Introduction  
Strain distributions in bone microstructure have been considered one of the key factors in determining the biological response of bone to mechanical loading. It has been suggested that the magnitude of threshold strain to develop microcracks is about 0.005 [1] in static tension, and to trigger remodeling is about 0.0025 [2] in cyclic tension. Currently, the strain gage method is widely used to measure strain in engineering materials. While the length of a typical strain gage varies in the range of 3 to 6 mm, in bone the diameter of typical microstructural features is 10 µm for osteocyte lacuca and 40 µm for Haversian canals. Consequently, traditional strain gage methods are unable to assess local strain at the level of the microstructure in bone.

This study explores the use of an optical method (DISMAP: microdisplacements by machine vision photogrammetry) to measure the global and local strain field of bone in controlled uniaxial tensile loading. To apply uniform global stress on the desired region in test specimens, a special testing method was designed. Based on this work we asked two primary research questions: (1) Is the present new microtensile testing method comparable with traditional methods to determine the Young’s modulus of bone? (2) Are there differences between the magnitudes of local strain vs. global strain in bone under uniform global tensile stress?

Methods  
Three cortical bone specimens were prepared from transverse mid-diaphyseal sections of three canine femurs. Bow-tie shape microtensile specimens were fabricated by a micromilling machine. The specimen had a thickness of 0.22 mm. The top surface of the sample was polished with alumina slurries of 0.05 µm. Each micro-tensile specimen was mounted in aluminum alloy grips that were also prepared by the micromilling machine to ensure a close mating between bone and grip. Finite element analysis showed uniform stress at the middle of the specimen in tension. The microtensile tester consisted of a load cell and computer-controlled linear actuator (Ultramotion, NY) mounted on the stage of a reflected light microscope. Tension was applied under displacement control at a rate of 1 µm/sec. All experiments were performed at room temperature. Images of the bone surface were taken at specific load steps (Fig. 1a). The size of the area for the global measurement of strain was determined (Fig. 1b) by comparing the images taken from the unloaded and loaded test specimen. Local displacement points were established by electronically laying rectangular grids over the images; displacements were measured at the nodes of these grids. Corresponding to the displacements, maximum principal microstrain (εhmax) at each node was computed in the x, y, and xy direction following Williams et al. [3]. The global displacements in the x (tensile axis) and y direction were calculated by subtracting the displacement data between the nodes on the end-most grid lines in each direction. To estimate the global strain across the entire image area, the global displacements were divided by the global length of the grid (gage length). The global strain in xy (yx) direction was calculated dividing the global displacement in x (y) direction by the gage length in y (x) direction. Using the global strain components calculated in each direction, the global maximum principal strain (εhmax) was computed.

Results  
For strain analysis, images were taken from eight loading steps, four from specimen 1 (two of the four images in Fig. 2a,b), two each from specimen 2 and 3. The pixel counts were classified to be the local strain values at specific strain ranges as shown in histograms. The

Discussion  
The Young’s modulus value determined from our specimens closely agreed with the value of the transverse modulus in canine bone (8.23 GPa) [4] measured by traditional strain gage methods. Although a linear isotropic finite element analysis predicted a uniform stress field throughout the gage region of our test specimens, the complexity of microstructure in bone has been shown in this study to produce a non-uniform strain field in the gage region with many local strain gradients. Around osteocyte lacuca we observed local strain values that exceeded the putative threshold magnitudes for microcracks and remodeling even though the global strain was still below the putative microcrack/remodeling threshold. Accordingly, this study helps explain how it might be that cell-level strain levels could be ten or more times larger than the values measured by strain gauges.

References  

Fig. 2 The maximum principal strain distribution and histogram for specimen 1. (a) applied stress: 11.8 MPa and (b) 34.7 MPa. Interestingly, the global maximum principal strain showed strong relationship with applied stress (Fig. 3). The Young’s modulus value determined from our specimens closely agrees with the value of the transverse modulus in canine bone (8.23 GPa) [4] measured by traditional strain gage methods.