Introduction: Bone remodeling is a predictor of fracture risk independent of bone mineral density [1]. Although it is unclear how bone remodeling influences cancellous bone strength, it is believed that cavities formed during remodeling play a role [2-4]. A challenge in determining the biomechanical importance of resorption cavities is that quantitative methods of detecting and measuring resorption cavities have not been developed. Resorption surfaces are typically identified based on surface texture (so-called 'scalloped surface'). Because detection of a feature in an image requires a pixel size half that of the feature [5], a high resolution technique is required to identify bone surface irregularities characteristic of resorption cavities. A recently developed imaging technique known as serial milling can provide 3D images of bone at a resolution less than 1 micron/pixel [6][7]. However, image processing techniques associated with serial milling have so far only been used to measure volumes, and have not yet been refined to detect variation in bone surface texture. Here we present image processing and visualization techniques capable of identifying and quantifying resorption cavities in cancellous bone.

Materials and Methods: Specimens of cancellous bone are embedded undecalcified and imaged using serial milling [6][7]. Serial milling involves repeatedly trimming away the top of a specimen and collecting a mosaic of images via epifluorescence. The technique provides 3D images of bone and fluorescent markers at a resolution of 0.7 x 0.7 x 5 microns per voxel. A series of image processing steps are used. First, because epifluorescence microscopy is commonly associated with non-uniform specimen illumination (dark image corners), signal intensities in each image are normalized using a quadratic approximation to flatten the illumination profile. Second, each cross-section of the specimen consists of an array of images that must then be tiled to create a complete mosaic. While previous processing techniques utilized camera positioning to achieve tiling, in the current study pixel size is smaller than camera positioning accuracy, requiring automated tiling using normalized pixel cross-correlation. Similarly, the positioning of each cross-section in the vertical direction must also be corrected. A fiduciary marker is used to achieve vertical alignment. Another challenge of imaging through serial milling is subsurface fluorescence, observed as blurring along bone surfaces. To remove subsurface fluorescence, the underlying image is blurred, scaled, and subtracted, revealing the true cross-section. The images are then deconvolved using an optical point-spread function. Lastly, images are segmented to differentiate bone from marrow space using a local iterative thresholding algorithm [8]. A 3D image of the bone is then generated and visualized through Amira (Mercury Computer Systems). Functions in Amira allow direct measurement of features in the 3D image.

Results: The revised image processing techniques provide improved representation of bone surfaces (Fig. 1). Eroded surfaces are easily identifiable in the new 3D images (Fig. 2). Examination of the raw images was used to confirm identification of eroded surfaces (Fig. 3). Examination of the raw images suggests that surface irregularities characteristic of eroded surfaces are typically 3-4 micron in size.

Discussion: Precisely how remodeling affects bone biomechanics independent of bone mass is not known, although resorption cavities are believed to play a role. Here we present a quantitative technique for identifying individual resorption cavities in cancellous bone. Although our image processing techniques were developed for serial milling, our results suggest that bone surface irregularities characteristic of resorption cavities may be detected using any other 3D imaging modality with voxel size of 1.5 microns or smaller (half the size of eroded surface texture features). An advantage of serial milling, however, is in that it can examine specimens of almost any size (i.e. the technique is not limited in field-of-view) and can also obtain images of fluorescent markers of bone formation and microscopic tissue damage. Future work with this technique will be used to determine the spatial relationship between resorption cavities and microscopic tissue damage.

Acknowledgements: NIH/NIAMS AR054448, Case Western Reserve Univ.