Relationships developed from experiments using vertebral trabecular bone specimens are provided (Table 1.5 and Figure 1.18).
Table 1.5: Modulus-density relationships for human vertebral trabecular bone cubes. Modified from [53] with permission of Pergamon; permission conveyed through Copyright Clearance Center, Inc.

<table>
<thead>
<tr>
<th>Author, year [reference]</th>
<th>Number of specimens, loading</th>
<th>Specimen</th>
<th>Wet apparent density range (g/cm³)</th>
<th>Relationship (E in MPa)</th>
<th>Measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keaveny, 1997 [138]</td>
<td>9, compression</td>
<td>Lumbar, Cylinder, D = 8, L = 16</td>
<td>0.09 to 0.28</td>
<td>$E = 1540 \rho_{\text{app}}^{-58}$</td>
<td>Extensometer attached to the end caps</td>
</tr>
<tr>
<td>Keller, 1994 [139]</td>
<td>199, compression</td>
<td>Lumbar, Cube, 10x10x10 mm</td>
<td>0.05 to 0.33*</td>
<td>$E = 1134\rho_{\text{app}}^{1.92}$</td>
<td>Platen displacement</td>
</tr>
<tr>
<td>Kopperdahl, 1998 [15]</td>
<td>22, compression</td>
<td>T10-L4, Cylinder, D = 8, L = 15 mm</td>
<td>0.11 to 0.26</td>
<td>$E = 2100\rho_{\text{app}}^{-80}$</td>
<td>Extensometer attached to the end caps</td>
</tr>
<tr>
<td>Morgan, 2003 [25]</td>
<td>61, tension and compression</td>
<td>T10-L5, Cylinder, D = 8 mm, L = 16</td>
<td>0.11 to 0.35</td>
<td>$E = 4730\rho_{\text{app}}^{1.56}$</td>
<td>Extensometer attached to the end caps</td>
</tr>
<tr>
<td>Ouyang, 1997 [140]</td>
<td>36, compression</td>
<td>T12-L4, Cube 10x10x24 mm</td>
<td>0.46 to 0.71</td>
<td>$E = 2383\rho_{\text{app}}^{1.88 \pm 0.07}$</td>
<td>Platen displacement</td>
</tr>
</tbody>
</table>

* Converted to apparent density with the relationship $\rho_{\text{ash}} = 0.6\rho_{\text{app}}$

Figure 1.18: Experimental relationships between Young’s modulus and apparent bone density. The dashed lines identified by the markers represent the relationships extrapolated for a wide range of apparent bone densities while the thicker line represents the range of the densities used to determine the specific relationship.

One limitation of the current literature is that the spine testing has been performed on the lumbar and thoracic vertebrae. The trabecular bone of the cervical spine has not been tested with
testing procedures consistent to other anatomical regions. One investigation used cervical vertebra samples to develop a power law relating the Young’s modulus to the mass of the samples but did not measure the apparent density of the bone [141].

Experimental parameters influence the relationship between the density and Young’s modulus. Testing bone from different anatomical locations results in different relationships [25]. The geometry of the samples influences the results with stiffness increasing in larger specimens compared to smaller specimens and in specimens with larger length to diameter ratios [142]. The relationship between Young’s modulus and density can be influenced by the alignment of the specimen relative to the physiological loading direction [143], a factor that is not always controlled [144]. Specimen preparation may also influence the results. For instance, high-resolution FE models were used to demonstrate that when trabeculae are cut, the load bearing ability of the bone is reduced resulting in an underestimation of the true Young’s modulus [145]. Furthermore, the loading rate and the specimen anatomical variation may result in different results among studies [53]. In summary, while modulus-density relationships are commonly used in development of specimen-specific models, the relationships depend on many factors, and these factors are difficult to fully account for experimentally.

1.6.3 Comparison between FE vertebral models and experiments

A summary of FE specimen-specific models in which the computational results were compared to experiments for axial compression is provided (Table 1.6). In these models, the Young’s modulus of the element varies depending on the bone density (Figure 1.19).

![Figure 1.19: Example of a specimen-specific vertebral body FE model with heterogeneous material properties. Republished from [49] with permission of Pergamon; permission conveyed through Copyright Clearance Center, Inc.](image-url)
The coefficient of determination of experimentally measured and computationally predicted strength ranged from 0.77 to 0.96. These strong correlations demonstrate the clinical value of computational models for predicting the strength of vertebrae.

In studies where stiffness was investigated, all found a statistically significant relationship between the experimental and FE stiffness. In some cases, calibration or tuning of the material properties was used; good agreement for the stiffness magnitude was achieved but required knowledge of the experimental outcomes to generate the models [59], [146], [147]. In other models, there was fair correlation but the FE stiffnesses did not agree in magnitude [58], [148], fair correlation using a more complex material model [149], [150] or poor correlation [49].

The difficulty in predicting stiffnesses may be a result of choices in the material model but may also be explained by experimental influences. For instance, there is compliance in the MTS resulting in incorrect linear variable displacement transducer (LVDT) measurements. When testing bone specimens, it is necessary to either use an extensometer or to correct for the compliance of the MTS, which has been done in more recent studies [58], [59], [147]. However, earlier studies may incorrectly report the experimental stiffness. Another factor that may affect the stiffness is the interaction of the potting cement (most commonly PMMA) and bone. In some studies, vertebral sections without endplates were used to eliminate the bone/PMMA interaction [58], [59], [147], but the removal of the vertebral endplates influences the loading of the vertebral body. However, computationally the removal of endplates was predicted to have a small influence on the failure force and damage distribution [151]. Compliance at the interfaces as well as in the MTS should be considered in all experimental setups, especially if the displacements are small meaning that incorrect measurement may result in large errors in stiffness.

In most studies in which specimen-specific FE models of vertebrae were created and compared to experiments, the posterior elements of the vertebrae were removed during testing and load was only applied through the vertebral body (Table 1.6). In the in vivo spine, there would be load transmitted through both the vertebral body and the facet joints. Removal of the posterior elements simplifies the experiments and the models but is not expected to affect the load-bearing ability of the vertebral body. However, loading through the facet joints may affect
the failure mode of the bone, and the posterior elements would change the bone response to non-axial loading.

While stiffness represents the global response of the bone, strain measured on the bone represents the local deformation response. Studies have investigated the ability of vertebral FE models to predict the strain on the bone surface [152]–[154]. One study used two strain gages on the center vertebra of a three-vertebra segment and found strong agreement in the peak principal strains measured [152]. However, only two strain gages on a single specimen were used in this investigation. In the work by Imai and colleagues [153], four strain gages were attached to the anterior, posterior and lateral surfaces of vertebral body specimens (n = 12). Strain was used as a measure to validate the computational models and the largest strains were measured on the anterior surface of the bone. The R² value for the model and experimental strain correlation was 0.70 (Figure 1.20). However strain was only measured at four discrete locations for each bone and it is not clear if the strain correlation would be similarly strong if comparing the strain at other locations on the bone. In another investigation, isolated vertebral bodies were tested in three loading configurations (compression, right torsion, and left torsion), and the strains on two vertebrae instrumented with eight strain gages were compared to FE models [154]. When both the minimum and maximum principal strains were included for all loading configurations, the R² value for the FE to experimental comparison was 0.69. These studies indicate that FE models are able to predict strain, but more strain measurement locations would improve model validation.

![Figure 1.20: Measured versus FE-predicted minimum principal strains for vertebral bodies. The strain was measured on the bone using four strain gages. Significant correlation was obtained. Republished from [153] with permission of Wolters Kluwer Health, Inc.; permission conveyed through Copyright Clearance Center, Inc.](image-url)
Table 1.6: Literature summary of axial loading of isolated vertebral bodies compared to specimen-specific FE models

<table>
<thead>
<tr>
<th>Author, year [reference]</th>
<th>Experimental specimens</th>
<th>Number tested</th>
<th>Compression rate</th>
<th>Finite element model and material</th>
<th>Material mapping relationship</th>
<th>Superior boundary conditions</th>
<th>$R^2$ strength</th>
<th>$R^2$ stiffness</th>
<th>$R^2$ strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silva, 1998 [148]</td>
<td>Thoracolumbar midsagittal section 10 mm thick</td>
<td>18</td>
<td>Load up to 20% strain at unspecified rate</td>
<td>Brick elements with elastic, perfectly plastic material with transversely isotropic elastic constants, isotropic yield strength</td>
<td>Keller, 1994 and Kopperdahl, 2002</td>
<td>Uniform displacement</td>
<td>0.93, yield force</td>
<td>0.79</td>
<td>-</td>
</tr>
<tr>
<td>Liebschner, 2003 [146]</td>
<td>Thoracolumbar</td>
<td>23</td>
<td>0.15 mm/s</td>
<td>Brick elements with anisotropic material properties and an explicit cortical shell</td>
<td>Calibration similar to Kopperdahl, 2002</td>
<td>Uniform displacement</td>
<td>0.79, fracture strength</td>
<td>0.81</td>
<td>-</td>
</tr>
<tr>
<td>Crawford, 2003 [136]</td>
<td>Lumbar</td>
<td>13</td>
<td>0.15 mm/s</td>
<td>Brick elements with anisotropic material properties and an explicit cortical shell</td>
<td>Kopperdahl, 2002</td>
<td>Uniform displacement with lateral constraint</td>
<td>0.86, fracture strength</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Imai, 2006 [153]</td>
<td>Thoracolumbar</td>
<td>12</td>
<td>0.5 mm/min</td>
<td>Tetrahedral elements with explicit cortical shell</td>
<td>Keyak, 1998</td>
<td>Uniform load</td>
<td>0.96, fracture strength</td>
<td>-</td>
<td>0.70</td>
</tr>
<tr>
<td>Buckley, 2007 [49]</td>
<td>Thoracolumbar</td>
<td>77</td>
<td>1 mm/min</td>
<td>Voxel mesh with transversely isotropic, linearly elastic–perfectly plastic material</td>
<td>Kopperdahl, 2002</td>
<td>Uniform axial displacement</td>
<td>0.80, force at 3% strain</td>
<td>0.28</td>
<td>-</td>
</tr>
<tr>
<td>Chevalier, 2008 [149]</td>
<td>Lumbar</td>
<td>12</td>
<td>5 mm/min</td>
<td>Voxel mesh, transversely isotropic with elasticity, plasticity, and damage</td>
<td>Constitutive model</td>
<td>Uniform displacement</td>
<td>0.77, fracture strength</td>
<td>0.64</td>
<td>-</td>
</tr>
<tr>
<td>Chevalier, 2009 [150]</td>
<td>Lumbar</td>
<td>12</td>
<td>5 mm/min</td>
<td>Voxel and surface meshes with transverse isotropic materials with damage</td>
<td>Constitutive model</td>
<td>Uniform displacement</td>
<td>0.89, fracture strength</td>
<td>0.72</td>
<td>-</td>
</tr>
<tr>
<td>Author, year [reference]</td>
<td>Experimental specimens</td>
<td>Number tested</td>
<td>Compression rate</td>
<td>Finite element model and material</td>
<td>Material mapping relationship</td>
<td>Superior boundary conditions</td>
<td>( R^2 ) strength</td>
<td>( R^2 ) stiffness</td>
<td>( R^2 ) strain</td>
</tr>
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<td>----------------</td>
</tr>
<tr>
<td>Zeinali, 2010 [155]</td>
<td>Thoracolumbar</td>
<td>9</td>
<td>0.5 mm/min</td>
<td>Brick elements with transversely isotropic properties with either linearly elastic/plastic or linearly elastic, perfectly plastic materials</td>
<td>Kopperdahl, 2002</td>
<td>Uniform displacement</td>
<td>0.94</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Dall’ara, 2010 [58]</td>
<td>Thoracolumbar sections, endplates removed</td>
<td>37</td>
<td>5 mm/min</td>
<td>Voxel model with elastic damage constitutive model</td>
<td>Rincon-Kohli and Zysset (2009)</td>
<td>Axial load but upper plate was allowed to rotate</td>
<td>0.79, fracture strength</td>
<td>0.49</td>
<td>-</td>
</tr>
<tr>
<td>Pahr, 2012 [147]</td>
<td>Thoracolumbar sections, endplates removed</td>
<td>35</td>
<td>5 mm/min</td>
<td>Tetrahedral mesh with explicit cortical shell</td>
<td>Rincon-Kohli and Zysset (2009), Calibration to get material properties</td>
<td>Axial load but upper plate was allowed to rotate</td>
<td>-</td>
<td>0.86</td>
<td>-</td>
</tr>
<tr>
<td>Pahr, 2014 [59]</td>
<td>Thoracolumbar sections, endplates removed</td>
<td>37</td>
<td>5 mm/min</td>
<td>Tetrahedral mesh with explicit cortical shell</td>
<td>Rincon-Kohli and Zysset (2009), Calibration to get material properties</td>
<td>Axial load but upper plate was allowed to rotate</td>
<td>0.92, fracture strength</td>
<td>0.68</td>
<td>-</td>
</tr>
</tbody>
</table>
1.6.4 Use of strain gages for validation of FE specimen-specific models

While the use of FE models to predict surface strain has been limited for vertebrae [153], [154], surface strains have been predicted with computational models on other bones including the pelvis [156], proximal femur [157]–[161], distal radius [162] and scapula [163] (Table 1.7). The surface strains on the bone were measured with either uniaxial strain gages or strain rosettes. In the studies where strain rosettes were used, the principal strains were calculated and compared. In one study, only the minimum strain was used in the correlation [153]. However, in other studies, the correlation was performed using both the minimum and maximum principal strains (Figure 1.21). This may bias the results to show a correlation since assuming normal material behavior, the minimum principal strain will be negative and maximum principal strain will be positive. In cases where uniaxial strain gages were used, the tensile and compressive areas on the bone may similarly influence the correlation.

![Strain comparison graph](image)

Figure 1.21: FE-predicted strains versus strain gage measurements on femora for models with heterogeneous properties assigned based on an experimental modulus-density relationship [25] developed for the femur. Modified from [159] with permission of Pergamon; permission conveyed through Copyright Clearance Center, Inc.
Table 1.7: Summary of comparisons of surface strains to FE-predicted strains in specimen-specific FE models

<table>
<thead>
<tr>
<th>Author, year [reference]</th>
<th>Bone</th>
<th>Strain gages</th>
<th>Correlation details</th>
<th>R² correlation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anderson, 2005 [156]</td>
<td>Pelvis</td>
<td>10 strain rosettes</td>
<td>Minimum and maximum principal strain</td>
<td>0.82</td>
</tr>
<tr>
<td>Bessho, 2007 [157]</td>
<td>Proximal Femur</td>
<td>12 strain rosettes</td>
<td>Minimum and maximum principal strain</td>
<td>0.93</td>
</tr>
<tr>
<td>Taddei, 2006 [158]</td>
<td>Proximal Femur</td>
<td>13 strain rosettes</td>
<td>Minimum and maximum principal strain</td>
<td>0.91</td>
</tr>
<tr>
<td>Schileo, 2007 [159]</td>
<td>Proximal Femur</td>
<td>15 strain rosettes</td>
<td>Minimum and maximum principal strain</td>
<td>0.91</td>
</tr>
<tr>
<td>Keyak, 1992 [160]</td>
<td>Proximal Femur</td>
<td>11 strain rosettes</td>
<td>Minimum and maximum principal strain</td>
<td>0.59</td>
</tr>
<tr>
<td>Trabelsi, 2011 [161]</td>
<td>Proximal Femur</td>
<td>5 uniaxial strain gages in direction of anticipated strains</td>
<td>Strains aligned with strain gage</td>
<td>0.95</td>
</tr>
<tr>
<td>Bhatia, 2014 [162]</td>
<td>Distal Radius</td>
<td>6 strain rosettes</td>
<td>Minimum and maximum principal strain</td>
<td>0.86</td>
</tr>
<tr>
<td>Gupta, 2004 [163]</td>
<td>Scapula</td>
<td>18 uniaxial strain gages</td>
<td>Strains aligned with strain gage</td>
<td>0.81</td>
</tr>
<tr>
<td>Imai, 2006 [153]</td>
<td>Vertebra</td>
<td>4 strain rosettes</td>
<td>Minimum principal strain</td>
<td>0.70</td>
</tr>
<tr>
<td>Roost, 2012 [154]</td>
<td>Vertebra</td>
<td>8 strain rosettes</td>
<td>Minimum and maximum principal strain</td>
<td>0.69</td>
</tr>
</tbody>
</table>
1.6.5 Use of DIC for validation of FE models

DIC provides a rich dataset for validation of FE models but the utility of DIC has only recently been used for this purpose.

FE models of composite bones have been developed and compared with DIC results [118], [119], [121]. For the pelvis, DIC and strain gages were used to measure the strain on the superior-posterior ilium surface for intact bones and bones implanted with an acetabular cup. The Von Mises strain predicted by linear elastic FE models showed strong agreement with the experiment for the intact ($R^2 = 0.91$) and the implant case ($R^2 = 0.88$) [118]. Similarly for the proximal femur, good agreement was found for the Von Mises strain measured experimentally with DIC and predicted computationally [119]. Another study also used composite proximal femora ($n = 6$) and loaded the bones quasi-statically until fracture. A correlation between the DIC-measured strain and the FE-predicted strain for the minimum and maximum principal strain was strong ($R^2 = 0.87$). However, this may be a stronger correlation than would have been found if only considering the minimum or maximum principal strain alone [121]. Composite bones, however, have less variation in bone geometry and material properties than real anatomical specimens.

DIC on cadaveric bones has been used to qualitatively validate specimen-specific models. In our laboratory, one cadaveric proximal femur was loaded dynamically in a sideways fall configuration and qualitative agreement was found in the pattern of strain in the experiment and FE model [164]. Another study used 22 cadaveric proximal femora and created 2D FE models based on projected geometry. However, the DIC was only compared qualitatively to the FE model [109]. While there is a large potential for using DIC as a tool in development of FE models of cadaveric bones, few quantitative comparisons have been performed to date.

One investigation performed quantitative evaluation of FE models using DIC-measured strains [165]. In this study, three femora were tested by fixing the shaft and applying load to the femoral head, parallel to the shaft. FE models were created with heterogeneous specimen-specific properties with a bilinear material model. The principal strains were compared between the model and experiment at a load equal to four times body weight. For the three bones together, the coefficient of determination was 0.94 (Figure 1.22). However, this correlation included both minimum and maximum principal strains, which may have increased the $R^2$ value.
The failure load was predicted within 2% in two bones; the load for the third bone was not compared because the end cap slipped during the experimental loading.

![Graph showing FE-predicted strains versus DIC-measured strains for three cadaveric femora.](image)

**Figure 1.22:** FE-predicted strains versus DIC-measured strains for three cadaveric femora. Modified from [165] with permission of Pergamon; permission conveyed through Copyright Clearance Center, Inc.

In summary, specimen-specific models of vertebrae have been developed. The models are typically validated for the overall stiffness and strength of the specimen. However, these FE models typically do not utilize strain measurements on the cortical surface. Studies that do use strain for validation have only used strain gages which only provide a single measurement for each transducer. On the other hand, DIC can provide full-field displacement and strain for hundreds of subsets on the bone for validation. To date, there has been limited validation of FE models of bones using DIC, and DIC has not been used on cadaveric vertebral bodies for FE model validation. Use of DIC for quantitative validation of FE models can help develop more accurate models of the bones that more accurately predict the bone response.

### 1.7 Research questions and objectives

The overall goal of this thesis was to investigate use of DIC in the laboratory to understand compression of vertebral bodies. DIC is a technique that has grown in use since its development, but the use of DIC to quantify bone surface displacement and strain has been limited. The studies in this thesis are both experimental and computational and demonstrate how DIC improves the synergy between these two methods (Figure 1.23).
The following paragraphs summarize the research questions and motivations for the four studies performed.

**Study 1: How similar are strain gage and DIC measurements for principal strains on the surface of porcine vertebral bodies during cyclic, QS loading? Do the two methods have similar levels of signal noise?**

Experimentally, vertebral bodies are commonly tested in compression. Typically, the response is quantified using strain gages, the current standard technique for strain measurement on the bone. DIC is an alternative measurement method that can provide full-field strains. However, the agreement between strain gages and DIC measurements has not been quantified for vertebral bodies or during cycling loading. In addition, the noise of the signal is relevant to choice of measurement method and was quantified in this investigation.

**Study 2: Do the locations of high strain correspond with the locations of vertebral fracture and what is the timing of local damage to the cortex relative to the yield force?**

Vertebral fracture is important clinically and providing methods for quantification of vertebral fracture in the laboratory can increase the value of the experiments. Using DIC, the fracture progression and strain measurements related to damage can be quantified.
DIC has not been previously used to investigate the location and timing of fracture in bone.

**Study 3: Are the patterns and magnitudes of the DIC-measured displacements on bones at slow and fast rates similar?**

The response of vertebral bodies depends on the rate at which the bones are loaded. Experimentally, bones are often tested at slow rates even though real-world injuries result from fast rate loading. Using a repeated measures design, DIC displacements for vertebral bodies tested at slow and fast rates were compared. DIC can provide more information about the magnitude and the pattern differences than was previously available.

**Study 4: Do specimen-specific FE models of vertebral bodies predict the DIC-measured surface displacement of the bone?**

FE models can be used to predict patient risk of vertebral fracture. Previously, these models have been validated using specimen stiffness derived from displacement measurements from the top to the bottom of the potting. However, this global measurement may not be representative of what occurs on the surface of the bone, which can be quantified using DIC. DIC can provide more detailed information about the surface of the bone than has been previously available, and this improved measurement can increase the ability to assess FE models.
Chapter 2: Comparison of strain measured with strain gages and DIC

2.1 Introduction

Strain measurements on the surface of bones are used to improve understanding of the mechanics of bones [86], [87], [114], [166], [167], inform development of orthopedic implants [168], [169], and validate computational models [153], [159]. Although strain gages are commonly used to measure strain on cadaveric bone surfaces, each strain gage only measures strain at one location and the size of a strain gage may be too large to detect high strain gradients. In addition, temperature changes and the quality of the gage attachment to the bone surface can negatively affect strain gage data. The adhesives used to attach strain gages may also reinforce the bone and thus lower the strains being measured [170].

DIC is an alternative method of measuring surface strain. It is an optical technique that tracks subsets of pixels in an image to determine the displacement of a material’s surface. The surface of an object is painted with a speckle pattern to provide unique pixel patterns. DIC can also measure bone surface strain in 3D using two cameras [171]. DIC’s benefits over strain gages include its ability to measure surface strains on irregular bone surfaces without direct bone contact, both of which are difficult or impossible using strain gages. However, DIC has limitations including that DIC assumes that the surface is a continuum and therefore may not accurately measure the displacements where this assumption is violated, such as near a crack, and the measurement quality is influenced by the speckle pattern and DIC correlation parameters.

Previous work has compared strains measured with strain rosettes and DIC on various bones or on engineering composites with mechanical properties similar to bone [113], [114], [118]. Sztefek et al. [113] loaded murine tibiae in compression and showed that a uniaxial strain gage and DIC measured similar strain magnitudes, but they did not directly compare the two techniques because the strain gage was too large to capture the local gradients. Ghosh et al. [118] found that strain rosette and DIC measurements were highly correlated for strains measured on the anterior surface of a composite human pelvis in compression. Gilchrist et al. [114] compressed cadaveric proximal femora and compared the strains measured using rosettes and

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DIC and found good agreement. To my knowledge, no previous studies have compared strains from DIC and strain gages on vertebrae.

The primary goal of this study was to compare the vertebral body bone strains measured using a strain rosette and DIC. Specifically, we compared the noise present in each method and quantified the differences in peak principal strains measured by each method during QS loading. We hypothesize that the strain gage will have less noise than DIC and that the average difference in the peak strains for the strain gage and DIC will be less than 15% of the measured strain.

2.2 Methods

2.2.1 Experimental procedure

Porcine thoracic and lumbar vertebrae (n = 15) were obtained from the fresh-frozen spine of one pig (Yorkshire-Landrace cross, female, 72 kg, 7 months) and stored at -20 °C prior to testing. Soft tissues were cleaned from the bones and the posterior elements were removed. Shortly before testing, two PMMA pieces were molded to the superior and inferior endplates to create parallel surfaces for applying a compressive force. Specimens were randomly assigned to have the strain rosette applied to an area on the left or right anterior surface of the vertebral body. A scalpel was used to remove all remaining tissue from the strain rosette area, and then the area was cleaned three times each with isopropyl alcohol and then acetone. A stacked triaxial strain rosette (FRA-2-11-3LT, backing diameter of 7 mm, Tokyo Sokki Kenkyujo Co. Tokyo, Japan) was applied using cyanoacrylate glue. The strain rosette was oriented with the central gage nominally in the superior/inferior direction and therefore we expected all gages to record compression during axial loading. While the glue cured, pressure was applied to the gage by hand using Teflon film.

The anterior surface of the vertebra, including the area over the rosette, was painted with a thin layer of white airbrush paint, and an airbrush was used to apply a black speckle. Finally, six black dots were painted on the anterior surface to create two equilateral triangles (~8 mm per side) located symmetrically across the sagittal centerline with one triangle centered over the strain rosette (Figure 2.1).
Figure 2.1: An example of camera view of the anterior surface of a prepared porcine vertebra. In this case, the strain rosette was applied on the right side. The bone was painted with a white layer with black speckle for DIC. Two sets of three dots were painted symmetrically about the centerline of the bone.

The vertebrae were then placed in line with the center of the actuator between the platens of a MTS (Electropuls E10000, Instron, Norwood, MA, USA). Two cameras (V12.1 Vision Research, Wayne, NJ, USA) with macro lenses (Nikon 105mm f/2.8 VR Micro-Nikkor, Tokyo, Japan and Sigma 105mm f/2.8 EX DG Macro, Kawasaki, Japan) were used to capture images of the anterior surface of the bone (1280x800 pixels, 24 frames/second). The cameras were positioned nominally 20 degrees apart; previous studies with two-camera DIC positioned the cameras 15 to 30 degrees apart [105], [112], [172]. The specimen was lit with two halogen lights (VRI-L250C, Vision Research, Wayne, NJ, USA). For each specimen, four load-controlled trials were performed (Figure 2.2). The first trial was used to as seat the PMMA on the vertebral endplates. Data from the second, third and fourth trials were analyzed. Each trial consisted of an initial 100 N hold for 3 s followed by three ramp loading/unloading cycles consisting of loading to 2050 N at 2000 N/s, a 0.5 s hold, unloading to 100 N at 2000 N/s and another 0.5 s hold. All strain gage measurements in the trials are relative to the strain measured after the start of the 100 N hold, when the first video image was captured. The strain rosette signals, as well as the trigger, load and position from the MTS, were collected at 20 kHz using a data acquisition system (PCI-6040E, National Instruments Corporation, Austin, TX, USA). More detailed experimental methods are provided in Appendix A. The DIC processing methods are provided in Appendix B and the accuracy of the DIC measurements are assessed in Appendix C. An alternative method for strain measurement, fiducial marker tracking (FMT), is detailed in Appendix D.
Figure 2.2: Time history of the loading for each specimen. The pre-test trial was used to ensure the bone was seated to the PMMA. The prescribed loading was the same for all trials.

2.2.1.1 Data processing

Three-dimensional DIC analysis was performed on the images using commercially-available software (StrainMaster 8.2, LaVision, Göttingen, Germany). The subset size was 31x31 pixels and the distance between the centers of adjacent subsets was 15 pixels. Image distortion was corrected using a pinhole camera model. On average, the images had a spatial resolution of 45 pixels/mm (range 38 to 57 pixels/mm). The first image for the trial, collected during the 100 N preload, was used as the reference state, and successive images for that trial were compared to the first image.

For the DIC, the time-history of the strain tensor components were zeroed over 2 seconds during the 100 N hold; for one specimen, the zeroing was only performed over 0.125 seconds (3 frames) as more video was not recorded. Each of these signals was low-pass filtered at 2 Hz. For the strain rosette, the strain signals from the three gages were zeroed over 2 seconds during the 100 N hold and similarly low-pass filtered at 2 Hz. The minimum and maximum principal strains were calculated from the zeroed and filtered strains.

Following zeroing and filtering, the noise associated with each strain measurement method was assessed by taking the root mean square (RMS) error of the minimum and maximum principal strain signals during the 100 N preload. Analysis of the RMS noise of the strain rosette was performed for trials without evidence of debonding. For each method the average of the six values of RMS noise for each specimen (minimum and maximum principal strain signals for three trials) was taken. The noise level of DIC was measured over the area of the strain rosette and over the triangular area contralateral to the strain rosette.

To compare the strain calculation methods, the time of the peak load was identified from the load cell signal for each loading cycle. The strains at the times of maximum load were
compared using Bland-Altman plots, which are used to compare two methods where neither is considered a gold standard.

To account for magnitude differences in the strains between the two methods, an additional value was calculated, termed normalized error. Normalized error (Equation 2.1), was calculated by dividing the difference, or bias, between the two methods (y-value on Bland-Altman plot) by the absolute value of the mean value of the two methods (x-value on Bland-Altman plot) for each peak and then taking the average of the absolute values; n is the number of peaks.

\[
\text{Normalized Error}\% = \frac{\sum_{1}^{n} \frac{|Peak_1 - Peak_2|}{|Peak_1 + Peak_2|/2}}{n} \times 100
\]  

Equation 2.1

Evidence of strain rosette failure or debonding, defined as loss of local adhesion between the rosette and the cortical bone, was observed in some trials. Four criteria were used to identify failure or debonding:

1. Visual identification: If two out of three observers saw rosette motion relative to the cortical bone or changes in the reflected light over the rosette in the high-speed video.
2. Tensile gage: If there was at least one gage in tension. Based on the strain gage mounting relative to the load, all gages were expected to be in compression.
3. Strain angle SD: If the SD of the principal strain angles at peak minimum principal strain (3 trials, 3 peaks/trial) was above 0.5 radians.
4. Strain SD for individual gages: If the SD of the peak strains (3 trials, 3 peaks/trial) from any gage in the rosette exceeded 700 με.

A trial that met any two of four criteria was excluded from the strain rosette to DIC analysis. Based on these criteria, 20 of 45 trials were excluded from the Bland-Altman analysis. An alternative method for identifying debonding is presented in Appendix E.

2.2.2 Statistics

A one-way analysis of variance (ANOVA) was used to evaluate if the average RMS noise was different for the strain rosette, the DIC over the strain rosette, and the DIC of the triangular area not over the strain rosette. A post-hoc Tukey test was used to determine if the RMS noise was significantly different. Bland-Altman plots were created to compare the rosette strain to the average DIC strain measured over a 2 x 2 mm region overlying the strain rosette. A
one-sample t-test was used to evaluate if the bias was significantly different than zero. P-values less than 0.05 were considered significant for all statistical tests.

2.3 Results

2.3.1 RMS noise

The mean ± SD RMS noise levels for strain measurement methods were different (ANOVA p<0.0001; Figure 2.3). The strain rosette noise (1 ± 1 με) was less than the DIC noise for the strain rosette area and the area with no strain rosette (30 ± 8 and 24 ± 6 με; Tukey p<0.0001 and p<0.0001). The RMS noise levels measured with DIC over the rosette and on the side without the rosette were not significantly different (Tukey p=0.41). The RMS noise and the average values of the minimum and maximum principal strains for each method are provided (Table 2.1).

![Figure 2.3: Comparison of the RMS noise of the principal strains on the surface of the vertebrae measured by the strain rosette and DIC.](image)

For the DIC, the noise was measured both over the strain rosette and over the triangular region. Means (± 1 SD) are shown and the raw data are plotted as points. The RMS noise was
defined as the RMS error of the strain during 2 seconds of the trial where the load was held constant at 100 N. The DIC RMS noise levels are significantly different than the strain rosette.

Table 2.1: Summary of results for the RMS noise and the average peak values of the minimum and maximum principal strain for each of the measurement methods

<table>
<thead>
<tr>
<th></th>
<th>Strain Rosette</th>
<th>Digital Image Correlation (Strain rosette area)</th>
<th>Digital Image Correlation (Triangle area, side without strain rosette)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RMS Noise</strong> (Average ± 1 SD) (με)</td>
<td>1 ± 1 με</td>
<td>29 ± 8 με</td>
<td>24 ± 6 με</td>
</tr>
<tr>
<td><strong>Average Peak Minimum Principal Strain (με)</strong></td>
<td>-2840 με</td>
<td>-2731 με</td>
<td>-2587 με</td>
</tr>
<tr>
<td><strong>Average Peak Maximum Principal Strain (με)</strong></td>
<td>514 με</td>
<td>568 με</td>
<td>678 με</td>
</tr>
</tbody>
</table>

2.3.2 Calculating strain using DIC compared to a strain rosette

Comparing the peak magnitudes of the minimum principal strain between DIC and a strain rosette, the normalized error was 10% (Table 2.2); for the individual trials, the normalized error ranged from 1.7 to 41.6%. The minimum principal strain error for trials without evidence of debonding was typically less than 600 με (Figure 2.4).

Table 2.2: Summary of results for the Bland-Altman plots including the bias ± 1 SD Normalized error was found by taking the average of the absolute differences between the methods of strain measurement (y-value on the Bland-Altman plot) divided by the average absolute values of the strain measurements (x-value on the Bland-Altman plot).

<table>
<thead>
<tr>
<th>Strain Rosette - DIC</th>
<th>Minimum Principal Strain</th>
<th>Maximum Principal Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bias ± 1 SD (με)</strong></td>
<td>-108 ± 351 με</td>
<td>-53 ± 166 με</td>
</tr>
<tr>
<td><strong>Normalized Error ± 1 SD (%)</strong></td>
<td>10 ± 8%</td>
<td>21 ± 14%</td>
</tr>
</tbody>
</table>
Figure 2.4: The difference at the loading peaks between the minimum principal strain measured by strain gage and DIC for trials (three peaks per trial, three trials). Peaks from trials that had no evidence of debonding are shown with circles while peaks from trials with evidence of debonding are shown as triangles.

Bland-Altman plots comparing the magnitudes of the peak values of the strains from the DIC and the strain rosettes had biases (± two-SD error bounds) of -108 με (±702 με) for the minimum principal strain and -53 με (±332 με) for the maximum principal strain (Figure 2.5). The biases were significantly different from zero for the minimum and maximum principal strains (one-sample t-test, p=0.009 and p=0.006, respectively). The mean bias of the minimum principal strain between during all time points when the load was greater than 500 N ranged from -999 to 257 με.
Figure 2.5: Bland-Altman plots of the magnitudes of the (a) minimum (compressive) principal strain and (b) maximum (tensile) principal strain measured using a strain rosette and DIC at the peak load. The horizontal axis is the average values of the strain measured using the rosette and the average value of the strains from DIC over the same area at the peak load. The vertical axis is the value measured using the rosette minus the strain from DIC. The solid lines are the average value of all the differences. The dashed lines are the average value of the differences ± 2 SD.

In the trials where debonding was observed, the strain measured with the rosette was of lower absolute magnitude than the strain measured with DIC (Figure 2.6).
Figure 2.6: Example time histories of the minimum principal strains measured with DIC and strain rosette in one trial from each specimen in which debonding of the strain rosette was observed. For all trials where debonding was observed, the DIC measured higher absolute minimum principal strains than the strain rosette. Listed on each plot are the criteria met that demonstrate debonding.

2.4 Discussion

Understanding the strain response of bone to loading is important in many research areas. In this study, we examined two methods for measuring strain on the surface of bone: strain rosettes and DIC. This study demonstrated that strain rosette signals have lower RMS noise (1 με), but could be influenced by factors we interpreted as debonding or poor initial bond quality. For DIC compared to the well-bonded strain rosettes, the bias ± 2 SD was -108 με ± 702 με for the minimum principal strain. We hypothesized the average difference between the methods
would be less than 15% of the strain measured. This was true for the minimum principal strain (10%) but was not true for the maximum principal strain (21%).

DIC had a higher RMS noise compared to the rosette. The noise in DIC is important to consider, especially when calculating the principal strains on the surface of the bone. The strain during the 100 N preload was expected to be zero since each component of the strain tensor was zeroed but noise in the raw strain signals resulted in a small negative minimum principal strain and a small positive maximum principal strain. This effect also exists when the bone is loaded and would contribute to an increased absolute magnitude of both the minimum and maximum principal strain.

Other studies have investigated noise in DIC measurements on bones. The average RMS noise in the DIC strain signal during a 100 N hold was 24 με over the triangular region, while previously, the noise of DIC on mouse tibiae in an unloaded state was reported as 500 με (300 με with filtering) [113]; the noise in the current study may be lower since vertebral bodies are less curved than a mouse tibia. For a flat plate imaged next to a cadaveric femur using stereo DIC, the maximum principal strain noise levels after filtering for three femora were found to be 19 με, 27 με, and 29 με [112]; the RMS noise on the vertebrae was similar.

Localized debonding of the strain rosette was visually identified in seven out of fifteen specimens and 20 out of 45 trials. The relatively high proportion of debonded gages could be due to the bone porosity, bone moisture, or mounting technique; however, there is nothing in the technique used that we suspect may be the cause of this. To identify debonded gages, we used four criteria that may not be available in loading scenarios that are not cyclic or not captured with high-speed video. In all specimens in which debonding was deemed to occur, the strain signals still followed the cyclic loading profiles but with a lower magnitude than that measured by DIC, a result expected for debonded gages [173]. Since the shape of the strain signal may appear normal and the gage returns to zero upon unloading, it could be difficult to assess if a strain gage partially debonds without evaluating the video or comparing its output to an independent measurement. While we do not know the rate, if any, of unrecognized debonding in prior studies using strain gages, the results indicate that users of strain gages need to carefully inspect their bonds before and after an experiment and ideally use high-speed video or DIC to confirm that debonding has not occurred.
Gilchrist et al. [114] reported that average values of the minimum principal strain error (DIC compared to strain rosette) on cadaveric femora ranged from -375 to 336 µε. In the current experiment, the average differences were larger in magnitude with values ranging from -999 to 257 µε. Ghosh et al. previously used DIC and rosettes to assess strain on the surface of a composite pelvis during a static hold [118]. They reported that a normalized error between the DIC and rosette strains was 11% for the minimum principal strain and 22% for the maximum principal strain, both of which are similar to the normalized error in the current study of 10% for the minimum principal strain and 21% for the maximum principal strain.

This work has limitations. Human bones may be more variable than the porcine vertebrae used in the current study. Overall, porcine vertebrae have similar characteristics to human vertebrae in terms of moisture, size, and surface curvature. However, bones from human donors are generally from elderly donors and may have surface abnormalities which would influence how well the DIC can reconstruct the bone topography and how well the strain gage can be bonded to the bone. Although we only measured surface strain, vertebra internal strains can be measured with DVC [126]. To exclude specimens and trials where the strain gages appeared to debond, human observation and criteria developed by examining the data were used.

The RMS noise levels, standard deviations, and errors presented in this paper are dependent on the speckle pattern, the region size, the subset spacing, the tracking dot shapes, and other imaging parameters. The influence of particular imaging parameters has been shown in literature; for example, speckle pattern, as well as the subset size used for analysis of speckle pattern, has been shown to influence the displacement accuracy [174]. It was not the goal of this paper to explore imaging parameters but instead to characterize the errors expected from a particular setup using different methods of strain measurement.

Optical techniques have both advantages and disadvantages compared with strain rosettes. DIC provides full-field strains and may reduce specimen preparation time compared to applying strain gages, but DIC requires cameras and lenses to collect images and strain can only be measured where the surface is visible. However, optical methods do not have the risk of strain gage debonding. Experimentally measuring strain on the bone is a useful technique for many biomechanical applications. When selecting a method to measure strain, the advantages and disadvantages of each technique should be considered.
Chapter 3: Identification of fracture using DIC

3.1 Introduction

Worldwide, it is estimated that osteoporosis causes nine million fractures annually [175]. Of all osteoporotic fractures, 27% occur to the vertebrae [33] and these spinal fractures negatively affect the quality of life of patients [176]. Characterizing fracture forces ex vivo can help us understand the relationship between structure and force and eventually allow us to identify individuals who may be at risk for fracture. Previously, experimental testing has been used to determine the compressive load tolerance of ex vivo vertebrae [27], [44], [47], [177], [178]. Typically, the failure force is determined as the maximum force that can be sustained by the vertebra. However, mechanical damage to the vertebra may occur prior to this point and identification of this damage can help better characterize the bone’s tolerance.

Various methods have been used previously to identify damage or fracture on cadaveric specimens during experiments including acoustic emission sensors [179]–[182], strain gages [183], [184], video analysis [185], [186], electro-conductive lines [187], [188] or DVC [126], [128]. Each of these methods has strengths and limitations. Acoustic emission sensors and strain gages can provide the time of injury but do not measure the location of damage. Electro-conductive lines can provide the location of the damage but are time intensive to apply, provide low resolution, and the technique may not be suitable for compressive fractures. Video can allow for qualitative evaluation of fracture location and propagation, but the limited ability of the human eye may limit its usefulness for detecting minor damage. DVC quantifies the volumetric deformation of bone but requires computed tomography (CT) images, and therefore, the temporal resolution is low. To address these limitations, we propose use of DIC for damage identification.

DIC is an optical technique that measures the displacement and strain on a surface using images collected by cameras. In the initial image, the surface is divided into smaller subsets, each with a unique array of pixel intensities. In subsequent images, the subsets can be identified using a pattern-matching algorithm providing the displacements, and therefore strains, on the surface. By using two or more views of the same object, the three-dimensional surface displacements and strains can be measured. DIC has been used on soft tissues, such as the IVD [105] and arteries [103], and on bones, such as the femur [112], [114] and the murine tibia [113].
DIC can yield hundreds of data points on the surface of the bone to understand the sample behavior instead of the global displacement or the strain at one location.

Strain is related to fracture of the bone since locations of high strain lead to bone failure. When bone failure occurs, there are then higher strains over the locations of the fracture. Since cortical bone has previously been shown to have heterogeneous properties [189], [190] and heterogeneous surface strains when loaded [108], [191], global experimental measurements such as axial force or displacement of the whole bone may not be appropriate for identification of local failure of the bone. DIC provides local strains on vertebral bodies and therefore may better detect failure of the cortex.

The objective of this study was to quantify strain progression using DIC on thoracolumbar vertebral bodies loaded in compression to failure. We predict that qualitatively the failure locations identified from video analysis will correspond with locations of high compressive strain on the anterior cortex at the time that the strain pattern on the bone stabilizes. Furthermore, we hypothesize that local variables related to damage, including DIC minimum principal strain pattern stabilization and a percentage of the DIC subsets reaching a cortical strain failure threshold, will occur prior to reaching yield force.

3.2 Methods
3.2.1 Experimental procedure

Fresh-frozen human cadaveric vertebrae (thoracic, n = 1 and lumbar, n = 5; male, n = 3, female, n = 1, and unknown, n = 2) were obtained (Table 3.1). The soft tissue and posterior elements were removed from the bones. The specimens were potted in PMMA using a custom potting rig to make parallel surfaces for compressing the bone. To provide unique pixel intensities for DIC, the bones were painted white and a black speckle pattern was applied using an airbrush (VL, Paasche, Chicago, IL, USA). To indicate loading direction, additional larger dots were painted on the surface of the bone.
Table 3.1: Summary of the vertebral body specimens tested. Two specimens (1-L2 and 1-L3) were from the same donor. U/K indicates the donor information is unknown.

<table>
<thead>
<tr>
<th></th>
<th>Sex</th>
<th>Donor Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-L2</td>
<td>U/K</td>
<td>U/K</td>
</tr>
<tr>
<td>1-L3</td>
<td>U/K</td>
<td>U/K</td>
</tr>
<tr>
<td>2-L1</td>
<td>M</td>
<td>51</td>
</tr>
<tr>
<td>3-T11</td>
<td>M</td>
<td>36</td>
</tr>
<tr>
<td>4-L1</td>
<td>F</td>
<td>89</td>
</tr>
<tr>
<td>5-L1</td>
<td>M</td>
<td>66</td>
</tr>
</tbody>
</table>

The specimens were loaded using a materials testing machine (Electropuls E10000, Instron, Norwood, MA, USA) with an attached platen. The potted specimen was positioned below the actuator and the inferior PMMA was fixed to be stationary. The bone was loaded at a rate of 0.1 mm/s up to 3 mm, resulting in fractures for all specimens. During the loading, two cameras (V12.1 Vision Research, Wayne, NJ, USA) with macro lenses (Nikon 105mm f/2.8 VR Micro-Nikkor, Tokyo, Japan with an extension tube and Sigma 105mm f/2.8 EX DG Macro, Kawasaki, Japan) were used to capture images of the anterior surface of the vertebra (1280 by 800 pixels; 100 frames/second) (Figure 3.1). The angle between the cameras was on average 22.3 degrees. The force and displacement from the materials testing machine were collected at 5 kHz. More detailed experimental methods are provided in Appendix A.

Figure 3.1: Images of the same vertebral body from the two cameras. The large black dots indicated by the arrows were painted on the midline of the anterior surface and were aligned with the loading direction. Two additional black dots were painted but not used in this study. The yellow outline indicates the approximate area where the DIC analysis could be performed and the red square indicates the relative size of the subset consisting of 31 x 31 pixels used for the DIC analysis.
3.2.2 Data processing

The images from the cameras were imported into the DIC software (StrainMaster 8.2, LaVision, Göttingen, Germany) and the images were calibrated based on multiple images of a calibration grid collected with the same camera configuration (See Appendix B). For the analysis, a subset size of 31 x 31 pixels with 15 pixel spacing between subsets was used (Range: 1713-4198 subsets) (Figure 3.1). Since DIC analysis requires that the surface be visible in both camera views, the displacement could not be measured at the lateral edges of the vertebra and at superior and inferior edge of the vertebra where the view was obscured by the PMMA. For each subset, the displacement and strain tensor signals were low-pass filtered at 20 Hz with a Butterworth fourth-order filter. Using the filtered signals, the magnitudes of displacement as well as the minimum and maximum principal strains were calculated.

The locations of fracture were identified in the video as visible failure of the anterior cortex. In some cases, this failure progressed to complete compromise of the cortical shell. In other cases, there was relative motion between cortical shell regions without cortical shell disruption; this type of failure was considered sub-catastrophic.

Three time points of interest were identified related to the hypothesis regarding the timing of the bone failure: the yield point, the point when the minimum principal strain pattern stabilized and the time when a proportion of the subsets reached a minimum principal strain threshold of 1.0%. These will be referred to as yield, strain stabilization, and 1% strain threshold, respectively, throughout the paper.

The yield force was defined using the 0.2% offset method [192] based on the average strain on the bone surface measured with DIC. To find the strain on the bone surface, for each subset located at the top edge of the DIC analysis area (Range: 61 to 106 subsets), a corresponding subset located inferriorly along the loading vector was identified (Figure 3.2). Since strains calculated over short distances would have higher errors, lengths were eliminated from analysis if the distance was less than 80% of the maximum distance for each specimen. To find strain, the relative displacement along these lengths was measured and the value was divided by the original length. These strains were averaged for each specimen. Then, the stiffness of the specimen was calculated for the force-strain curve using a linear fit of the points from 25% to 75% of the maximum force. Finally, the yield force was identified by offsetting the stiffness line by 0.2% and finding the intersection of the offset line and the force curve.
Figure 3.2: Schematic of a DIC analysis region showing the direction of loading, as defined by the alignment points. During loading, the strain was calculated for each superior edge subset relative to the inferior edge subset located along the loading vector. Lengths of less than 80% of the maximum length were excluded.

Strain stabilization was defined as the time when the pattern of high strain stabilized. This was found by identifying in each frame the 10% of the DIC subsets that had the largest minimum principal strains which ranged from 171 to 420 subsets, depending on the specimen. Then, each frame was compared to the next frame to quantify the percentage of subsets at the same location from one frame to the next. The percentage similarity time history signal was low-pass filtered at 2 Hz to reduce the noise, and the strains were considered stabilized when there was 98% similarity from one frame to the next for the location of the 10% of the DIC subsets with the largest minimum principal strains (Figure 3.3).
Figure 3.3: Example for specimen 5-L1 of the (A) unfiltered and (B) filtered overlap (%) from frame-to-frame of the locations on the bone surface with the largest minimum principal strains. To determine the time that the DIC strain stabilized, the signal from (A) was low-pass filtered at 2 Hz to get the signal in (B). The dot indicates the time when there was 98% similarity between the frames.

The 1% strain threshold was defined as the time when 10% of the DIC subsets had a minimum principal strain magnitude of 1.0% or greater. For this criterion, 10% was selected to ensure a sufficient number of subsets had exceeded the yield strain criterion, and the yield strain criterion of 1% was based on the yield strain previously found for cortical bone [70], [193], [194].

The sensitivity of these variables to the selected thresholds is explored in Appendix F.

3.3 Results

The largest compressive strains developed near the superior and inferior endplates (Figure 3.4).
Figure 3.4: The progression of the minimum principal strain on the anterior surface of the specimens as a percentage of the yield force. The strain signals were low-pass filtered at 20 Hz prior to calculation of the minimum principal strain.

The fractures seen in the video were similar to the locations of high strain on the bone surface at the point where the minimum principal strain stabilized to consistent locations (Figure 3.5). The fractures observed were generally horizontal fractures near the endplates. For this study, four of six bones fractured near the superior edge while two specimens fractured near the inferior edge. In three of the vertebral bodies, where fracture was observed at the superior edge, there was subcatastrophic failure at the inferior edge and in one vertebral body with fracture at the inferior edge, there was subcatastrophic failure at the superior edge.
Figure 3.5: Comparison of the video-identified fractures and DIC high-strain locations. For each specimen, the upper image is a video image. The outline indicates the area that was tracked using DIC. The white solid lines represent where the bone failed completely based on video analysis. The white dashed lines indicate where subcatastrophic failure was observed in the videos. The lower image provides the DIC map of the minimum principal strain and the color bar indicates the strain magnitudes. This image is at the time of strain stabilization where the strain stabilizes based on the criteria of 98% similarity between frames of high strain DIC subsets. The black points indicate the centers of 10% of the DIC subsets that had the largest minimum principal strains.

In each specimen, the first event was the strain stabilization followed by 10% of the DIC subsets reaching 1% minimum principal strain, and finally the global bone yield occurred (Figure 3.6). These values were tabulated (Table 3.2) along with the mean and SD of the strains at that point.
Table 3.2: Summary of important force levels for the specimens including strain stabilization, 1% strain threshold, and yield. The mean and SD of the minimum principal strains at that time are reported for the entire DIC measurement area and for the 10% of DIC subsets with the highest strain.

<table>
<thead>
<tr>
<th></th>
<th>DIC Strain stabilizes</th>
<th>1% strain threshold</th>
<th>Yield force</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Force (N)</td>
<td>Minimum Strain Characteristics (με)</td>
<td>Mean</td>
</tr>
<tr>
<td></td>
<td></td>
<td>All DIC subsets</td>
<td>10% highest strain subsets</td>
</tr>
<tr>
<td>1-L2</td>
<td>3482</td>
<td>-5649</td>
<td>2737</td>
</tr>
<tr>
<td>1-L3</td>
<td>1981</td>
<td>-4670</td>
<td>1888</td>
</tr>
<tr>
<td>2-L1</td>
<td>746</td>
<td>-4679</td>
<td>2131</td>
</tr>
<tr>
<td>3-T11</td>
<td>2186</td>
<td>-5250</td>
<td>2099</td>
</tr>
<tr>
<td>4-L1</td>
<td>993</td>
<td>-5919</td>
<td>2664</td>
</tr>
<tr>
<td>5-L1</td>
<td>1075</td>
<td>-2512</td>
<td>1248</td>
</tr>
</tbody>
</table>

|                  | Force (N)             | Minimum Strain Characteristics (με) | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
|                  |                       | All DIC subsets | 10% highest strain subsets | All DIC subsets | 10% highest strain subsets | All DIC subsets | 10% highest strain subsets | All DIC subsets | 10% highest strain subsets |
| 1-L2             | 3954                  | -6458 | 3254 | -13263 | 5552 | 291 | 10613 | -39843 | 12598 |
| 1-L3             | 2870                  | -6384 | 2697 | -12619 | 2555 | 113 | 10613 | -39843 | 12598 |
| 2-L1             | 1190                  | -6427 | 3286 | -14102 | 3883 | 328 | 11399 | -42681 | 13666 |
| 3-T11            | 2919                  | -6767 | 2746 | -13420 | 3095 | 274 | 11399 | -42681 | 13666 |
| 4-L1             | 1108                  | -6379 | 2910 | -12791 | 3597 | 291 | 11399 | -42681 | 13666 |
| 5-L1             | 3322                  | -6493 | 3631 | -14796 | 4902 |

|                  | Force (N)             | Minimum Strain Characteristics (με) | Mean | SD | Mean | SD | Mean | SD | Mean | SD |
|                  |                       | All DIC subsets | 10% highest strain subsets | All DIC subsets | 10% highest strain subsets | All DIC subsets | 10% highest strain subsets | All DIC subsets | 10% highest strain subsets |
| 1-L2             | 7210                  | -13291 | 10613 | -39843 | 12598 | 10613 | -39843 | 12598 |
| 1-L3             | 7179                  | -16153 | 12754 | -46861 | 17300 | 12754 | -46861 | 17300 |
| 2-L1             | 2212                  | -13476 | 11399 | -42681 | 13666 | 11399 | -42681 | 13666 |
| 3-T11            | 7019                  | -19440 | 11803 | -48744 | 11231 | 11803 | -48744 | 11231 |
| 4-L1             | 2805                  | -14841 | 11055 | -38754 | 20368 | 11055 | -38754 | 20368 |
| 5-L1             | 8130                  | -18270 | 15702 | -56530 | 16648 | 15702 | -56530 | 16648 |
3.4 Discussion

In this study, the progression of strain on the anterior cortex of vertebral bodies loaded in compression to failure was measured. The failure locations based on video analysis were in good qualitative agreement with the high-strain locations from DIC. In support of our hypothesis, the 10% of DIC subsets with the largest minimum principal strains stabilized to a consistent location on the bone and 10% of the subsets reached 1% strain prior to the yield force. Both the strain stabilization and the local strain are measures of damage to the vertebral cortex.

At the time of DIC stabilization, there was qualitative agreement between the locations of high-strain DIC subsets and the fracture locations observed in the video analysis. This indicates that at the time of strain stabilization the locations of failure can be predicted for vertebral bodies loaded in compression. In the current study, fractures occurred either near the superior or inferior endplate in all specimens. The location of the observed fractures were in agreement with previous studies showing that there is more high-risk tissue near the endplates [195] and that failure most commonly initiates near the endplates [126], [128].
In all specimens, the sequence of the events was similar and in agreement with our hypothesis. Based on the 98% threshold used to define strain stabilization, the high-strain locations stabilized at between 13 and 48% of the yield force, depending on the specimen. The 1% strain threshold was reached by 10% of the DIC subsets between 39% and 55% of the yield force. During whole bone testing, yield force typically is calculated for the entire structure. Based on the results in the current study, however, small regions of the cortical bone likely yield much earlier than the global structure appears to yield. At the yield force, the average minimum principal strains within the 10% of subsets with the highest values was 4.6%, much higher than the yield strain previously found for cortical bone samples in compression of between 0.7 and 1.3% [70], [193], [194]. When characterizing the tolerance of bone to compressive loading, the maximum force indicates the force the structure can sustain until catastrophic failure but likely does not characterize the force at which permanent damage to the cortical shell initiates.

Previously, DIC has been measured in studies where rat femora [111] or cadaveric human femora [112] were loaded to failure. It was shown that the failure occurred at locations of high maximum principal strains [111] or where the sum of the magnitudes of the maximum and minimum principal strains were highest [112]. These findings are similar to the current study where the failure locations qualitatively corresponded with the locations of high compressive strain. DIC has also been used to detect damage on small cortical bone samples [196], [197].

For spatial identification of the fracture location, the most common methods are video analysis [186], [198], [185] and DVC [128], [129]. Video requires the observer to identify where the cortical shell is being compressed or pulled apart, essentially identifying where the observer sees strain on the bone; this is difficult for subtle but injurious strains. Using DIC for fracture identification can detect subtle changes not possible by eye and allows for quantification of the magnitude of the strain. DVC, compared to DIC, provides a more complete quantification of bone failure throughout the volume of bone. However, DVC requires a high-resolution CT scanner and time-consuming scans of the specimen following step-wise loading and the loading rig must fit within the scanner. These requirements limit the size of bones that can be loaded and the types of experiments that can be performed using DVC. DVC also uses the natural bone texture to match the volumetric subsets, meaning that the bone microstructure influences the accuracy of the DVC. Use of DIC to investigate bone failure can complement ongoing DVC research regarding vertebral body fracture. DIC allows for fracture quantification during
complex experimental conditions that would not be possible within a CT scanner. Furthermore DIC analysis, unlike DVC, can be performed at physiologically relevant rates since the only temporal limitation for DIC is the rate at which images can be collected by the cameras.

Like all *ex vivo* experimental studies, this study has limitations. First, the bones were loaded through PMMA, not an IVD. Loading through PMMA is less physiologic and may result in a higher ultimate force [199]. However, loading through PMMA creates a consistent boundary condition independent of the IVD degeneration. Second, DIC only measures the surface displacements; failure internally or on an exterior portion of the bone where the DIC was not measured could not be quantified. For example, in specimen 4-L1, there was fracture on the anterior cortex but it was outside of the DIC measurement area; the subsets of high minimum principal strain identified in the DIC stabilization did not fully represent the fracture location. In other specimens, the fracture may have initiated outside of the DIC measurement area but could only be quantified when fracture was present within the measurement area. However, for fractures on the anterior cortex, DIC improves the identification of fractures. Third, the current investigation was only performed for vertebral bodies in one loading scenario. For bones from other anatomic regions and loading scenarios, it is not clear if the timing relative to the yield point would be similar.

In this study, strain progression was quantified for six thoracolumbar vertebrae under compressive loading, and the anterior cortex fractures occurred near the superior and inferior endplates. Two variables were defined which are related to cortex damage, the time of strain stabilization and the time when 10% of the DIC subsets reached a local strain threshold, to evaluate the relative timing of local damage to the timing of global yield. In all specimens, the strain stabilized and 10% of the subsets reached a minimum principal strain of 1% prior to the yield force. This work demonstrates the utility of DIC for fracture identification on bones, and shown that the time of the vertebral body reaching the yield force or the maximum force is not representative of the initiation of local failure.
Chapter 4: Using DIC to understand rate-dependent effects of vertebrae

4.1 Introduction

The risk of vertebral fracture is high, particularly among the elderly. For instance, studies show the prevalence of vertebral fractures among women ages 70 to 75 is 15-27% [200]–[202]. Vertebral fractures are often due to compressive loading and may occur as the result of traumatic loading, such as a fall, or non-traumatic loading, such as during activities of daily living like bending forward and lifting, particularly in patients with low bone mineral density [203], [204]. Vertebral compression fractures most often occur at the T12 or L1 level [34]. In the laboratory, cadaveric vertebrae can be tested to investigate the similarities or differences between these non-traumatic (slower) and traumatic (faster) loading rates and the associated injury mechanics.

The viscoelasticity of bone has been shown with isolated cortical bone [68], [70] and trabecular bone samples [71], [72]. Viscoelasticity of bone can be observed in creep tests, relaxation tests, evaluation of structural stiffnesses at different rates, or in the hysteresis during cyclic loading indicating dissipation of energy [205]. Previous whole bone experiments have shown that human cadaveric vertebrae are viscoelastic and demonstrate loading rate-dependent effects; in particular, vertebrae are stiffer and have higher ultimate strength when loaded at higher displacement rates [50], [75], [76]. Typically the stiffness of the vertebra is measured using a LVDT and a load cell in a MTS. This global measurement of stiffness includes the compliance within the MTS (Appendix G) and the potting material. Thus the true viscoelastic response of bone may not be accurately captured using global measurements of displacement.

To measure the displacement over the surface of the bone itself, an optical technique called DIC can be used [206]. DIC is an algorithm that tracks motion of a surface. To do this, the surface of interest must either have a natural texture or be painted with a speckle pattern for contrast. In a reference image, the surface is divided into small square subsets, each with an array of pixel intensities. These subsets are then located in the deformed images using pattern matching algorithms. By using two or more views of the same object, the three-dimensional surface displacement can be measured. Given the displacement of the surface, strain can also be calculated. DIC has been used to characterize surface deformation on soft tissues, such as the IVD [105] or arteries [103], or on bones, such as the femur [112], [114] or the murine tibia [113]. DIC can measure strain or displacement at hundreds of data points on the surface of the bone instead of measuring the overall average strain using the global displacement measured with a
LVDT or the strain at one specific location using a strain gage. To date, no studies have used DIC and its ability to provide full-field measurements of the bone surface to investigate the effect of rate on the viscoelastic response of human vertebrae.

The goal of this paper was to use DIC to evaluate the mechanical response of isolated human cadaveric vertebral bodies tested first at a QS rate in a MTS and then at a dynamic rate with a drop weight. In accordance with viscoelastic theory, we hypothesize that 1) the stiffness in the direction of loading measured with DIC on the anterior cortex of the vertebral body will be greater in the dynamic testing than in the QS testing 2) full-field displacements observed in the QS testing will be correlated on a subset-by-subset basis with the displacements observed in the dynamic testing and 3) when comparing subset-by-subset, the dynamic loading displacement measurements on the anterior vertebral body will be lower than the displacements measured during QS loading.

4.2 Methods

4.2.1 Experimental procedure

Fresh-frozen human cadaveric lumbar vertebrae were obtained (n = 9) (Table 4.1). Specimens did not have severe bony deformity or evidence of previous spine surgery. Prior to testing, soft tissue and posterior elements were removed from the bones by cutting through the pedicles. The specimens were potted in PMMA using a custom rig to create parallel surfaces between which to compress the bone. The specimen was aligned so that the anterior edge of the superior endplate was horizontal. During potting, modeling clay was wrapped around the superior and inferior edges to only allow the PMMA to contact the endplates but ensure the PMMA did not wrap around onto the vertical surfaces of the vertebral body. To track the vertebral body surface with DIC, the bones were first painted white and then a black speckle pattern was applied using an airbrush (VL, Paasche, Chicago, IL, USA). Additionally to identify the loading direction, two points were painted vertically along the anterior midline using a vertical laser line for alignment.
Table 4.1: Summary of lumbar vertebral body specimens used in testing. The number of the specimen ID indicates the donor and the second part indicates the spine level of the specimen.

<table>
<thead>
<tr>
<th>Specimen ID</th>
<th>Donor Age</th>
<th>Donor Sex</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-L1</td>
<td>55</td>
<td>M</td>
</tr>
<tr>
<td>1-L2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2-L2</td>
<td>63</td>
<td>M</td>
</tr>
<tr>
<td>2-L3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-L1</td>
<td>55</td>
<td>M</td>
</tr>
<tr>
<td>3-L3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-L1</td>
<td>63</td>
<td>F</td>
</tr>
<tr>
<td>4-L2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-L3</td>
<td>U/k</td>
<td>U/k</td>
</tr>
</tbody>
</table>

U/k: Unknown

Two non-destructive tests, one QS and one dynamic, were performed sequentially on each specimen. For the QS loading, the potted specimen was placed in the MTS (8874, Instron, Norwood, MA, USA). The specimen was positioned beneath the actuator and was attached to a six-axis load cell (4366J, Denton, Plymouth, MI, USA). Two high-speed cameras (V12.1 Vision Research, Wayne, NJ, USA) with macro lenses (Nikon 105mm f/2.8 VR Micro-Nikkor, Tokyo, Japan and Sigma 105mm f/2.8 EX DG Macro, Kawasaki, Japan) were used to image the anterior surface of vertebra at a rate of 100 frames/second and a resolution of 1280 by 800 pixels (Figure 4.1). For the loading, an initial preload of 50 N was applied. The specimen was compressed at 0.05 mm/s until a load of 1200 N was reached and then the specimen was unloaded. The forces and moments from the load cell were collected at 50 kHz along with the displacement and force from the materials testing machine.
Figure 4.1: Example camera image of the anterior surface of the vertebral body. The large black dots indicated by the arrows were painted on the approximate midline of the anterior surface aligned with the loading direction. The dots on the PMMA were unused.

For the dynamic loading, a custom impact rig with a mass of 0.96 kg was attached to a drop rail (Figure 4.2). The rig had a flat aluminum impactor with a diameter of 50 mm oriented parallel to the lower platen. The potted specimen was positioned 10 cm below the impactor, again with the load cell below the specimen. The height of the impactor was chosen based on pilot specimens to result in a non-injurious impact. The two high-speed cameras were used to image the vertebra, this time at a rate of 10,000 frames/second and a resolution of 800 by 600 pixels. Load cell data and a trigger signal, which was based on a circuit closed at the time of contact between foil on the impactor and foil on the specimen, were collected at 50 kHz. The velocity immediately prior to impact was measured with a velocity gate consisting of two infrared sensors placed 25 mm distance apart and a flag attached to the impactor. The velocity failed to collect in one test. For the remaining eight tests, the average velocity was 1.37 ± 0.06 m/s. More detailed experimental methods are provided in Appendix A.
Figure 4.2: Test setup used to impact the vertebrae for the dynamic test. For the tests, the impactor rig was released from a height of 10 cm above the specimens.

4.2.2 Data processing

The images from the high-speed cameras were imported into the DIC software (StrainMaster 8.2, LaVision, Göttingen, Germany) and the two cameras were calibrated based on multiple images of a calibration grid (See Appendix B). For the analysis, a DIC subset size of 31 x 31 pixels with 15 pixel spacing between subsets was used. Image distortion was accounted for based on a pinhole camera correction. The average spatial resolutions were 22.25 ± 0.39 and 20.41 ± 0.41 pixels/mm for the QS and dynamic testing, respectively. The displacement on the anterior surface of the bone was calculated by finding the relative displacement between each DIC subset along the superior edge of the measurement region (QS: 50 to 76 subsets; dynamic: 39 to 52 subsets) and the corresponding DIC subset located along the inferior edge in the direction of the loading vector. These data were low-pass filtered with a fourth-order Butterworth filter at 20 Hz for the QS tests and 2000 Hz for the dynamic tests. Each displacement was normalized by the original length between the superior and inferior subsets to account for the original distance between these points being different.

Using the normalized surface displacements and the axial force applied to the specimen, the stiffness was calculated for each specimen between 25 and 75% of the maximum load for the QS loading (300 to 900 N, QS, full range), for the dynamic test between 25 and 75% of the maximum load (dynamic, full range), and for the dynamic range using a reduced force range to match the force range in the QS stiffness calculation (300 to 900 N, dynamic, reduced range).
The filtered displacements of the surface at 1000 N were found relative to the displacements at a load of 300 N for the QS and dynamic load cases. The forces of 300 and 1000 N were selected because they represented a linear portion of the loading curve and the forces were reached in both the QS and dynamic testing. The surface from the dynamic test was transformed (rotation and translation) to the location of the surface from the QS test based on the coordinates of the two painted points and two additional distinct speckle points that could be uniquely identified in both data sets, collectively referred to as alignment points (Figure 4.3). The anterior surface displacements were compared on a subset-by-subset basis by taking each subset from the dynamic test (728 to 1270 subsets) and identifying the closest subset in the QS test. The average distance between the corresponding QS and dynamic subsets was 0.29 mm.

![Figure 4.3: Example of the alignment of the QS and dynamic surfaces showing the individual subsets. The alignment points were used to register the two surfaces for comparison.](image)

### 4.2.3 Statistics

Since the variances of the stiffnesses were high, a non-parametric Friedman’s test was used to evaluate if the stiffnesses for the three rates were different. Post-hoc, the Wilcoxon
signed-rank test with Bonferroni correction was used for pairwise comparison. Linear correlations between the QS and dynamic displacements at corresponding locations were created. To evaluate the similarity of the displacement patterns, the $R^2$ for each specimen was reported. Hypothesis testing was used to evaluate for each specimen if the slope was different from one. Statistical analyses were performed in Statistica 7.1. Values of $p$ were considered significant if $p < 0.05$.

4.3 Results

Force-normalized displacement curves for two representative specimens are provided to show the two distinct QS displacement progressions observed (Figure 4.4). In Specimen 5-L3, the displacement and force steadily increased, and this behavior was observed in seven of nine specimens. In Specimen 1-L1, there was a force plateau while the displacement increased; this behavior was observed also observed in Specimen 2-L2. In Specimen 1-L1, sub-catastrophic fracture of the bone was observed at the time of the plateau in the high-speed video. In Specimen 2-L2, there was no visual evidence of partial bone failure but it may have occurred where it was not possible to visualize the failure. Due to the abnormal force traces indicating damage, analyses were performed with and without these two specimens included.
Figure 4.4: Example force vs. displacement plots used to calculate the stiffnesses. The displacements were measured using DIC on the anterior vertebral body surface relative to a preloaded bone state of 100 N and normalized by the original lengths between measurement points. The region for which the stiffness was calculated is indicated on the plots by a thick line. For the QS tests, the stiffness was calculated between 25 and 75% of the maximum load. For the dynamic tests, the stiffness was calculated in two ways, (1) based on 25 to 75% of the maximum force in the dynamic test (full range) and (2) using the same force values as in the QS test for that specimen (reduced range).

When considering all specimens, the stiffness magnitudes normalized to the original lengths (Figure 4.5 and Table 4.2) had significant differences (Friedman’s test, p < 0.001). Specifically, there was a significant increase from the QS stiffnesses to the full-range dynamic stiffnesses (Bonferroni corrected alpha = 0.017, Wilcoxon, p = 0.008) and reduced-range dynamic stiffnesses (Wilcoxon, p = 0.008). The reduced-range dynamic stiffnesses were also significantly lower than the full-range dynamic stiffnesses (Wilcoxon, p = 0.008). When performing the pairwise comparison excluding Specimen 1-L1 and 2-L2, the differences between the stiffnesses groups were slightly higher than the Bonferroni-corrected significance level for all comparisons (Wilcoxon, p = 0.018).
Figure 4.5: Comparison of the normalized stiffnesses calculated for the QS tests, the full-range dynamic tests, and the reduced-range dynamic tests. Each color and symbol represents a different specimen. Significance between groups is indicated by the asterisks.

The aligned surfaces (Figure 4.6) were compared on a subset-by-subset basis (Figure 4.7). The average of the $R^2$ values was 0.88 (Table 4.2). For each specimen, the slopes were significantly less than one based on 95% confidence intervals.
Figure 4.6: An example of the aligned DIC displacements for Specimen 4-L1 during QS and dynamic loading at a force of 1000 N relative to the displacements at a load of 300 N. The displacement magnitudes at corresponding locations were compared.

Figure 4.7: Subset-by-subset comparison of the DIC-measured displacements on the anterior surface of the bone at an axial force of 1000 N, relative to a preload of 300 N. The linear fit is plotted for the displacements and the dashed line is unity. The $R^2$ value indicates how well correlated the QS displacements are with the dynamic displacements. Points below the unity line indicate lower displacement for the dynamic loading.
Table 4.2: Summary of results for the normalized stiffnesses and the correlations comparing displacements subset-by-subset for QS compared to dynamic loading rates. The two shaded rows indicate the specimens where there was damage to the vertebral body during QS loading as identified by the force trace.

<table>
<thead>
<tr>
<th></th>
<th>Normalized stiffness (N/(mm/mm))</th>
<th>Correlation summary</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>QS</td>
<td>Dynamic, full range</td>
</tr>
<tr>
<td>1-L1</td>
<td>4.37E+05</td>
<td>8.03E+05</td>
</tr>
<tr>
<td>1-L2</td>
<td>4.37E+05</td>
<td>7.17E+05</td>
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<td>2.20E+06</td>
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<td>2-L3</td>
<td>3.76E+05</td>
<td>3.75E+06</td>
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<tr>
<td>3-L1</td>
<td>4.73E+05</td>
<td>1.74E+06</td>
</tr>
<tr>
<td>3-L3</td>
<td>5.10E+05</td>
<td>1.74E+06</td>
</tr>
<tr>
<td>4-L1</td>
<td>2.34E+05</td>
<td>5.76E+05</td>
</tr>
<tr>
<td>4-L2</td>
<td>3.10E+05</td>
<td>9.91E+05</td>
</tr>
<tr>
<td>5-L3</td>
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<td>1.25E+06</td>
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<tr>
<td>Mean</td>
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<td>1529757</td>
</tr>
<tr>
<td>SD</td>
<td>101390</td>
<td>997058</td>
</tr>
</tbody>
</table>

4.4 Discussion

DIC was used to demonstrate that the anterior cortical shells of lumbar vertebral bodies exhibit different mechanics when loaded at a dynamic rate (initial rate of 1370 mm/s) compared to a QS rate (constant rate of 0.05 mm/s). This was evaluated based on linear measurements on the anterior surface of the bone as well as a subset-by-subset comparison between the full-field DIC QS and dynamic surface displacements. As hypothesized, the stiffness on the anterior surface was higher for the dynamic testing, as compared to the QS testing. The QS displacement patterns were strongly correlated with the dynamic displacements with an average $R^2$ value of 0.88. When comparing the surface displacements on a subset-by-subset basis, the QS surface displacements were significantly higher than the dynamic displacements.

Two specimens exhibited unexpected behavior in the QS loading displacements (Figure 4.4), which may be evidence of sub-catastrophic failure. Exclusion of these specimens does not change the overall findings of the study although the differences in the stiffnesses are not significant when these specimens are excluded due to reduced statistical power. These specimens were included in the analysis because there was approximately linear force-displacement behavior for the range over which the stiffness was calculated and over which the displacement
was compared. No other specimens showed evidence of sub-catastrophic failure in video analysis.

Unlike studies that measure displacement with a LVDT, use of DIC on the bone to quantify displacement excludes the influence of compliance of the PMMA [63] and the MTS [207]. This allows for calculation of the stiffness independent of the system compliances. An increase in vertebral body stiffness was observed between the QS and dynamic testing when considering both the full-force and reduced-force ranges. This finding was in agreement with our hypothesis. Additionally, the full-force range stiffnesses were higher than the reduced-force range stiffnesses. This highlights that the force range for stiffness calculations should be considered when comparing between loading rates.

For the linear regressions between the QS and dynamic displacements, the average $R^2$ value was 0.88 indicating strong agreement in the pattern of displacement for the two rates. However, Specimen 2-L3 had a $R^2$ of 0.66. This specimen had a lower maximum DIC displacement than the other specimens, and the lack of correlation is likely due to the low DIC signal relative to the noise. In all the specimens, the slopes of the regressions were significantly less than 1 and all points were below the unity line. This indicates that on a subset-by-subset basis, the QS displacements were significantly greater than the dynamic displacements, and supports the hypothesis that at the same force, there is more displacement during the QS loading compared to the dynamic loading as expected for viscoelastic materials and structures.

Previous studies have investigated the influence of rate on cadaveric vertebral bodies. In one study, cadaveric thoracic vertebral bodies were loaded in compression at rates of 0.09 mm/s to 900 mm/s and there was a significant increase in the stiffness with increasing rate [76]. Another investigation performed compressive loading of lumbar vertebral bodies (n = 53) and found a trend (p-value = 0.06) towards greater stiffness at high impact rates when comparing specimens impacted at an average velocity of 600 ± 400 m/s to specimens impacted at 2500 ± 800 m/s [50]. The lack of significance in this study may be due to the relatively similar loading rates or the small sample size. The current study used a QS loading rate of 0.05 mm/s while the dynamic impact occurred at an average initial velocity of 1370 mm/s. These rates represents a 27,400 fold difference in the initial rate, on the order of the investigation with thoracic vertebral bodies [76].
For trabecular cores in compression, one study showed that the strain rate ($\dot{\varepsilon}$) increased the Young’s modulus and strength by a factor of $\dot{\varepsilon}^{0.06}$ [71]. Based on the initial velocity of 1370 mm/s in the dynamic test, the ratio of the dynamic stiffness to the QS stiffness is expected to be 1.85. Since the force is the same, the ratio of the QS to the dynamic displacement is also expected to 1.85. For each specimen, the ratio of the QS to the dynamic displacement was found by calculating the inverse of the slope of the correlation (Table 4.2). On average, the ratio of QS to dynamic displacement magnitudes ($\pm$SD) was 1.98 ($\pm$0.66). This is in good agreement with the effect of rate expected indicating that for the current vertebral body experiments, a multiplier of $\dot{\varepsilon}^{0.06}$ captures on average the expected decrease in the displacement magnitudes for the dynamic compared to the QS. However, the high SD also demonstrates that the specimen response was variable and that the response likely depends on bone material or structural properties, although it was not the aim to investigate such differences in this study.

The rate-dependency of vertebral bodies using the same bone boundary conditions were evaluated with a repeated measures design. Previous investigations have investigated the rate-dependency of lower-limb bones using a similar study design [80], [81]. One investigation [80] used strain gage measurements and found the bones were stiffer at the higher loading rate but the viscoelastic effect was more moderate than in the current study. This may be the result of the use of more similar loading rates for the high-strain and low-strain loading than for the current investigation. In another study performed in our lab [81], no effect of rate on stiffness was identified for femora (n = 16) loaded in a sideways fall configuration at a QS rate (0.22 kN/s ) and a dynamic rate (150 kN/s) based on whole bone compliance-corrected displacements. However in the dynamic tests, specimens that were stiffer had significantly higher bone mineral density (BMD) than the specimens that were less stiff. The finding of rate not affecting stiffness may result from displacements associated with subtle sub-failure mechanical damage occurring on the specimens with low BMD, confounding the displacement measurements. DIC-measured minimum principal strain was compared on the femoral neck for these same specimens [208]. Comparing the slow to the fast loading in that study, for a given specimen there was a difference in the strains but there was no consistent trend for all the specimens. This conclusion is different than the comparison of DIC-measured displacements in the current study where it was found that for all specimens, the surface displacements were smaller for the dynamic testing than for the QS
testing. This difference may be because the current investigation compared displacements as opposed to strains or due to the method of aligning the DIC surfaces.

For the current study, the repeated measures design was both an advantage and a limitation of this investigation. The repeated measures design allowed for direct comparison of the surfaces during the QS and dynamic loading. However, two tests were performed on each specimen and undetected but subtle damage from the QS test may have influenced the dynamic test. For the QS loading, the load was expected to be below the specimen’s damage threshold. However, in one specimen (1-L1) sub-catastrophic damage on the anterior cortex near the superior edge was observed during the QS loading and for another specimen (2-L2) there was suspected damage. It is possible that damage occurred in other specimens, although no evidence of damage was visible in the high-speed videos or inferred based on the force-displacement curves.

DIC is a potentially useful measurement technique that has been validated for measurements on vertebral bone [206] but also has limitations. DIC measurements can be noisy [112] and may be sensitive to factors including the subset size and speckle quality [94], [97], [196]. Another limitation is that for the alignment of the QS and dynamic surfaces, there was an average error of 0.29 mm. However, given the continuity of the displacement field, this error is expected to have a minimal effect on the results. Additionally, for displacement measurements on the bone surface, a straight line was assumed between the superior and inferior points of the DIC, although the bone surfaces had a slight concavity.

In conclusion, the displacement pattern for the QS loading was strongly correlated with the displacement pattern for the dynamic loading rate in the majority of the specimens. This study found that for a linear displacement measurement on the anterior surface, the vertebral body is stiffer for the dynamic loading rate than the QS, in agreement with viscoelastic theory. Furthermore, this study demonstrates a novel use of DIC to compare the full-field anterior surface displacements on vertebral bodies at two loading rates. Use of DIC provides measurements of the bone surface independent of the compliance of the MTS, the PMMA, or the PMMA-endplate interface and allows for better characterization of the bone surface response.
Chapter 5: Using DIC to validate vertebral body FE models

5.1 Introduction

Vertebral fractures are associated with serious consequences, such as pain, disability, or neurological deficits, and deleterious changes in quality of life [42], [176], [209], [210]. Vertebral fractures can occur from a single traumatic loading or, in the context of osteoporosis, from damage accumulated during repeated activities of daily living. Specimen-specific FE models are an important tool to help researchers understand the relationship between vertebral loading and fracture. These models account for vertebral geometry, bone density and density distribution and better predict vertebral strength and stiffness as compared with dual-energy x-ray absorptiometry (DEXA) [58], [136]. Numerous studies have compared specimen-specific FE models of vertebral bodies loaded in compression to experimental measurements. In general, the models have predicted the experimental strength well ($R^2 = 0.79$ to $0.96$) [58], [59], [146], [150], [153] while whole bone stiffnesses are predicted less well ($R^2 = 0.49$ to 0.86) [58], [59], [146], [147], [150]. Studies that predict strength do not use consistent yield or failure metrics. Furthermore, the stiffnesses have been derived based on displacements measured for the entire tested specimen including the potting. The platen-potting and potting-bone interfaces may influence the stiffness, and inaccurate bone properties may have been derived to compensate for compliance at these interfaces. More precise and specific validation metrics may improve the assessment of the quality of specimen-specific FE vertebral body models.

One validation metric that has not yet been used is surface displacement of the vertebral bodies. This can be measured using DIC, a non-contact optical method for measuring the displacement on the surface of a material or structure during loading. Images of the surface deformation are analyzed based on pattern matching criterion to determine the motion of pixel subsets. The use of DIC for validation of FE models has been performed for composite bones [121], [119], [109], [118], but to our knowledge only one study has compared DIC results to specimen-specific FE models of cadaveric bones [165]. In that study, three FE models were created of proximal femora and compared to the experimental DIC strains from cadaveric testing. Strong correlation ($R^2 = 0.92$ to 0.95) was found on the femora for the FE to DIC principal strains, and the failure load and location were predicted well by the FE models. While these results are promising, the sample size was small and, to date, no investigations have been performed using DIC measurements from vertebrae loaded ex vivo for FE model validation of
predicted displacements and derived stiffness values. This is an important step to determine an appropriate modulus-density relationship, necessary for validation of yield or failure criteria in bone generally and specifically in the context of vertebral fracture prediction.

The purpose of this study was to compare the surface displacements predicted by specimen-specific vertebral body FE models with the displacements measured by DIC during QS compressive loading of *ex vivo* specimens. We hypothesize that agreement will be seen between the predicted and observed bone surface displacements and also for the derived stiffness values.

### 5.2 Methods

#### 5.2.1 Experimental procedure

Human fresh-frozen cadaveric thoracolumbar vertebrae (*n* = 6) were obtained from a body donation program (Table 5.1). The experimental results in this study were from the same testing reported in Chapter 3. The specimens were scanned in air at an isotropic resolution of 246 microns in a high-resolution peripheral quantitative computed tomography (HR-pQCT) scanner calibrated with a hydroxyapatite phantom (XtremeCT and Q1 Phantom, Scanco Inc., Switzerland). During preparation, the specimen was kept hydrated using saline. The soft tissue including the periosteum was removed using a scalpel. Care was taken to not cut into the cortical shell while removing the soft tissue. The posterior elements were removed from the vertebrae by cutting through the pedicles. Each vertebral body was potted using PMMA to create parallel surfaces. For the DIC analysis, the anterior cortex was blotted dry with paper towel and painted with a thin layer of white paint. Then an airbrush was used to create a speckle pattern with black paint. Following this, four registration points were painted on the bone; these dots were used to register the DIC surface to the surface of the vertebra from the CT scans (Figure 5.1).

**Table 5.1: Summary of the vertebral body specimens tested.** To determine the volumetric average bone mineral density (vBMD) for each specimen, the volumetric average of the ash density was found for FE elements that had no external faces in the model. U/K indicates the information was unknown.

<table>
<thead>
<tr>
<th>Sex</th>
<th>vBMD (g/cm³)</th>
<th>Donor Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-L2</td>
<td>U/K</td>
<td>0.26</td>
</tr>
<tr>
<td>1-L3</td>
<td>U/K</td>
<td>0.27</td>
</tr>
<tr>
<td>2-L1</td>
<td>M</td>
<td>0.14</td>
</tr>
<tr>
<td>3-T11</td>
<td>M</td>
<td>0.23</td>
</tr>
<tr>
<td>4-L1</td>
<td>F</td>
<td>0.17</td>
</tr>
<tr>
<td>5-L1</td>
<td>M</td>
<td>0.22</td>
</tr>
</tbody>
</table>
Figure 5.1: Image of a prepared specimen potted in PMMA and painted with a speckle pattern and points for registering the DIC surface to the segmented geometry. The outline indicates the area for which the DIC displacements were measured.

The inferior PMMA was rigidly attached via screws to a metal plate (Figure 5.2). The specimen was placed in a MTS (Electropuls E10000, Instron, Norwood, MA, USA) and compressed to a preload of 100 N. A 3D point digitizer (Optotrak Certus, Northern Digital Inc., Waterloo, ON, Canada) was used to measure the coordinates of the four registration points, the loading vector, and points on the vertebral body to use for registration with the geometry segmented from CT (113 to 131 points per specimen). Two video cameras (V12, Vision Research, Wayne, NJ, USA) were positioned an average of 22.2° (±0.5°) apart to image the anterior surface of the vertebra and the specimen was illuminated with halogen lights. Camera images were recorded at 100 frames per second with an image resolution of 1280 by 800 pixels. A monotonic loading ramp at a rate of 0.1 mm/s was applied up to 3 mm, a displacement that resulted in failure for all specimens. The load was recorded at 12.5 kHz.
Figure 5.2: Specimen in the experimental setup and a specimen-specific FE model. The specimens were loaded in compression to failure. The FE modeling was performed in two steps. First, the bone was loaded with a constant displacement on the superior PMMA to determine the force distribution on the endplate. Second, that force distribution was used to apply 1000 N on the superior endplate, excluding the PMMA from the model.

5.2.2 Computational procedure

Specimen-specific models of each vertebral body were developed. To create the geometry, open-source software [211] was used to interactively segment the vertebral bodies from the CT scans. Each slice of the CT scan was visually checked to ensure consistent vertebral body segmentation. A convergence investigation was performed for nominal element edge lengths of 1, 2, 3, and 4 mm; an edge length of 2 to 2.5 mm was selected based on the convergence of the average displacement of the whole bone and of the superior endplate (See Appendix H.5). The volumes were meshed with 10-node quadratic tetrahedrons using commercial software (V15, ANSYS Inc., Canonsburg, PA). The PMMA was modeled by extruding the endplates a nominal height of 10 mm. The PMMA was assigned linear elastic material properties (E = 2.9 GPa, ν = 0.3) [63] (Figure 5.2).

Bone material properties were assigned based on five different Young’s modulus-density relationships previously experimentally determined for vertebrae (Table 5.2 and Figure 5.3). These relationships were previously reviewed [53]. The ash density (ρ_{ash}) in each CT voxel was related to the calibrated bone mineral content (mgHA) using ρ_{ash} = (mgHA/1000 + 0.09)/1.14 g/cm³ and to apparent density (ρ_{app}) with ρ_{app} = ρ_{ash}/0.6 [137].
Table 5.2: Summary of the modulus-density relationships used for specimen-specific models of vertebral bodies. All relationships were based on experiments using vertebral trabecular bone. Each modulus-density relationship was given a label for identification (MDR-#).

<table>
<thead>
<tr>
<th>Label</th>
<th>MDR-1</th>
<th>MDR-2</th>
<th>MDR-3</th>
<th>MDR-4</th>
<th>MDR-5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relationship</td>
<td>[ E = 1540\rho_{app} - 58 ]</td>
<td>[ E = 1890\rho_{ash}^{1.32} ]</td>
<td>[ E = 2100\rho_{app} - 80 ]</td>
<td>[ E = 4730\rho_{app}^{1.30} ]</td>
<td>[ E = 2383\rho_{app}^{1.88} ]</td>
</tr>
</tbody>
</table>

\( E \) in MPa, \( \rho_{app} \) and \( \rho_{ash} \) in g/cm\(^3\).

* Rate dependent component was not included since testing was performed at a quasi-static rate.

Figure 5.3: Graphical comparison of the modulus-density relationships used for assignment of material properties to the FE elements. The dashed line and markers represent the different relationship extrapolated for a wide range of apparent bone densities while the thick line represents the range of the bone densities tested experimentally to determine the specific relationship.

Linear elastic material properties from the empirical modulus-density relationships were mapped to the FE elements using Method B from a recent study [212] (See Appendix H.5). This method accounted for the partial volume effect by removing the voxels at the air-bone boundary and expanding the unaffected layer of voxels outward. The Young’s moduli were then calculated.
for each voxel and the moduli of the voxels were used to calculate the moduli of the elements. The density was used to determine an isotropic Young’s modulus for the element, and the Poisson’s ratio for the bone was set to 0.3. To determine the volumetric bone mineral density (vBMD), the ash density for each specimen was determined by finding the volumetric average of the internal elements (elements with no external faces) (Table 5.1).

The models were solved using an implicit solver (V17, ANSYS Inc., Canonsburg, PA). The simulations were performed in two steps, in order to perform a relevant and consistent comparison of experimental and simulation data. First, the model with the PMMA was solved, applying a uniform vertical displacement of 1 mm to the superior surface of the PMMA while constraining the transverse motion of the superior PMMA. The inferior PMMA was fixed to be stationary and the superior endplate nodes and the adjacent PMMA nodes were tied, providing the force distribution at the superior PMMA-vertebra interface. The nodes were tied since this best simulated the experimental conditions in which the PMMA was well attached to the endplate. For comparison, the simulations were also performed using sliding contact between the PMMA and the endplate; sliding contact resulted in an average $R^2$ value of 0.87 for the correlation compared to 0.88 with the tied contact but slightly improved stiffness agreement (See Appendix H). Second, the model was simulated without the PMMA, using the determined force distribution to apply a total force of 1000 N to the superior endplate, again constraining transverse motion of the superior endplate, while the inferior endplate was constrained to be stationary. This ensures that the FE to DIC comparison was carried out at the same load and that the FE model had a reasonable estimate of the force distribution at the endplates. This method isolates the response of the bone, given that compliance between the bone and the PMMA is unknown.

### 5.2.3 Data processing

Images were processed using commercially available DIC software (StrainMaster 8.2, LaVision, Göttingen, Germany). For processing, a subset size of 31 by 31 pixels was used with a spacing of 15 pixels between the subset centers. The displacements were low-pass filtered at 10 Hz with a fourth-order Butterworth filter. The average spatial resolution of the reconstructions was 32.9 pixels/mm. To account for the vertical displacement of the inferior PMMA-bone interface during loading, all DIC values were reported relative to a plane perpendicular to the loading vector at the inferior edge of the DIC measurement area.
To compare DIC and FE measurements, results were transformed to the experimental location of the bone (points digitized during the experiment while the bone was preloaded) (Figure 5.4). For the DIC surface, the x-, y- and z- coordinates of the registration points were exported from the DIC software. These coordinates were aligned to the digitized control point coordinates, minimizing the sum of squared errors between the corresponding control points. To align the segmentation from the CT to the experimental bone location, an iterative closest point registration algorithm without scaling was used to transform the CT surface coordinates to the digitized points resulting in the two surfaces (FE and DIC) in the same experimental coordinate system. For the center of each DIC subset, the ten closest exterior FE nodes were found and averaged, giving the FE displacement at a force of 1000 N that corresponded to the DIC displacement. The goodness of the alignment was assessed by the average distance between the points on the DIC surface and the corresponding point on the FE surface.

Figure 5.4: Graphic showing the alignment of the FE and DIC measurements by transforming both surfaces to the surface of the bone that was digitized during the experiment. By aligning the surfaces, it was possible to compare the FE and DIC measurements on a point-by-point basis.

Stiffnesses were calculated for each specimen based on the DIC displacements. For the DIC-measured displacements, the time histories of the displacements of the superior DIC subsets
were found relative to the inferior subset displacements, aligned by the loading vector. Since the original lengths varied across specimens and between specimens, the displacements were normalized to the original lengths. The axial force applied was plotted against the normalized DIC displacements. The stiffnesses for each subset were calculated by fitting a line to the force-displacement curve between 25 and 75% of the maximum force reached during the testing. The specimen’s DIC stiffness was defined as the average of all the subset-derived stiffnesses. For comparison with the DIC-derived stiffness, the displacements predicted by FE were found at the same locations as the experimental measurements; the stiffness was calculated by dividing the applied force by the average of the displacements, normalized to the original lengths.

5.2.4 Statistics

Linear regressions were used to compare the DIC-measured displacements with the FE displacement predictions location-by-location for the bone at a compressive load of 1000 N. The coefficient of determination ($R^2$) was used to quantify how well a linear model described the FE to DIC relationship, and the concordance correlation coefficient (CCC) [213] was used to measure the similarity of the measurements to the unity line ($x = y$). Values of the CCC range from -1 to 1 with 1 representing perfect agreement. The agreement between the FE and experimental stiffnesses were assessed with $R^2$, and relationships were considered significant for $p < 0.05$. A linear regression between experimental and FE-predicted stiffnesses was performed, and the slope and the intercept of the regression line was determined as well as the coefficient of determination ($R^2$). Hypothesis testing was used to evaluate whether the slope was different from unity and intercept different from zero, with $p < 0.05$ (Matlab R2015b, The Mathworks, Inc., Natwick, MA, USA).

5.3 Results

The average distance between the DIC surface points and the FE nodes after registration was 0.88 mm, when the bone was preloaded to 100 N. The maximum distance between the DIC surface point and FE node for all points was 2.24 mm.

The DIC displacements for the specimens at a load of 1000 N were plotted, demonstrating differences in the displacement distributions between specimens (Figure 5.5).
Figure 5.5: For each specimen, the DIC-measured displacement magnitudes (mm) on the anterior surface of the vertebral body specimens are provided for an applied compressive load of 1000N; displacements were measured relative to a plane perpendicular to the loading vector at the inferior edge of the specimen.

The correlation between the FE-predicted displacements and the DIC-measured displacements was quantified using the coefficient of determination and the CCC for the five modulus-density relationships (Table 5.3). The relationship with the highest $R^2$ was MDR-5 which had an average value of 0.88 and CCC of 0.80. The results for each specimen using MDR-5 are presented (Figure 5.6).

Table 5.3: Summary of the CCC and $R^2$ values for local displacement comparisons between DIC and FE results. The comparison was carried out for each specimen and each modulus-density relationship.

<table>
<thead>
<tr>
<th></th>
<th>MDR-1</th>
<th>MDR-2</th>
<th>MDR-3</th>
<th>MDR-4</th>
<th>MDR-5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$R^2$</td>
<td>CCC</td>
<td>$R^2$</td>
<td>CCC</td>
<td>$R^2$</td>
</tr>
<tr>
<td>1-L2</td>
<td>0.93</td>
<td>0.92</td>
<td>0.93</td>
<td>0.30</td>
<td>0.93</td>
</tr>
<tr>
<td>1-L3</td>
<td>0.92</td>
<td>0.41</td>
<td>0.93</td>
<td>0.61</td>
<td>0.89</td>
</tr>
<tr>
<td>2-L1</td>
<td>0.87</td>
<td>0.85</td>
<td>0.91</td>
<td>0.18</td>
<td>0.87</td>
</tr>
<tr>
<td>3-T11</td>
<td>0.83</td>
<td>0.81</td>
<td>0.80</td>
<td>0.30</td>
<td>0.83</td>
</tr>
<tr>
<td>4-L1</td>
<td>0.89</td>
<td>0.92</td>
<td>0.93</td>
<td>0.30</td>
<td>0.89</td>
</tr>
<tr>
<td>5-L1</td>
<td>0.75</td>
<td>0.85</td>
<td>0.75</td>
<td>0.21</td>
<td>0.75</td>
</tr>
<tr>
<td>Average</td>
<td>0.86</td>
<td>0.79</td>
<td>0.88</td>
<td>0.32</td>
<td>0.86</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>0.07</td>
<td>0.19</td>
<td>0.08</td>
<td>0.15</td>
<td>0.06</td>
</tr>
</tbody>
</table>
Figure 5.6: Point-by-point comparison of the anterior surface displacements at 1000 N predicted by the specimen-specific vertebral body FE models using MDR-5 relationship compared to the displacements measured using DIC during experimental loading. The load distribution on the superior endplate was determined based on tied contact between the PMMA and endplate nodes.

The FE stiffness was compared to the experimental stiffnesses for MDR-5 on the surface of the bone (Figure 5.7). The $R^2$ value was 0.90 and the relationship was significant ($p = 0.004$). The slope was not significantly different from one (Confidence Interval (CI): 0.48 to 1.36), and the intercept was not different from zero (CI: -2.3e5 to 1.9e5).
Figure 5.7: Comparison of the FE stiffnesses to the experimental stiffnesses of the bone for MDR-5. The load distribution on the superior endplate was determined assuming tied contact between the PMMA and endplate nodes. The stiffness was calculated based on the displacement measured with DIC and predicted by FE on the anterior cortex. The experimental stiffnesses were found by fitting a line to the force-displacement curve between 25 to 75% of the maximum force. The FE stiffnesses were calculated by dividing the applied force of 1000 N by the average of the normalized displacements corresponding to the locations of the DIC measurements. The slope and intercept were not significantly different from 1 and 0, respectively.

5.4 Discussion

The goal of this paper was to compare the surface displacements and stiffnesses predicted by specimen-specific FE models of lumbar vertebral bodies with DIC measurements of the vertebral surface displacement and derived stiffnesses during compression. Five modulus-density relationships characterizing vertebral trabecular bone were evaluated. The modulus-density relationship that resulted in the strongest agreement between the model and experimental displacements (MDR-5, [140]) had an average $R^2$ of 0.88 and an average CCC of 0.80. Considering all specimens, the specimen-specific FE model stiffnesses derived from vertebral surface displacements using MDR-5 were significantly correlated with the stiffnesses derived from experimental DIC-measurements ($R^2 = 0.90$, $p = 0.004$).
For the point-by-point displacement comparisons at a force of 1000 N, the displacements between FE and experiment were generally highly correlated (Figure 5.6). The $R^2$ values ranged from 0.75 to 0.93. However, one specimen, 4-L1, had a concave shape to the displacement correlation. The displacements for this specimen were generally similar for low values but for higher displacements, the DIC displacements were higher than the FE displacements. It is not clear why the superior cortex experienced more displacement in the experiment than predicted in the model but it may be a result of subtle damage to the bone prior to reaching 1000 N (or pre-existing damage). To evaluate the similarity of the displacement magnitudes, the CCC was used. The modulus-density relationship with the highest average CCC (MDR-5) had greater than 0.8 CCC for five out of six specimens while one specimen (1-L3) had a CCC value of less than 0.5. Specimen 1-L3 was stiffer in the FE model than in the experiment, which again may be the result of some damage to the bone that was not accounted for in the FE model. Although there were some outliers for the shape and magnitudes of the displacement correlation, the correlation between predicted stiffness and derived stiffness on the surface of the bone was significant ($R^2 = 0.90$, $p = 0.004$). The strong agreement between the models and experiments indicates that the selected modulus-density relationship and boundary conditions resulted in specimen-specific FE models that were highly predictive of the anterior cortex experimental mechanics.

To compare the FE and DIC results, a method was developed to align the relatively feature-less anterior surface of the vertebral body obtained from DIC to the segmented FE geometry using points digitized during the experiment. Good alignment of the DIC and FE surfaces was obtained. To compare the FE and DIC measurements, previous studies used registration of the DIC surface to the FE geometry for the femur [165] or landmark alignment for the pelvis [118]. However, the anterior surface of the vertebral body has less distinct geometry than other bones, and therefore, the additional step of aligning both the DIC surface and FE geometry to the experimental point cloud was required.

Comparing the results for local displacement correlation directly to previous work is difficult, due to methodological differences. However, one study compared FE predictions and DVC measurements for vertebral displacements of three-vertebra segments loaded in compression [214]. DVC is a method similar to DIC but DVC uses volumetric subsets instead of 2D subsets and utilizes the natural trabecular bone structure for pattern matching instead of an applied speckle pattern. The study found some qualitative agreement in the displacement patterns.
but quantitatively, the displacement errors were high (12 to 279%). While both DIC and DVC provide additional measurements that can be used to validate FE models, the increased number of experimental measurements elucidates differences between the model and experiment that could not previously be quantified.

Previous studies have used DIC for validation of FE models of composite bones [121], [119], [109], [118] and cadaveric femora [165]. These studies have all used strain to quantify the agreement between the experiments and the models. These previous investigations were performed on the femur [109], [119], [121], [165] or pelvis [118] while the current investigation loaded vertebral bodies in compression. Loading in the previous studies resulted in bending stresses and strains on the bone while the current study only applied direct compression to the vertebral body. The loading applied in these previous studies may create more distinct displacement and strain patterns on the femurs and pelvises than were created on the vertebral bodies.

Previously, the FE-experimental agreement for stiffness was measured using a LVDT and was somewhat lower than the values in the current study ($R^2 = 0.49$ to 0.86) [58], [59], [146], [147], [150]. Other studies have not quantified the stiffness based on surface displacement measurements on the bone, as was performed using DIC in this study. This improvement over previous work can most likely be attributed to the approach for isolating the bone response in the FE models using first a simulation to determine the load distribution on the superior endplate by applying displacement on the PMMA cap and then applying that load distribution in a second simulation where only the bone response was modelled. Using this method, the response of the bone was isolated, eliminating the necessity to know the compliances between the platen and the PMMA and the PMMA and the bone. Previously, the issue of compliance between the bone-PMMA and PMMA-platen has been addressed by removing the endplates to create parallel surfaces, eliminating the need for using PMMA experimentally [58], [59], [147], however at the expense of biofidelity. These studies, along with the current study, show that compliance may negatively affect the comparison between experiments and FE models.

This study demonstrates that the full-field displacement on the surface of bone can be predicted for an applied load of 1000 N. Future work should use DIC for validation of computational models that incorporate failure and more complex materials properties. Previously, it was shown that compared with use of aBMD, compressive strength assessed by FE
models is more predictive of vertebral fracture risk [215]–[217]. Continued use of DIC for validation is important clinically to ensure the highest quality models are used to assess fracture risk in patients.

All studies which incorporate both experimental and computational models of biological tissue have limitations. This study used a small sample size; testing more specimens may result in improved confidence in the FE models for predicting displacements and stiffnesses. Nevertheless, strong and significant correlations were demonstrated. The current study compressed the bone through PMMA. Although this loading is not as physiologic as loading the vertebral body through the IVD, this approach was taken because the PMMA is likely more consistent than the IVDs of elderly donors. Another limitation is use of isotropic linear elastic bone properties. Given the range of loading, it is not expected that the bone would be outside the linear range but this material model may not fully capture the complexity of bone compression. The PMMA contact with the circumferential element faces at the superior and inferior edges of the bone were not modeled; the PMMA contact was only modeled for the vertebral endplates. However, the effect of the added constraint on these elements is expected to be small since the endplate nodes are tied to the PMMA nodes in the first simulation step. Finally, the cortex was not explicitly modeled. However, the elements containing cortical bone had higher stiffnesses than the interior elements meaning the stiffening effect of the cortex was captured. Previous work has shown that the predictions of global stiffness and strength are improved with explicit cortical shell modeling [150] but the stiffness agreement in the current study was strong, even without modelling an explicit cortical shell, implying that its influence is captured in the chosen material mapping strategy.

In summary, specimen-specific FE models were created and a modulus-density relationship was identified that accurately captured the local cortical response quantified experimentally using DIC. For this study, the surface displacements were compared on a point-by-point basis using a novel method for alignment of the DIC surface and FE segmented geometry and good agreement was found for the displacements. The stiffnesses predicted by FE strongly agreed with those derived from experimental measurements ($R^2 = 0.90$). Use of DIC allows for a more comprehensive characterization of surface deformation than has previously been performed and this information enables improved validation of specimen-specific FE models.
Chapter 6: Integrated discussion

6.1 Overview

In this thesis, the overall goal was to perform several detailed investigations of the mechanics of vertebrae in compression using DIC. DIC, unlike strain gages or markers on the bone, can provide the full-field displacement patterns, which can then be used to calculate the full-field surface strain. In Chapter 2, the similarity between strain gages and DIC for measurement of strain in porcine vertebral bodies was evaluated. Strain gages are commonly used in laboratory testing of bones but the similarity of DIC and strain gage measurements on bone had not been quantified. In the study it was determined that DIC is an appropriate and valuable tool for measuring bone displacement and strain. In Chapter 3, DIC was used for fracture identification on the anterior cortex of vertebrae loaded in compression since DIC provides quantitative measures of the strains which are related to cortex damage. In Chapter 4, DIC was used to evaluate the effect of loading rate in vertebral body specimens compressed quasi-statically and dynamically. The full-field DIC displacements were used to characterize the degree of similarity in the loading patterns and to measure the stiffness. Finally, in Chapter 5, DIC was applied for validation of FE models of vertebral bodies, evaluating the similarity of the displacements measured with DIC and predicted by FE.

6.2 Summary of findings

The noise level for the DIC strains was higher than the strain gages. Although the strains were filtered, the DIC noise 29 με, higher than the strain rosette which had a noise level of 1 με for the minimum and maximum principal strain during application of a nominal 100 N preload.

Noise in the DIC strain tensor components resulted in an increase in the maximum principal strains and a decrease in the minimum principal strains. The minimum and maximum principal strains are calculated from the strain tensor components (ε_xx, ε_xy, ε_yy). For typical materials, the minimum principal strain is negative and the maximum principal strain is positive. Therefore, when noise is present, there is an increase in the maximum principal strain and a decrease in minimum principal strain.

Criteria including the high-speed video observation, strain angle, SD between peaks, and tensile gages were capable of detecting strain gage rosette debonding. Another method for detecting gages that are improperly bonded is evaluating the linearity between the applied force and the
strain gage response. A value of $R^2 > 0.98$ has been suggested for acceptable bonding of the strain gage [86], [166]. Both the criteria proposed in Chapter 2 and the gage linearity method identified gages with poor bonding.

The strain gage and DIC-measured strains were similar. The agreement was better for the minimum principal strains. The average difference between the strain gage and the DIC for the minimum principal strain was 10% while the average difference for the maximum principal strain was 21%.

The locations of high strain on the surface of the bone corresponded qualitatively to subcatastrophic and catastrophic fracture locations seen in the videos. On the anterior cortex, the minimum principal strains were quantified with DIC and compared to visual observation of the video; strong agreement between the locations of high strain and fracture was found. Based on observation, areas of high strain led to fracture, and when fracture occurred more strain resulted.

The strain on the anterior cortex stabilized and the local cortical bone yield strain is reached before the global yield force is reached. Typically, the failure force is considered the maximum force, which can be sustained by the vertebra. However, this study indicates that there is likely irreversible damage that occurs prior to the maximum force.

Vertebrae are viscoelastic, but the degree of viscoelasticity varies by specimen. The stiffness of the bone was measured on the surface of the bone using DIC. Previous studies used the global stiffness to characterize the mechanical response of the bone. As expected, on the surface of the bone there was a significant increase in the bone stiffness from the QS loading to the dynamic loading. The ratios of QS stiffness to dynamic stiffness ranged from 0.10 to 0.61 for the specimens. On a subset-by-subset basis and comparing at the same force magnitude, the slopes of the correlation curves ranged from 0.31 to 0.82. The ranges of the stiffness ratios and slopes demonstrate an inconsistent effect of rate on the mechanical response of the bone.

The displacements at QS rates are correlated with the displacements at dynamic rates. The average $R^2$ value for the QS and dynamic displacements was 0.88 indicating the patterns on the bone were similar between the two rates.
The displacements predicted by specimen-specific FE models were similar to displacements measured experimentally. The $R^2$ values ranged from 0.75 to 0.93 for the point-by-point comparison between FE and DIC displacement magnitudes.

The FE models predicted the experimental stiffnesses well. When comparing the predicted and experimental vertebral surface stiffnesses, there was strong correlation ($R^2 = 0.90$). This demonstrates that when the response of the anterior cortex is isolated experimentally, specimen-specific FE models can capture the experimental behavior.

6.3 Implications

Recently, the use of DIC in laboratory has become more common. Use of DIC in the investigations presented in this thesis has provided insights about experimental methodology with implications for testing of vertebral bodies and other bones.

Compared to strain gages, use of DIC provides more comprehensive measurements of the surface of the bone. Chapter 2 compared strain gages with DIC to establish the validity of DIC measurements and quantify the noise in the two methods. This basic study can provide justification for future use of DIC, a technique that provides full-field displacement and strain maps.

In this work, cortical bone damage and fractures occurred prior to complete vertebral collapse and are not necessarily associated with a change in the force trace. The failure force is commonly considered to be the maximum force. Since damage has likely already occurred on the bone at this point, the injury tolerance of lumbar vertebrae to axial compression may be lower than is commonly reported in literature. In terms of computational models, FE models of vertebrae are often validated based on agreement with the experimental maximum force. However, it should be considered that this force is not representative of the force at which damage begins to occur to the cortical bone.

With regards to loading rate, this work showed that as expected when comparing displacements at the same force, there was less displacement for the dynamic loading compared to the QS loading. However, the slope of the correlation between QS and dynamic displacements was more variable than expected. The assumption that an increase in rate has a similar effect on all specimens is incorrect. Repeated measures of vertebral bodies at different rates have not been previously reported, and further work is needed to understand why the variability was observed, perhaps due to differences in trabecular architecture or bone health. This is important for the
basic understanding of the mechanics of bone and for FE models of vertebral bodies that incorporate rate dependency.

The boundary conditions applied to the bone influence the bone’s response, and DIC can be used to ensure consistency for the applied loading. Potting the bones in PMMA, dental stone, or Wood’s metal is commonly used and the difference in materials has been reported [63], [64]; however, meaningful detail about the potting procedure is less commonly reported. In the studies presented in this thesis, a custom potting rig was used for preparation of specimens (Appendix A.4) in Chapters 3, Chapter 4, and Chapter 5. Use of the custom rig provided a method for creating parallel planes for compression. In earlier pilot testing, it was observed that in specimens where the potting was not highly parallel, there were abnormal DIC displacement patterns demonstrating non-uniform loading of the bone (Figure 6.1). Without DIC, this unevenness may be difficult to detect. Potting of bones is important for biomechanical testing, and care should be taken to ensure high experimental standards for preparation of the bones to obtain meaningful results. DIC can be used to identify specimens with abnormal boundary conditions and improve the overall consistency in experimental testing.

Figure 6.1: Example of a map of the displacement magnitude for vertebral body loaded in compression. This specimen demonstrates an abnormal displacement pattern, identified using DIC.

Another experimental finding was the presence of interface compliance, which can occur at the bone-PMMA interface or the PMMA-platen interface. Compliance resulted in higher than expected DIC-measured displacement at the inferior edge of the DIC measurement region. Some motion at the inferior edge is expected due to compliance of the PMMA material itself and the compression of the bone inferior to the location of measurement. However, the measurements at the inferior edge were higher than the predictions made by the FE model for a vertebral body with PMMA using the input measured from the machine compliance-corrected LVDT.
displacement. This is illustrated for a representative specimen from Chapter 5 (5-L1). At a load of 1000 N, the LVDT displacement corrected for machine compliance (0.15 mm) was applied to the top PMMA surface, and the displacement magnitudes are provided (Figure 6.2 and Figure 6.3).

**Figure 6.2:** Experimental measurements of the displacement magnitude for Specimen 5-L1.

**Figure 6.3:** FE predictions of Specimen 5-L1 response for a uniform applied displacement on the superior PMMA. The displacement applied was measured experimentally by the LVDT and corrected for MTS compliance. Note that the color scale is not the same as the experimental displacements.

At the inferior edge, there was a nominal displacement of 0.06 mm for the DIC measurements, while in the FE model the corresponding inferior edge had a nominal displacement of 0.03 mm. In the FE model, the reaction force for this displacement was 2631 N, higher than the 1000 N measured experimentally for the same applied displacement. This could indicate that either the material properties of the bone or the PMMA are incorrect or there is compliance in the experiment that is not captured in the model. Since the vertebral body FE-
predicted stiffness was in agreement with the experimental stiffness for the vertebra (Chapter 5) and PMMA is a well-characterized material, the higher force predicted by the model is likely caused by compliance in the experiment between the bone and PMMA or the PMMA and the platen.

The amount of compliance is small (nominally 0.03 mm inferiorly in this case). However, given that the stiffnesses are measured based on relatively low displacements, this error is relevant to consider. When compressing vertebrae, even with the best experimental practices, compliance of interface materials or apparatuses is likely. Full-field DIC measurements of the bone provide a more accurate quantification of stiffness. The methods presented here demonstrate that using DIC, the bone response can be isolated independent of the system compliance. Furthermore, future experiments could use DIC and FE together to evaluate assumptions about boundary conditions and quantify compliances.

In Chapter 5, specimen-specific finite element models were developed that had good correlation with the surface displacements and were capable of predicting the stiffness well. The goal of these models is to develop a clinically applicable technique for evaluating risk of vertebral fractures in patients. Previously, it was shown that compared with use of aBMD, compressive strength assessed by FE models is more predictive of vertebral fracture risk [215]–[217]. Use of FE models can provide a more accurate tool to evaluate patient risk and will result in better targeting treatments and preventative measures to patients with the highest risk.

Our specimen-specific FE models demonstrate that DIC measurements can be used for validation. Previously, validation of FE models was largely focused on the global stiffness and ultimate strength. While these are important, recent work with DVC has shown that local deformation response may not be predicted well by FE models [214]. The increased spatial density of measurements provided by DIC has been and should continue to be applied for validation of vertebral body response. These improved models can be used to better predict the vertebral fracture risk among patients. More complete validation will improve the robustness and quality of the FE models, which hopefully will increase the clinical acceptance of these methods.

Although the FE specimens presented in Chapter 5 were not modeled to the point of failure or with strain-rate dependent materials, the data presented in Chapter 3 and 4 have relevance for FE modeling of fracture and dynamic loading. For instance, the average magnitude of difference between the QS and dynamic rate displacements could be used to validate generic
FE models with rate dependent material properties. Additionally, creating specimen-specific models of the failure specimens could validate failure prediction criteria. Future FE models will benefit from the full-field measurements that can be collected with DIC.

6.4 Limitations

In addition to the limitations presented for each study, there are over-arching considerations to keep in mind when considering these investigations.

All the testing was performed on vertebral bodies with the posterior elements removed from the specimens. The transmission of load to the bone occurred only through the endplate, while *in vivo* load is also transmitted through the facet joints. However, the load through the facet joints is less than through the IVD. For FSUs from donors under the age of 50, it was found that the facet joints generally carried less than 20% of an axial force applied, the majority of the load going through the IVD [55]. The effect of cutting the pedicles was examined for the middle vertebra of a three-vertebra segment in four specimens [87]. Using strain gauges to measure the response, the only significant change in strain seen was at the base of the pedicle but not at other locations on the bone indicating that removal of the posterior elements is an acceptable simplification.

The vertebral bodies in these investigations were tested in compression. For isolated vertebral bodies, more complex loading configurations are difficult to setup experimentally. Tensile and torsional testing of isolated vertebral bodies is not commonly performed since the instrumentation required to hold the vertebra would in many cases affect the response of the specimen. Furthermore, compression fractures commonly occur in real world situations due to osteoporosis [219] and trauma [39], [41], [42], which provides rationale for the use of compressive testing. The DIC methods presented here are also applicable to testing longer spine segments in more complex loading scenarios such as lateral bending [54] or flexion/extension [167], [220].

All testing was performed for specimens embedded in PMMA. The response of specimens loaded with PMMA is different than the response of specimens loaded through an IVD [126], [195], [221]. However, loading through an IVD resulted in less consistent strains than loading through a potting material [221]. This may be a result of the age and degree of degeneration of the IVD which affect the mechanical properties of the IVD [6], [222]–[224]. With age and degeneration, there are changes in the extracellular matrix and hydration of the
nucleus as well as weakening of the annulus which affect the loading experienced by the vertebral body [225] and the failure patterns [177]. Since all the cadaveric specimens were from elderly donors, use of the IVD with varying degeneration would have reduced the mechanical consistency of testing. Although loading through PMMA is less physiologic, it provides a consistent medium for applying load to the vertebrae.

6.5 Future work

Following from this thesis, there are a number of possible next steps that would provide more answers about vertebral bodies loaded compressively. One test would be to evaluate the effect of removal of the posterior elements on the strain on the bone. This would experimentally confirm the finding that the posterior elements have minimal effect on the response of the anterior cortical shell. Another test of interest would be to test the same vertebral body multiple times to a load thought to be non-injurious, repotting between each testing. The similarity of the strain pattern would measure the influence of the potting on the strain, and therefore load, experienced by the cortical shell. A study could be performed to measure DIC displacement and strain on the potting to evaluate compliance. This would elucidate the effect of potting on the stiffness of the specimen when using a global stiffness measurement. Rate dependency was identified in the vertebrae but the effect of rate varied between specimens. It would be interesting to look into the bone architecture to see if structural features of the bone predict if the bone will behave more or less viscoelastically. For instance, does the orientation of the trabeculae or the number of micro-cracks prior to testing influence the degree of viscoelasticity? Finally, previous work has shown that the highest strains on the bone occurred at the base of the pedicle as well as near the endplates on the posterior side [85], [87]. DIC could be used to verify and expand on these results by measuring the full-field strains, perhaps with sequential imaging of different areas to quantify all cortical surfaces visible in an axial loading scenario [226]. Combined with morphological assessment of the bone structure, this type of investigation could relate changes in trabecular structure to the cortical response.

As discussed in the limitations, these investigations have focused on testing vertebral bodies embedded in PMMA but the displacement and strains under other loading configurations can be quantified with DIC. More work should be done to characterize the displacement and strain response of longer spine segments. The quantification of the displacement and strain
response can be used to validate the bone response in FE models of the spine under various loading conditions such as lateral bending or flexion-extension.

Previous testing of isolated cortical bone samples was performed in compression and tension to characterize the mechanical response, and strain was measured using a global extensometer measurement [12], [69], [70]. However, it has been shown using DIC that localized strains occur on cortical bone during loading, and regions of high strain are often the result of microstructural features of the bone, such as osteophyte lucunae [196]. Although there has been work using DIC to characterize bone properties [106], [197], [227], a large scale experiment to characterize cortical sample properties using DIC would be beneficial. Use of DIC could identify boundary condition issues such as fixture slipping, previously reported as a problem in these types of tests [12]. DIC could also be used to identify, and possibly exclude, specimens with abnormal local strains due to microstructural features. Therefore, use of DIC may provide more consistent experimental conditions for characterizing cortical bone response.

DIC can improve the understanding of the effect of implants or bone fixation devices on the cortical bone response. Previously, strain gages were most commonly used to evaluate the biomechanical effect of implants on cortical bone [169], [228], [229]. More recently, DIC has been used to understand the cortical bone response to implantation in composite bones [118], [120], [124], [230] as well as limited work using cadaveric bones [122]. Future work should continue to use DIC to characterize the cortical response to implementation, particularly in cadaveric bones, which are more biofidelic than composite bones.

One advantage of DIC is that the rate at which analysis can be performed is only dependent on the rate at which images can be collected, assuming that there is no camera motion [231]. In the dynamic experiment (Chapter 4), images were collected at 10,000 frames/second using the high-speed cameras. These rates allow for collection of dynamic events relevant to real world injuries. Although some of the data presented here were collected at QS rates, DIC is relevant for identification of fractures and quantification of motion at higher rates as well. Future work using DIC can capitalize on its high-frequency measurement for providing biomechanical insights.

The FE models presented here were implicit simulations and the bone was considered a linear elastic material property. For dynamic loading scenarios, explicit simulations could be performed, and the high-rate capabilities of DIC could be used for validation. Explicit FE models
that incorporate rate dependent material properties and failure may more accurately reflect the real-world situations under which vertebral fracture occurs. Use of DIC provides more validation data to ensure that these more complex models are similar to experimental measurements.

6.6 Contributions

In this thesis, four studies were performed which provide new insights and methodology for use of DIC on bone. The thesis integrates experimental and computational approaches to study compressive loading of vertebral bodies in the laboratory with implications for clinical vertebral fractures. To summarize, the main contributions of this work are as follows:

A comprehensive quantitative comparison of strain gage and DIC measurements on bone. This is important for future studies using measurements with DIC on bone instead of strain gages, the typical approach to strain quantification on bones. Chapter 2 shows the similarity of the measurements with strain gages and DIC, and also identifies debonding for some strain gages, an experimental issue that can result in incorrect measurements.

Novel investigation regarding use of DIC to identify fracture on the cortical surface of an organ-level bone. Previously, DIC fracture analysis has been performed on rocks [232] or stainless steel [233] as well as small cortical samples [196], [197] but fracture identification for whole bones has not performed. Use of DIC to identify fracture provides new insights about the timing of damage to bone, which occurred much earlier than the global yield force in the vertebral specimens tested. Previous methods to detect fracture such as AE sensors, strain gages, force traces, and video analysis do not provide the same quantitative information about the timing and location of damage to the cortical shell.

Quantification of the effect of rate on stiffness based on displacement measured directly on the surface of the vertebral body. Previously, the influence of rate on vertebral bodies has been measured using the stiffness which is measured between the superior and inferior potting surfaces [50], [75], [76]. In this investigation, the displacement was measured directly on the bone, thus excluding the influence of compliance within the MTS, between the platen and potting, and between the potting and the bone. By measuring displacement on the anterior cortex, the viscoelasticity of the vertebral bodies was demonstrated.
Repeated measures investigation of the displacement on the vertebral body. This was the first study to perform repeated measures testing on vertebral bodies. The similarity in the displacements on the surface of the bone when loading at a slow and fast rate was shown.

Development of a robust method for alignment of the vertebral surface to the DIC surface for comparison with FE model results. Previous work to align DIC measurements to FE predictions has been largely qualitative [109], [119], [164]. Alignment of DIC and FE surfaces performed quantitatively have either been performed for the pelvis based on landmarks [118] or for the femur based on iterative closest point registration [165], [208]. However, these methods were not possible due to the lack of features on the anterior cortex of vertebral bodies. Instead, registration points were used to align the segmented CT geometry to the points digitized experimentally.

Demonstration of the ability of FE models to predict stiffness on vertebral surface. Compliance is present in most experimental setups for bone loading. This work isolated the response of the vertebral body surface. There was good agreement between the surface stiffnesses of vertebral bodies loaded experimentally in compression and predicted by FE models.

6.7 Conclusions

In summary, the work presented in this thesis demonstrates the utility of DIC for improved understanding of bone response to loading. In Chapter 2, the comparison between DIC and strain gages was presented showing that although strain gages had lower signal noise, the measurements were similar and DIC provided full-field measurements of the strain. In Chapter 3, the quantitative measurements of strain for six vertebrae were presented and used to demonstrate that high local strains on the bone occur prior to the yield force and correspond with the locations of damage from the video. In Chapter 4, vertebrae were tested at a slow and fast rate showing that the subset-by-subset comparison for the displacements had similar patterns, but the magnitudes of the dynamic displacements were lower than the QS displacements. Finally in Chapter 5, specimen-specific FE models were created. The point-by-point DIC-measured and FE-predicted displacements were similar, and the FE models predicted the surface stiffnesses well. DIC is a valuable but underutilized biomechanical tool. Future use of DIC can improve experimental methods and computational models of vertebrae.
Bibliography


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Appendices

Appendix A  Testing procedures

A.1 DIC and strain gage porcine experiments (Chapter 2)

Specimen Preparation:
- Thaw specimen prior to dissection
- Dissect soft tissues and muscle from the bone
- Use a scalpel to scrape the bone clean
- Use bone saw to remove posterior elements
- Remove specimen from freezer 12-24 hours prior to testing place in refrigerator

Potting (updated in later experimental procedures):
- Mix a small amount of PMMA and allow to set slightly (1 scoop powder:1 glass vile liquid ratio)
- Form the PMMA into a ball
- Place on the lower portion of the potting rig
- Position the vertebra such that the anterior plane of the superior endplate is parallel to the ground
- Let the PMMA set completely
- Repeat the process for the upper potting, using a level to ensure the upper plate of the potting fixture is parallel to the lower plate

Strain gauges:
- Clean strain gage area again with the scalpel and then with sandpaper
- Apply a layer of ethanol with a q-tip, let dry
- Apply a layer of acetone and 2-propanol with a q-tip, let dry
- Bond the strain gage using cyanoacrylate and the Teflon tape to apply the gage

DIC preparation:
- Paint the bone with layer of white paint
- Apply even black speckle pattern across the surfaces using the airbrush
- Paint six dots on the bone to create equilateral triangles

Instron setup:
- This testing was performed at the Centre for Hip Health and Mobility (CHHM) bioengineering laboratory
- Setup loading profile in Wavematrix
- Connect BNC cables for the trigger, load, and position to the DAQ
- Set up Instron position and warm up as per standard operating procedure and training
- Check the gain settings
- Change the zero point of the analog output sensitivity to the displacement of the LVDT when the actuator just contacts the specimen
- Setup the Phantom V12s with the macro zoom lenses
- Connect the cameras with a BNC to f-sync
- Setup one camera as master (internal) and one camera as slave (external)
  - Ensure that EDR is 0 and that the exposure is identical for both cameras
- Perform current session reference in Phantom
- Ensure EDR is set to zero and that the exposure for the two cameras is equal
Position the cameras so that they are both imaging the anterior bone surface
Focus the cameras and ensure sufficient lighting
Check trigger in Phantom
Record the camera settings
Set the Phantom collection rate to 100 frames/second
Position the specimen in the Instron and apply a ~50 N load (compression) to the specimen
Ensure the strain gage is connected

Running the test:
- Set capture in Phantom; ensure the message in the bottom left reads, “Waiting for trigger”
- Run Labview program and press record button
- Turn on lights
- Start Wavematrix loading profile
- Save the videos
- Collect calibration images of the grid for image distortion and calibration of DaVis

Cleanup:
- Autoclave plates
- Wipe down Instron
- Wipe down anatomy lab

General comments:
- Be sure to take still photos of each test
- Keep specimen hydrated throughout prep and testing

A.2 Cadaveric failure experiments (Chapter 3 and Chapter 5)

Specimen Preparation:
- Dissect soft tissues and muscle from the bone
- Use a scalpel to scrape the bone clean
- Use the bone saw to remove posterior elements

Scanning:
- Double bag specimen (clean on the outside)
- Place in clean cooler for transport
- Scan in the xTreme CT scanner at 246 micron resolution

Potting:
- Turn on the fume hood and put down plastic in the fume hood
- Get necessary supplies
  - PMMA powder/liquid
  - Paper cups
  - Tongue depressors for mixing
  - Gloves
  - Allen key set
  - Stand and clamp to hold bone
- Refer to Appendix A.4 for the potting method and photos

DIC preparation:
- Paint the bone with layer of white paint
- Apply even black speckle pattern across the surfaces using the airbrush
- Paint four dots on the bone, two aligned in the loading direction and two arbitrary points

Instron setup:
- Setup loading profile in Wavematrix
- Connect BNC cables for the trigger, load, and position to the DAQ
- Turn on Instron
- Balance the load cell
- Adjust the actuator height
- Check the PID settings
- Change the output values to be about the current position of the Instron
- Setup the Phantom V12s with the macro zoom lenses
- Connect the cameras with a BNC to sync
- Setup one camera as master (internal) and one camera as slave (external)
- Perform current session reference in Phantom
- Ensure EDR is set to zero and that the exposure for the two cameras is equal
- Position the cameras so that they both are imaging the anterior bone surface
- Focus the cameras and ensure sufficient lighting
- Check trigger in Phantom
- Record the camera settings
- Set the Phantom collection rate to 100 frames/second
- Position the specimen in the Instron and apply a 100 N load (compression) to the specimen

Running the test:
- Set trigger in Phantom
- Start Labview DAQ
- Turn on lights
- Start Wavematrix loading profile
- Save the videos
- Collect calibration images of the grid for image distortion and calibration of DaVis

Cleanup:
- Autoclave plates
- Wipe down Instron
- Wipe down anatomy lab

A.3 Rate-dependency experiments (Chapter 4)

Specimen Prep:
- Dissect soft tissues and muscle from the bone
- Use a scalpel to scrape the bone clean
- Use bone saw to remove posterior elements
- Remove specimen from freezer 12-24 hours prior to testing place in refrigerator

Potting:
- Turn on the fume hood and put down plastic in the fume hood
- Get necessary supplies
- PMMA powder/liquid
- Paper cups
- Tongue depressors for mixing
- Gloves
- Allen key set
- Stand and clamp to hold bone
- Refer to Appendix A.4 for the potting method and photos

**Painting:**
- Paint the bone with layer of white paint
- Apply even black speckle pattern across the surfaces using the airbrush
- Paint four dots on the bone that will be visible in the videos to align the surfaces from the Instron and the drop tower

**Instron Setup:**
- Setup loading profile in Wavematrix
- Setup the six-axis load cell under the actuator
- Connect BNC cables for the trigger, load, and position to the DAQ
- Turn on Instron
- Balance the load cell
- Adjust the actuator height
- Check the PID settings
- Change the output values to be about the current position of the Instron
- Setup the Phantom V12s with the macro zoom lenses
- Connect the cameras with a BNC to sync
- Setup one camera as master (internal) and one camera as slave (external)
- Perform current session reference in Phantom
- Ensure EDR is set to zero and that the exposure for the two cameras is equal
- Position the cameras so that they both are imaging the anterior bone surface
- Focus the cameras and ensure sufficient lighting
- Check trigger in Phantom
- Record the camera settings
- Set the Phantom collection rate to 100 frames/second
- Attach specimen to plate
- Position the specimen in the Instron and apply a ~50 N load (compression) to the specimen

**Running the Test:**
- Perform tests
  - Arm the camera trigger in Phantom
  - Turn on lights
  - Start Labview program and hit record
  - Run loading profile
  - Save videos and record frames collected
- Collect grid and checkerboard images

**Drop Rail Setup:**
- Flip the head of the tripods upside-down
- Setup the Phantom V12s with the macro zoom lenses
• Perform current session reference in Phantom and camera steps mentioned above
• Position the cameras so that they both are imaging the anterior bone surface
• Focus the cameras and ensure sufficient lighting
• Set the Phantom collection rate to 10,000 frames/second
• Attach specimen to plate
• Attach plate to load cell
• Put foil trigger on specimen
• Adjust velocity gate height
• Check trigger in Phantom
• Raise impactor to 20 cm above specimen
• Check cameras and lights

Running the Test:
• Perform tests
  o Reset velocity gate
  o Arm the camera trigger in Phantom
  o Turn on lights
  o Run Labview program and hit record
  o Impact specimen
  o Record velocity of impact
  o Save videos and record frames collected
• Collect grid and checkerboard images

Cleanup:
• Autoclave plates
• Wipe down Instron and drop rail
• Wipe down anatomy lab
A.4 Vertebral body potting procedure

1. Tape a plastic ring for potting to the base of the potting fixture. Put screws into the base so that they will hold the PMMA. Grease the inside of the ring, the base of the metal plate, and around the screws.

2. Level the base of the potting fixture using the adjustable feet. Use a stand and clamp to position the bone relative to the base. Level the anterior superior endplate of the vertebra. Lower the vertebra until the inferior endplate is below the top of the plastic ring.

3. Mix PMMA with a ratio of 1 scoop powder to 2 glass measurements full of the liquid. Pour the PMMA into the plastic ring and let harden.
4. Check that the base is still level.

5. Tape a plastic ring to the upper piece of the test fixture. Grease the ring.

6. Put one collar on each rail of the test fixture. Put the upper piece onto the rails. Locate and level the upper piece by adjusting the set collars. Put on the other set of collars. Put on the adjustable feet.
7. Flip the fixture over and level the fixture using the adjustable feet. Pour the PMMA into the plastic ring.

8. Lift the PMMA so that it contacts the bone. Tighten the lower set collars. Let harden.
Appendix B  Steps for DIC

This appendix documents the DIC procedures used to process the images throughout the thesis in the StrainMaster software, version 8.2 (LaVision, Inc., Göttingen, Germany). The steps are as follows:

1. **Prepare the specimen**
   Clean the specimen using a scalpel to remove the periosteum. Paint the specimen with white paint and a black speckle pattern (or vice versa) using an airbrush.

2. **Collect images using the high-speed cameras**
   To perform 3D DIC, two cameras imaging the same area are required. If using the Phantom software (Vision Research, Wayne, NJ, USA), ensure that the cameras are synced using f-sync, have the same exposure, and that the extreme dynamic range (EDR) value is 0 for both cameras. Use the highest possible image resolution and maximize the size of the object relative to the image.

3. **Collect images of a grid pattern in multiple positions**
   This step can be done before or after the experiment as long as the cameras are not moved or the settings changed between the experiment and collecting calibration images. The grid pattern should have a known spacing between the grid points and mark dimensions. For the experiments in this thesis, a mark size of 2 mm was used with a spacing of 7.2 mm. For the calibration within the StrainMaster software, it is necessary to identify a specific mark on the grid pattern in each image so an identifying box around one mark should be added (Figure B.1) by the arrow. While capturing calibration images, the grid pattern should be moved keeping the grid in focus for both camera views.

![Figure B.1: Example of an image from the calibration. The arrow indicates the mark that has a box around it which was used to consistently identify this mark in each calibration image.](image)

4. **Convert videos to .tiff files**
To do this in the Phantom software, open the Phantom software go to “File” and then “Convert & Process .cine”. Browse to the .cine of interest. Select the .cine and then click “Open”. On the next screen, enter the file name. For naming the files for each camera view, one possible naming structure is specimen#_+4_00 or specimen#_+4_01 where the +4 automatically increments the images. Select for the save as type “TIFF 8, 24 images”. Browse to the folder to save the .tiff files and click “Save”.

5. **Select .tiff files to use for calibration**

Select about 20 .tiff images and then check all the images to make sure they are acceptable to use for calibration. If any images need to be deleted, the images need to be renumbered to make sure there are not gaps in the sequential numbering (although it is not important to keep the order the same as they were collected). There should be a minimum of 10-12 images for the calibration.
6. **Load the calibration images into DaVis**

Open the DaVis software. Click “New” to start a new project. Specify the type of project to be “Strain” (not the default which is “Imaging”). Click “Import” to import the calibration images. Browse to the “Cal” folder where the good .tiff images of the grid are located. Make sure the import mode is “Multi-frame images” and select the first image and click “Add to list”. Under the import list, select the file just added, and then change the wildcard such that * is in the location of the frame number where it says, “Select wildcard” and are the file range is from 1 to the number of calibration frames. Change the wildcard strings to be “00” and “01” or the strings used to specify the two different cameras. Click “Import data”.

![Image of DaVis software interface](image)
7. **Perform the calibration**

Click “Calibrate”. Select “2 cameras (mapped e.g. stereo)” and then click next. Change the number of views to correspond to the number of calibration frames used and then click next. Define the grid pattern used by selecting the 2D grid in the bottom left corner and entering the spacing between marks and the size of the mark; click “Next”. For the image acquisition step, click on the “Images” button with the file folder icon. Browse up one level in the dialog box by clicking on the name of the project. Select the .set file called “Cal”, or name of the folder with the calibration images, and click open. Specify Frame 0 to be Camera 1 and Frame 1 to be Camera 2. Click “Ok”. Enter the pixel size for the cameras (For the V12.1 Cameras by Vision Research, this was 20). Click “Next”.

For the mark definition (Step 5), click “All cameras/views”. Use the optimal button (located in the upper right corner, icon with a star wand) to adjust the image. Click on three consistent grid points for each specimen. The grid points should be selected in order; first, the origin point (same for each frame) followed by a grid point immediately to the right of the origin and then a grid point immediately above the origin. Once the points have been selected for all frames, click next. Click “Start Calibration”. The program will find the grid points (green boxes) and the origin (blue box) and provide the average deviation to the marks. The average deviation should be low (Nominal 0.2 to 0.5 pixels). Then click “Next”. The program will calculate a corrected image and provide the grid points. Click “Finish” and the option to overwrite and rescale images.
8. **Import the images collected during the experiment**

Click “Import”. Browse to the .tiff files for the experiment. Again, select multi-frame images for the import mode and import data by identifying the wildcard strings as 00 and 01. Once the images are imported, click “Exit”. Select the experiment .tiff files. Click Processing. Under the operation list, select, “Add camera attributes” and for Frames 0 and 1 select Cameras 1 and 2 as shown. Process the images. Exit from the Processing window.

9. **Run the DIC processing**

Under the file name, select the files that say “AddCameraAttribute”. These images are assigned Frame 0 and Frame 1. Click Surf + Vectors icon at the top of the screen. If that icon is not visible, close the project window and ensure a “strain” project was created by right clicking on the project and clicking “Convert project…”. In the Surf + Vectors window, click optimal to adjust the image viewing. Under define mask in 1st image, select the mask type and select the region for which the DIC analysis will be performed. Insert three to five seed points on the mask. Enter the subset size and step size. For the correlation mode, use “Relative to first” and enter in the maximum expected deformation (nominally 5 to 10 pixels). To check if the masked area and the seed points are acceptable, click test processing. Change the view of Surface Height to be 3D to ensure that the reconstruction of the object seems reasonable. Once satisfied with the test processing, click “Start Processing” to process all the frames (This step might take a while depending on the size of the file). Once that is complete, exit the Surf + Vectors window.
10. Explore or exporting the processed data

Once all the images have been processed, there are a few ways to explore or export the data.

- **Plotting**
  
  To plot the average of a variable, select the processed images from the main menu. Then click plot. Use the “Rect” tool to select the area of interest on the image over which the values will be averaged. For the Y-axis Group select the value of interest (for instance displacement magnitude or min normal strain on surface). For the x-axis select “File Number” for both the group and function. For store mode, either enter a name or use default. Click start processing to plot values.

- **View the full-field data**
  
  To look generally at the spatial distribution of a variable, select the processed images from the tree (default name should be something like “Strain_LSM_TS(##x## stepsize ##)”). Use the blue slider bar to advance the frames or enter the image number directly. Use the optimal tool to adjust the scale on the image to fit the displayed values. Right click to display settings to change the variable displayed, or adjust the scale.
• Export the surface

To export the coordinates of the DIC subsets, right click on “Surface” under the processed files and click export. Change the export type to .dat (or .txt if preferred). Select the frames to export the surface, specify the output location, and click export.

• Export the data

To export the data such as the minimum principal strain, select the processed data from the tree menu. Click on “Processing” on the home screen. For group, select the type of variable to extract (e.g. Extract Scalar Field: strain) and under operation, select the variable (e.g. Exx). Then start processing the data. After it is done processing, exit this screen. Now under the data file, there should be a new file (e.g. Exx). Right click on the file and export it as a .dat file or .txt file. These files can then be read into Matlab, Excel or another program for further processing.
Appendix C  DIC accuracy

The accuracy of the camera system used for all studies in this thesis was measured. To do this, an aluminum plate painted white with black speckle was attached to a translation table. Two high-speed cameras were used to measure the motion of the plate with the same lenses used in the specimen testing and the image size was 1280 x 800 pixels (Figure C.2). The cameras were positioned such that the translation of the plate was approximately along the axis of the camera views. The scaling for this test was 20.5 pixels/mm. DIC was calculated for regions of 31x31 pixels with a spacing of 15 pixels between regions, similar to the DIC parameters used throughout the thesis. An example of a pair of calibration images from the two cameras is provided (Figure C.1).

Figure C.1: A pair of calibration images from the two cameras. Approximately 15 sets of images were used to calibrate the cameras for the DIC processing.

For comparison, the displacement was also measured with a laser displacement sensor (Dynavision Model LTS 15/2.9, LMI Technologies, British Columbia, Canada). The sensor’s accuracy is 2 μm and the resolution is 0.2 μm. The sensor was aligned using a mirror to be normal to the motion of the plate.
Two trials were performed in which the linear table was translated approximately 2 mm. During this time, the displacement sensor collected displacement at 10,000 Hz and video frames were recorded at 100 frames/second. The collections were aligned with a trigger signal.

The displacement was applied with the linear table and the measurements of displacement from DIC and the laser displacement sensor were compared (Figure C.3).
Figure C.3: The DIC-measured displacement compared with the laser displacement sensor measurements for two trials. The rate of displacement was controlled manually.

The average absolute difference between the two measurements was 11.4 μm. The precision of the measurements, found by taking the SD of the differences between the laser and DIC measurements, was 13.1 μm. This work quantified the agreement of the DIC displacement and high-accuracy laser transducer for the camera setup used in the presented studies as well as the DIC parameters.
Appendix D  Comparison of fiducial marker tracking to DIC and strain gage results

D.1  Introduction

Another alternative to measuring strain on the bone besides strain gages or DIC is using fiducial marker tracking (FMT). In FMT, strain is measured optically by tracking the displacement of fiducial markers applied to a tissue’s surface and then calculating the Green-Lagrange strain tensor from these displacements. FMT has been applied to soft tissues such as the facet joint capsule [234], [235] and muscle [236], but is less commonly used for bone, likely due to the small marker displacements relative to the accuracy with which the markers can be tracked. To our knowledge, a direct comparison of DIC and FMT on bone has not been published.

In addition to the comparisons made in Chapter 2 between DIC and strain gages, the noise and the peak strain values for FMT to DIC and strain gages for the same vertebral compression tests were compared.

D.2  Methods

The experimental methods are presented in Chapter 2. On all the bones previously presented, six black dots (fiducial markers) of approximately 0.5 mm diameter were also painted on the anterior surface to create two equilateral triangles (~8 mm per side) located symmetrically across the sagittal centerline with one triangle centered over the strain rosette (Figure D.1).

![Figure D.1: An example of one camera view of the anterior surface of a prepared porcine vertebra. Two sets of three dots were painted symmetrically about the centerline of the bone for calculating the finite strain with the fiducial marker tracking (FMT) method.](image)

For FMT, the x- and y-coordinates of the markers defining the triangular regions were measured (center of gravity tracking algorithm, TEMA Motion, Image Systems Motion Analysis, Linköping, Sweden); the average fiducial area was 370 pixels, and the time history of the
centroid of each point was tracked using semi-automatic tracking. The 3D motion of each fiducial was reconstructed using 11 direct linear transformation (DLT) parameters that were established using a calibration block with 10 to 24 points, depending on the number of points visible in both camera views. The 11-parameter DLT algorithm incorporated the camera optical parameters and linear lens distortion factors [237]. The tracked fiducials were transformed to express their motions relative to the plane formed by the three fiducials in the reference configuration, i.e., during the 100 N preload. Plane strain was assumed, and the 2D displacement gradient was calculated for the three fiducials from which the Green-Lagrange strain tensor was found using custom code.

The general steps for finding the Green-Lagrange strain tensor are presented. The coordinate points for an undeformed (X and Y) and deformed (X' and Y') triangle are shown (Figure D.2). The spatial coordinate directions are defined as l₁ and l₂ are aligned with the sides of the triangle in the undeformed state. The displacements of the nodes in the spatial coordinate directions are u and v.

![Figure D.2: Coordinate definition for a triangle in an undeformed state (X, Y, shown in blue) and a deformed state (X', Y', shown in purple)](image)

First, the coordinates of the undeformed triangle are found in the spatial coordinates:

\[
\begin{bmatrix}
\frac{\partial X}{\partial l_1} & \frac{\partial Y}{\partial l_1} \\
\frac{\partial X}{\partial l_2} & \frac{\partial Y}{\partial l_2}
\end{bmatrix}
\begin{bmatrix}
X_1 \\
Y_1
\end{bmatrix}
= \begin{bmatrix}
X_2 - X_1 & Y_2 - Y_1 \\
X_3 - X_1 & Y_3 - Y_1
\end{bmatrix}
\]

Equation D.1

Next, the deformations of the triangle coordinates are calculated in the spatial coordinates:
\[
\begin{bmatrix}
\frac{\partial u}{\partial l_1} & \frac{\partial v}{\partial l_1} \\
\frac{\partial u}{\partial l_2} & \frac{\partial v}{\partial l_2}
\end{bmatrix}
\begin{bmatrix}
\frac{\partial u}{\partial X} & \frac{\partial v}{\partial X} \\
\frac{\partial u}{\partial Y} & \frac{\partial v}{\partial Y}
\end{bmatrix}^{-1}
\begin{bmatrix}
\frac{\partial u}{\partial l_1} & \frac{\partial v}{\partial l_1} \\
\frac{\partial u}{\partial l_2} & \frac{\partial v}{\partial l_2}
\end{bmatrix} = \mathbf{F} = \begin{bmatrix}
\frac{\partial u}{\partial X} & \frac{\partial u}{\partial Y} \\
\frac{\partial v}{\partial X} & \frac{\partial v}{\partial Y}
\end{bmatrix}
\]

Equation D.2

\[
= \begin{bmatrix}
(X'_2 - X_2) - (X'_1 - X_1) & (Y'_3 - Y_3) - (Y'_1 - Y_1) \\
(X'_3 - X_3) - (X'_1 - X_1) & (Y'_3 - Y_3) - (Y'_1 - Y_1)
\end{bmatrix}
\]

Then, the matrix \( \mathbf{F} \) is found which expresses the deformations in terms of the triangle coordinates, \( X \) and \( Y \):

\[
\begin{bmatrix}
\frac{\partial X}{\partial l_1} & \frac{\partial Y}{\partial l_1} \\
\frac{\partial X}{\partial l_2} & \frac{\partial Y}{\partial l_2}
\end{bmatrix}^{-1}
\begin{bmatrix}
\frac{\partial u}{\partial l_1} & \frac{\partial v}{\partial l_1} \\
\frac{\partial u}{\partial l_2} & \frac{\partial v}{\partial l_2}
\end{bmatrix} = \mathbf{F} = \begin{bmatrix}
\frac{\partial u}{\partial X} & \frac{\partial v}{\partial X} \\
\frac{\partial u}{\partial Y} & \frac{\partial v}{\partial Y}
\end{bmatrix}
\]

Equation D.3

This can then be used to calculate the Green-Lagrange strain tensor as follows:

\[
\varepsilon_{xx} = \frac{\partial u}{\partial X} + \frac{1}{2} \left( \left( \frac{\partial u}{\partial X} \right)^2 + \left( \frac{\partial v}{\partial X} \right)^2 \right)
\]

Equation D.4

\[
\varepsilon_{yy} = \frac{\partial v}{\partial Y} + \frac{1}{2} \left( \left( \frac{\partial u}{\partial Y} \right)^2 + \left( \frac{\partial v}{\partial Y} \right)^2 \right)
\]

Equation D.5

\[
\varepsilon_{xy} = \frac{1}{2} \left( \frac{\partial u}{\partial Y} + \frac{\partial v}{\partial X} \right) + \frac{1}{2} \left( \frac{\partial u}{\partial X} \frac{\partial u}{\partial Y} + \frac{\partial v}{\partial X} \frac{\partial v}{\partial Y} \right)
\]

Equation D.6

From the strain tensor, the principal strains can be calculated for the element.

For FMT, the time-history of the strain tensor components were zeroed over 2 seconds during the 100 N hold; for one specimen, the zeroing was only performed over 0.125 seconds (3 frames) as more video was not recorded. Then, each of these signals was low-pass filtered at 2 Hz. For the FMT strain noise and the FMT to DIC comparison, the strain was calculated over the triangular region on the side with no strain gage.

The repeatability of FMT was evaluated by tracking the video images from three trials five times. The tracking and the DLT parameters from the calibration images were used to reconstruct the displacements of the fiducials and the SDs were calculated. Over the entire loading cycle, the average SD from the point reconstruction, i.e., the tracking error was 0.0037 mm in the direction perpendicular to the load and 0.0007 mm in the direction aligned with the load. During loading, the average change in length of the sides of the triangles was 0.0196 mm, larger than the tracking error.
A one-way ANOVA was used to evaluate if the average RMS noise was different for the three strain measurement methods. A post-hoc Tukey test was used to determine which strain-measuring techniques generated significantly different levels of RMS noise relative to one another. Bland-Altman plots were created to compare the finite strains measured by FMT on the vertebra to the average strain over the same area measured with DIC. P-values less than 0.05 were considered significant for all statistical tests.

D.3 Results

The mean ± SD RMS noise levels for strain measurement methods were different (ANOVA p<0.0001; Figure 3). The strain rosette noise (1 ± 1 με) was less than the DIC noise for the strain rosette area and the side with no strain rosette (29 ± 8 and 24 ± 6 με; Tukey p = 0.0014 and p = 0.0016) and less than the FMT noise level (67 ± 25 με; Tukey p=0.0002). The DIC RMS noise levels over the strain rosette and on the side with no strain rosette was also less than the FMT noise (Tukey p=0.0002 and p = 0.0002).

![Figure D.3: Comparison of the RMS noise of the principal strains on the surface of the vertebrae measured by the strain rosette, DIC, and FMT. For the DIC, the noise was measured both over the strain rosette and over the triangular region without the strain rosette. Means (± SD) are shown and the raw data are plotted as points. The RMS noise was defined as the root mean square error of the strain during 2 seconds of the trial where the load was held constant at 100 N. The DIC and FMT RMS noise levels were significantly different than the strain rosette and the DIC RMS noise levels were significantly different than the FMT RMS noise, as indicated by the stars.](image-url)
Comparing the peak magnitudes of the minimum principal strain between DIC and FMT, the normalized error on the side with no strain gage was 21% for all trials; for the individual trials, the normalized error ranged from 2.7 to 42.7% (Figure D.4).

The magnitudes of the principal strain from FMT was compared with the DIC principal strain at the peak load and it was found that the Bland-Altman plots had biases of -480 με for the minimum (compressive) principal strain and 48 με for the maximum (tensile) principal strain (Figure D.5). For the compressive strains, which are negative by convention, the negative bias indicates that FMT measures higher absolute minimum principal strains compared to the DIC method. For the tensile strains, the positive bias indicates that the FMT method measures higher magnitude peak maximum principal strains compared to DIC. The bias for the minimum principal strain was significantly different than zero (one-sample t-test; p < 0.0001) while the maximum principal strain bias was not significantly different (p = 0.33). The two-SD error bounds for the maximum principal strain were ±1170 με; this spread is on the order of the tensile strain signals being measured (average: 702 με). The two-SD error bounds for the minimum principal strain were ±1138 με, generally smaller than the minimum principal strain signals being measured (average: 2822 με).
Figure D.5: Bland-Altman plots for the (a) minimum (compressive) principal strain and (b) maximum (tensile) principal strain measured using fiducial marker tracking (FMT) and DIC at the peak loads. The x-value is the average of the strain measured using FMT and DIC over the same area. The y-value is the strain value measured using FMT minus the peak strain from DIC. The solid lines are the average value of all the differences (bias). The dashed lines are the bias ± 2 SD.

D.4 Discussion

In this study, the feasibility of using FMT to evaluate the strain on bones was investigated and compared it to DIC and strain gages. The optical methods of FMT and DIC both had higher levels of RMS noise compared to the rosette. Similar to DIC, the high level of noise in the FMT signal may result in a small negative minimum principal strain and a small positive maximum principal strain, even when the bone is unloaded. The bias of the Bland-Altman was -480 με for the minimum (compressive) principal strain and 48 με for the maximum (tensile) principal strain.

FMT is an alternative to DIC and can also be used to optically track surface strain between points on a bone. Even in the test with the highest error, the overall shape of the minimum principal strain response was still similar between DIC and FMT (Figure D.4). However in the Bland-Altman plots for the FMT and DIC (Figure D.5) there is a trend of negative error at low average strains and positive error at high average strains. This might be explained by the variability in the FMT. While it was expected that the maximum principal strain would always be positive (tensile strain is expected perpendicular to the load application), in some cases FMT measured a negative value of the maximum principal strain. This leads to a negative error and a low average strain value. This variability in the FMT is expected to also affect the minimum principal strain, resulting in the upward sloping trend in the Bland-Altman plots.
There are limitations specific to the FMT method. A direct comparison was not made between the rosette and FMT because the fiducials represented a larger area than the strain rosette. For FMT calculation of strain, the surface of the bone was assumed to form a flat plane between the three points but the surface was actually curved. FMT measurements are limited by the accuracy and precision of measuring the calibration block point coordinates and the fiducial marker centroids. Both of these errors affect the tracking error; the tracking error was approximately one order of magnitude smaller than the displacements measured but may partially explain the discrepancies between the DIC and the FMT techniques. For the FMT images, no image distortion was performed but the effect of distortion was explored. For the lenses, there was minimal distortion and imaging was performed in the center of the images where the radial distortion is lowest; therefore, error as a result of distortion would be small relative to other errors.

To summarize the investigation of using FMT to track strain on bones, the noise is higher in FMT than for DIC or strain gages. Furthermore, although FMT captures the cyclic loading applied, the bias between DIC and FMT was large for the compressive strain and the Bland-Altman indicates an upward trend. For this type of setup although it is feasible to measure strains with FMT, caution should be taken in using the principal strains calculated with FMT for compressive loading of bones.
Appendix E  Strain gage linearity

Following publication of Chapter 2, it was suggested that linearity of the force and strain signal may be another alternative to assess the quality of the bonding of the strain rosette to the bone. It was recommended that if the linearity had an $R^2$ greater than 0.98, the strain gage bonding was acceptable [86], [166].

Based on this, the data was reanalyzed (Figure E.1- Figure E.4). In general, the debonding identified by the criteria in Chapter 2 was also identified through linearity analysis (6/7 specimens) (Table E.1). In one specimen where debonding was observed, there was no evidence of non-linearity. Four of eight specimens where no debonding was observed showed evidence of non-linearity. However, in all four specimens only one gage on the rosette had a linearity of less than 0.98 and therefore, the bond quality still may have been acceptable with a less stringent metric. Therefore, the conclusions on gages with debonding are similar to the criteria proposed in Chapter 2 if using a metric of no more than one gage with an $R^2$ less than 0.98 and all gages greater than 0.9. In future publications, the importance of verification of the linearity of strain gages should be emphasized.

Table E.1: Specimens that have debonding based on Chapter 2 criteria and based on linearity. Specimens that only had one gage less than 0.98 are indicated with * and the $R^2$ value of that gage is provided. The shaded columns indicate similarity between the two methods.

<table>
<thead>
<tr>
<th>Specimen</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
<th>11</th>
<th>12</th>
<th>13</th>
<th>14</th>
<th>15</th>
</tr>
</thead>
<tbody>
<tr>
<td>Debonding based on Chapter 2 criteria</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Debonded based on linearity ($R^2 &lt; 0.98$)</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
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<td>✓</td>
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<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>If only one gage ≤ 0.98, what is the $R^2$ value</td>
<td>0.93</td>
<td>0.97</td>
<td>0.95</td>
<td>0.97</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

* Only one gage had a $R^2$ value of less than 0.98.
Figure E.1: Linearity of the strain-force plots for each gage on the rosette, Specimens 1-4. The plots outlined in red indicate that the $R^2$ value is less than 0.98.
Figure E.2: Linearity of the strain-force plots for each gage on the rosette, Specimens 5-8. The plots outlined in red indicate that the $R^2$ value is less than 0.98.
Figure E.3: Linearity of the strain-force plots for each gage on the rosette, Specimens 9-12. The plots outlined in red indicate that the $R^2$ value is less than 0.98.
Figure E.4: Linearity of the strain-force plots for each gage on the rosette, Specimens 13-15. The plots outlined in red indicate that the $R^2$ value is less than 0.98.
Appendix F  Damage variable thresholds

For analyzing the damage on the anterior cortex using DIC, user-defined variables were created to define the strain stabilization point and the point when a percentage of subsets reached a local strain threshold. In this appendix, the sensitivity of these variables to selected levels is presented.

The strain stabilization was evaluated by considering the 10% of subsets with the highest minimum principal strains. The percentage overlap from frame-to-frame was used to determine the timing of the stabilization. The timing for different percentages of overlap was evaluated (Figure F.1).

Figure F.1: The overlap in highest strain subsets versus time (low-pass filtered at 2 Hz) for when the strain on the anterior cortex stabilizes for frame-to-frame comparison. The dots represent when the given specimen reaches 96 to 99% consistency from frame-to-frame. A threshold of 98% was selected for this variable in the fracture investigation in Chapter 3.

For the study, an overlap of 98% was selected since this value was after the signal reached a plateau for all specimens and represents a conservative threshold for the timing of the strain stabilization.
The other user-defined variable developed was the percentage of subsets reaching a local strain threshold. Two local strain criteria were considered based on literature, either 1% or 1.3% [70], [193], [194]. The force at which each specimen had a certain percentage of subsets reached the local strain threshold was evaluated (Figure F.2).

Figure F.2: The force at which a percentage of subsets reached a minimum principal strain threshold of (A) 1% or (B) 1.3%. For the current study, a criterion of 10% of the subsets reaching a minimum principal strain of 1% was selected.

All the specimens exhibited relatively linear behavior for the force rise as a function of the percentage of subsets. Since the rise was consistent, a value of 10% of the subsets reaching 1% minimum principal strain was selected. Although different thresholds for the percentage of subsets could be selected, the finding that many subsets reached the local strain criteria prior to yield force remains the same.
Appendix G  MTS compliance characterization

When using any MTS, it is important to consider the machine compliances since the components of the actuator are not infinitely stiff. Machine compliance means that the displacement measured from the LVDT (Disp_{overall}) is the combination of both the machine displacement (Disp_{machine}) and the specimen displacement (Disp_{specimen}):

\[ Disp_{overall} = Disp_{machine} + Disp_{specimen} \]

Equation G.1

The displacement is a function of force so the more force applied, the more displacement of the machine that would be expected.

To characterize this compliance, a rubber specimen was loaded into the MTS (Instron 8874, Norwood, MA, USA), and the platen displacement was measured using a dial gage (Figure G.1). To set the zero point of the MTS, the crosshead was lowered until a small load (~5 N) was registered. The reading from the dial gage was recorded at this point. Then, the specimen was loaded at increments of 50 N up to 300 N and then at increments of 200 N up to 4500 N. The load was applied at 10 N/second. After each loading, the specimen was allowed to sit for approximately 30 seconds and then the reading from the dial gage and the MTS software was recorded. The testing was performed three times.

![Test setup for characterizing the machine compliance in the MTS](image)

**Figure G.1: Test setup for characterizing the machine compliance in the MTS**

The machine displacement was calculated subtracting the dial gage reading (Disp_{specimen}) from the displacement given by the MTS software (Disp_{overall}). The average displacement ± one SD was plotted as a function of the load (Figure G.2). The average values of the compliance were fit with a second-order polynomial going through (0,0).
Figure G.2: Machine displacement plotted as a function of the load. The circles represent the average displacement for the three tests and the bars indicate ± 1 SD.

The equation relating the load measured in Newtons (x) to the machine displacement in mm is as follows:

\[ Disp_{mach} = -1.99 \cdot 10^{-9} \cdot x^2 + 1.69 \cdot 10^{-5} \cdot x \]  

Equation G.2

This equation can be used to correct the LVDT measured displacement as a function of the force and demonstrates the magnitude of influence that the machine compliance has on the displacement measurements.
Appendix H  Additional experimental and FE results

Additional figures are reported for the specimens in Chapters 3 and 5 including both experimental and computational investigations. In Section H.5, the sensitivity of the conclusions to the PMMA-bone boundary conditions, the mesh size, the method used to map the bone material properties, and the DIC parameters were evaluated. These investigations were used to ensure the choices made for the models in Chapter 5 would not influence the conclusions.

H.1  Experimental results

The force-DIC displacement curves that were used to determine the stiffnesses in Chapter 3 and Chapter 5 are presented for each specimen (Figure H.1).

Figure H.1: Force versus DIC displacement measured on the anterior cortex for the DIC analysis region. The cyan lines show the linear fit from 25 to 75% of the maximum force. These values were used for the stiffness comparison reported in Chapter 5.

H.2  LVDT-FE stiffnesses

In the LVDT measurements, there is compliance from the MTS as well as the bone-PMMA and PMMA-platen interfaces. Use of DIC displacements eliminates the influence of the compliance but for comparison, the stiffnesses were evaluated using the LVDT measurements
Due to the compliance, the experimental stiffnesses are lower than the FE stiffnesses and the relationship between the experimental and FE stiffnesses is weaker, although still significant.

![Graph showing comparison of FE and LVDT stiffnesses]

\[ R^2 = 0.54 \]
\[ FE = 1.24(DIC) - 4068 \]

**Figure H.2**: Comparison of the stiffness from the FE models based on the average superior endplate displacement with the stiffness from the experiments based on the LVDT displacement. The goodness of fit and the fit equation are provided. Note that the stiffnesses measured by the LVDT were not corrected for machine compliance.

**H.3 Element moduli distribution**

Specimen-specific models were created for each vertebral body, and histograms of the distribution of the element Young’s moduli can be used to demonstrate this qualitatively (Figure H.3).
Figure H.3: Histograms showing the frequency distribution of element Young’s moduli for each specimen.

H.4 FE-DIC strain comparison

In Chapter 5, the DIC-measured displacements on the vertebral bodies were compared with the FE-predicted displacements. However, other researchers have evaluated the strains [165]. For comparison, the FE-predicted and DIC-measured minimum principal strain maps (Figure H.4 and Figure H.5) are provided. The strains were compared on a point-by-point basis (Figure H.6), and the $R^2$ values ranged from 0.54 to 0.73, lower than the agreement for the displacements.
Figure H.4: FE-predicted minimum principal strains at a load of 1000 N for the specimens based on the MDR from Ouyang et al. [140]. The points plotted here correspond to the DIC measurement area.

Figure H.5: DIC-measured minimum principal strains at a load of 1000 N. Each point represents the center of a DIC subset.
Figure H.6: Point-by-point comparison of the minimum and maximum principal strains measured by DIC and predicted by FE. The goodness of fit is provided. The x- and y-axes as well as the unity line are provided for reference.

H.5  Sensitivity

H.5.1  PMMA-bone boundary sensitivity

The influence of the boundary condition between the PMMA and the endplates was evaluated since that interface affects the load distribution on the superior nodes, used as input for the second step of the simulation. For comparison with the results presented in Chapter 5 based on a tied interface, the results are presented for sliding contact between the endplate nodes and the PMMA for the point-by-point displacement correlation (Figure H.7) and the specimen stiffnesses (Figure H.8). Overall similar results were seen with the sliding interface between the endplate and PMMA as compared to the tied interface.
Figure H.7: Point-by-point comparison of the anterior surface displacements at 1000 N predicted by the specimen-specific vertebral body FE models using MDR-5 relationship compared to the displacements measured using DIC during experimental loading. The load distribution on the superior endplate was determined based on sliding contact between the PMMA and endplate nodes.

Figure H.8: Comparison of the FE stiffnesses to the experimental stiffnesses of the bone for MDR-5. The load distribution on the superior endplate was determined assuming sliding contact between the PMMA and endplate nodes. The stiffness was calculated based on the displacement measured and predicted by FE on the anterior surface. The experimental stiffnesses were found by fitting a line to the force-displacement curve between 25 to 75% of the maximum force. The FE stiffnesses were calculated by dividing the applied force of 1000 N by the average of the normalized displacements corresponding to the locations of the DIC measurements.
H.5.2 Mesh sensitivity

To investigate the effect of the mesh element size, a convergence investigation was performed on five specimens. Nominal element edge lengths of 1, 2, 3, and 4 mm were used to create 10-node quadratic tetrahedral meshes. The number of elements for each specimen is provided along with the average displacement on the superior endplate for a load of 1000 N evenly distributed over all nodes. For this investigation, one modulus density relationship was used [140].

![Graph showing comparison of average displacement on superior endplate for meshes with nominal edge lengths of 1 mm, 2 mm, 3 mm, and 4 mm for five selected specimens.]

Figure H.9: Comparison of the average displacement on the superior endplate for meshes with nominal edge lengths of 1 mm, 2 mm, 3 mm, and 4 mm for five selected specimens.
Figure H.10: Comparison of the R2 values for the FE to experimental displacement correlation for meshes with nominal edge lengths of 1 mm, 2 mm, 3 mm, and 4 mm for five selected specimens.

The mesh size does influence the predictions for the displacement on the superior endplate and anterior surface. In general for the displacement of the superior endplate, the displacements were similar for the different mesh sizes for each specimen. The largest change in the superior endplate displacement between the 1 mm mesh compared to the 2 mm mesh represented a change of 2.4% of the total displacement. Similarly, the largest change in the $R^2$ value from the 1 mm mesh to the 2 mm mesh was 3.0%. Given these smaller differences between the 1 mm and 2 mm mesh and the increase in computational resources for the 1 mm mesh, a mesh size of 2 mm was selected for the analysis.

H.5.3 Material mapping methods sensitivity

Material mapping is how the voxels from the bone CT scan are translated to the element material properties. The way in which the materials are assigned influences the predicted bone response. For the investigation, the modulus-density relationship proposed by Ouyang et al. was used [140]. The comparison between the FE and DIC was made for elements with nominal edge length of 2 mm and with DIC analysis parameters of 31 x 31 pixels for the subset size and a step size of 15 pixels.
The effect of element mapping method was investigated for two mapping strategies. In the first method (Material Mapping Method (MMM) 1), the voxels within the segmented bone geometry were identified. To ensure that there was sufficient CT information at the edges of the bone geometry, the CT voxels at the edge of the bone were expanded outward three times and the value of the old voxel was replaced if the new value of the voxel had a higher HU corresponding to a higher density. This provided sufficient information to use tri-linear interpolation to determine the Young’s modulus at each FE node from the CT voxel densities.

The second method (MMM 2) was similar but the outermost layer of the CT voxels at the edge of the bone was removed to correct for the partial volume artifact which occurs at the boundary where voxels contain both air and bone. After the layer was removed, the CT voxels at the edge were again expanded outward three times. Finally, given the moduli of the FE nodes, each element was assigned a modulus value with the nodes closer to the center having more influence on the element density. These two mapping strategies were compared for the vertebral body specimens.

![Figure H.11: The $R^2$ values for the FE to experimental comparison of the displacement on the anterior surface of the vertebral body specimens. There was a significant decrease in the $R^2$ value for MMM 2 ($p = 0.014$, CI: 0.0014 to 0.0094) but the decrease represented only about a 1% change in the correlation.](image)
Figure H.12: The CCC values for the FE to experimental comparison of the displacement on the anterior surface of the vertebral body specimens. The change in CCC for the MMM was not significant (p = 0.86) since the CCC increased for some specimens and decreased for others with the material mapping.

The selection of MMM does influence the correlation between the FE and experimental displacements. The $R^2$ values are slightly higher using the Material Mapping Method 1 but the effect size is small. The effect of MMM on the CCC was variable but MMM 2 resulted in a higher average CCC. For the current investigation, MMM 2 was selected since it accounts for the partial volume artifact.

**H.5.4 DIC parameters sensitivity**

The selection of DIC parameters influences the displacement patterns. There are two main DIC parameters, the subset size and the subset step size (the distance between subset centers). To evaluate the influence of these parameters, analyses was performed with three different subset sizes and step size combinations: 61 x 61 pixels with step size of 31 pixels, 31 x 31 pixels with step size of 15 pixels, and 25 by 25 pixels with step size of 5 pixels.
Figure H.13: $R^2$ values for the correlation of the FE to experimental displacements for different DIC subset sizes and step size between subsets. An ANOVA was used to evaluate if there were differences between the DIC parameters and no significant relationship was found ($p = 0.0515$) although more specimens may have identified a trend.

Figure H.14: CCC values for the correlation of the FE to experimental displacements for different DIC subset sizes and step size between subsets. An ANOVA was used to evaluate if there were differences between results using different DIC parameters and no significant differences were found ($p = 0.68$).

Three sets of DIC parameters were evaluated to determine the influence they had on results. While the different DIC parameters did not produce identical results, the results from different DIC parameters are generally similar.