INTRODUCTION
Osteoarthritis can evolve from numerous factors, including traumatic injury to cartilage. After progression to advanced OA, most therapies target symptom management. Recently, there has been a growing trend to detect early cartilage damage, facilitating intervention that may prevent or slow the progression of OA. However, current medical imaging methods such as magnetic resonance imaging (MRI) and computed tomography (CT) do not have the resolution to detect small structural or cellular changes, nor the ability to detect tissue viability. Multiphoton microscopy (MPM) has emerged as a method for imaging live intact tissue and may provide the submicron resolution needed to detect very early changes in damaged cartilage. The goal of this study was to use MPM to detect changes in tissue structure and cell viability immediately after impact injury, with a long term goal of adaptation for in vivo use.

METHODS
Tissue collection and traumatic injury. Osteochondral (OC) blocks from the distal 3rd metacarpus were collected from 6 normal horses aged 4-6 years and incubated in phenol-red free MEM media prior to and following injurious mechanical compression. OC blocks were mounted between a custom-designed specimen-chamber and a 2.25 mm diameter impactor on an EnduraTEC ELF3200 test frame (EnduraTec, Minnetonka, MN), subjected to a single traumatic stress of 30 MPa over 1 sec, and then incubated in the described media for 1 h at 37°C.

Multiphoton imaging. OC blocks were placed in 1 µM fluorescein in PBS to evaluate viability using a Ti:sapphire laser (Newport, Irvine, CA) at 780 nm excitation. Emission spectra was collected with 20x/0.95NA water immersion lens during a 3 mm x 3 mm x 100 µm tile scan, or with a 3 mm thick 27x/0.7NA water immersion lens designed for in vivo use.

Image analysis. MPM tile scan data was quantified in MATLAB to determine chondrocyte viability. The calculated difference between control and injured viability was assessed with a one-sample T test using Statistica 9.0 software.

RESULTS
MPM imaging was able to resolve cellular and structural changes in intact cartilage within 1 h of injury (Figure 1). MPM data was quantified to detect decreased chondrocyte viability at the injury site and neighboring tissue in 3 dimensions (Figures 2, 3, 4).

Fig. 1. Chondrocytes in control cartilage (a,c) were absent of fluorescence. After injury, chondrocytes accumulated fluorescein (b,d) and developed autofluorescent structures(b). Images were acquired in the transverse plane from the 40x(a,b) and 27x arthroscopic(c,d) lenses.

Fig. 2. MPM at the articular surface was used to detect chondrocyte death 100 µm deep into the intact (non-sectioned) dense tissue. Data indicated decreased viability after injurious compression (p<0.001). Each point represents the mean viability of all chondrocytes located in one 328 µm x 432 µm scan-section from a parent 3 mm x 3 mm area. Data shown as 30 points per z-depth, per horse, for each injury and each control.

Fig. 3. Data from MPM demonstrated an increased, non-uniform distribution of chondrocyte death after injurious compression. Viability also appeared reduced in neighboring areas that were not compressed. Each point represents the mean viability within one 328 µm x 432 µm section. Non-imaged sections of x=206 µm and y=202 µm between scanned sections resulted in 48% scan-coverage of the 3 mm x 3 mm region.

DISCUSSION
MPM imaging was able to discern cellular and structural changes in intact cartilage within 1 h after injury. These changes are outside the detection capabilities of clinical and research-grade MRI and CT. MPM images demonstrated high resolution that was able to detect cellular and structural changes in intact, unprocessed cartilage that appeared to radiate outward from the injury site in an asymmetrical pattern. This non-uniform viability distribution may result due to a relationship between stress dispersion from the injury site and collagen arrangement in the matrix, warranting further study. MPM offers a novel method for identifying early cartilage damage which can then be used to apply therapies and lifestyle management that may prevent the progression of OA.

SIGNIFICANCE
Identifying early cartilage damage is necessary in order to implement preventative therapies and alter the natural disease course of OA. MPM can discern early cellular changes immediately after injury, providing an opportunity to understand and intervene with disease progression.

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REFERENCES