INTRODUCTION
Bone cells respond to fluid shear loading by activating various biochemical pathways, mediating a dynamic process of bone formation and resorption. The whole-cell volume dilatation [1] and regional deformation of intracellular structures [2] may be able to directly activate and modulate relevant biochemical pathways. Therefore, understanding how bone cells deform under fluid flow can help elucidate the fundamental mechanisms by which mechanical stimuli are able to initiate biochemical responses. Most studies on cell deformation have focused only on cell deformation in the plane parallel to the substrate surface. Height-dependent cell deformation has not been well characterized even though it may contribute greatly to mechanotransduction mechanisms. Traditional techniques to obtain this additional height information of a cell-body, such as confocal and deconvolution microscopy, are inherently limited by the timescale under which the deformational information can be visualized. Previous studies have investigated cell adhesion to substrate under flow using a single view side-view imaging technique [3, 4]. In this study, we present a novel technique that is able to image a single cell simultaneously in two orthogonal planes to obtain real-time images of a cell at a millisecond timescale. Thus, the objectives of this study were to: (1) develop an imaging technique to visualize the depth-directional information of a cell simultaneously with the traditional 2-D view; (2) map out the strain fields of the cell by image analysis; and (3) investigate the viscoelastic behavior of osteoblasts under steady fluid flow.

MATERIALS AND METHODS
Microscope
A custom-designed dual-microscope system consisting of an IX-71 inverted microscope and BX-51 upright microscope was used in this study. A custom-built device was employed to align a mirror at 45 degrees in the light path of the BX-51 microscope to obtain “side view” images of a cell [4] (Figure 1). The IX-71 microscope was used to obtain the “bottom view” image of the cell body. Fluorescence images were synchronously recorded for both microscopes using two cooled CCD cameras, GFP filter sets, and one Lambda DG-4 xenon lamp.

Cell Culture
MC3T3-E1 osteoblastic cells were loaded with 2µM calcein-AM or transfected with an actin-EGFP plasmid using Lipofectamine 2000 and then plated on a gold-coated .6mm x .15mm x 50mm microslide that was patterned using microcontact printing and self-assembled monolayer technologies as previously described [5]. The microslide was then placed into a .70mm x .70mm x 50mm square microtube to form a chamber for imaging and flow studies.

Fluid Shear Experiments
A syringe pump was employed to generate 30 dynes/cm² of laminar flow inside the chamber. The steady flow was imposed for 45 seconds and then turned off. Cells were simultaneously imaged using both microscopes during the flow experiment and for 45 seconds afterwards.

Image Analysis
Digital image correlation was used as previously reported [6] to obtain the history of displacement fields of the cells in both bottom and side views. To obtain the strain field, the displacement fields were further smoothed using a thin-plate spline smoothing (TPSS) algorithm [7]. Lagrangian strains fields were then calculated by numerical differentiation of the displacement fields.
RESULTS
Localized regions of high strain were observed in the cell in both views in response to steady fluid flow (Figure 3, 4). The y axis is in the flow direction while the z axis is the height direction. Three different strain components were obtained from each image: $E_{xx}$, $E_{yy}$, and $E_{xy}$ were obtained from the bottom view of the cell (Figure 3), and $E_{zz}$, $E_{yy}$, and $E_{zy}$ were obtained from the side view of the cell (Figure 4). Clearly, creep and creep recovery was seen in the bottom third of the cell proximal to flow in both views of the cell. It is interesting to observe that creep deformation in certain parts of cell did not recover to zero. Therefore, this emphasizes the dynamic and active nature of bone cell deformation under fluid shear.

CONCLUSION
The strain fields of the bone cell indicate a high level of inhomogeneity in mechanical properties throughout a cell. Similar to previous studies on cells under fluid shear [2], significant localized strain in the cell was observed. Additionally, height-dependent cell deformation was quantified in this system by the usage of the side-view microscope. The time-dependent and active characteristics of cell deformation under fluid shear demonstrate the need for a real-time 3D imaging technique of bone cells under fluid shear. This novel real-time imaging technique should reveal in-depth knowledge of the mechanotransduction mechanisms of bone cells. Using more relevant intracellular markers such as actin or microtubule-GFP proteins to measure cellular deformation is a future area of interest.

REFERENCES