Calibration of a SPIM System for Studying Chondrocyte Mechanotransduction

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Disclosures: Dr. June owns stock in Beartooth Biotech. Drs. June and Brahmachary own stock in OpenBioWorks. Neither company was involved in this study.

Introduction: Osteoarthritis (OA) is a debilitating whole joint disease that affects ~600 million adults worldwide. There is currently no cure for OA. Aging, trauma, obesity, and joint inflammation contribute to OA pathogenesis[1]. Mechanical loading of the joint is crucial to maintaining healthy, normal articular cartilage and chondrocyte function[2]. In an attempt to better understand the mechanisms that underlie chondrocyte mechanotransduction, we previously assembled a SPIM (selective plane illumination microscope) system with the ability to apply uniaxial compression to cell-laden agarose gels. The primary objectives of this study are to (1) quantify functional imaging parameters of the SPIM and (2) assess the ability of the SPIM system to perform live-cell fluorescence imaging in primary chondrocytes. This study lays the foundation for both future studies in examining chondrocyte mechanotransduction through live-cell fluorescent imaging and a better understanding of chondrocyte response to mechanical stimuli.

Methods: Laser alignment and pixel size (0.33 micron) were previously determined. To develop a point spread function (PSF) for the system, sub-resolution 500 nm fluorescent beads (ex/em: 488/525) were embedded into physiologically stiff agarose (4.95% w/v). Because agarose can provide physiologically stiff microenvironments, agarose gels were situated into the compression platens of the SPIM system for imaging. The imaging sample chamber was then submerged in phosphate buffered saline (PBS). Utilizing the 4D motor axis of the SPIM system, a 3D image stack was acquired with 3-micron steps between images. Fluorescent beads were excited using a 488 nm laser at an approximate power level of 100 mW (50% total power) and images were acquired using a 488 nm notch filter (488/15), 525 nm emission filter (525/39), with a 500 ms exposure time through the µOpenSPIM software. Fiji (ImageJ) was used to analyze the resulting image stacks. In preparation for imaging chondrocytes exposed to mechanical stimulation, we piloted a baculovirus mediated gene delivery system (BacMam) expressing cmv-mNeonGreen fluorescent protein in primary bovine chondrocytes, the purpose of which was to verify system sensitivity between transduced and non-transduced fluorescent cell intensity. Controls included agarose gels with non-transduced bovine chondrocytes (n=3). Acquisition parameters were similar, except for the exposure time of 5000 ms. The 4D motor axis of the SPIM system was again utilized to generate image stacks with 3-micron steps between images and Fiji (ImageJ) was used to analyze the resulting image stacks.

Results: Utilizing the DeconvolutionLab2[3] plugin in Fiji and the fluorescent bead image stacks, an experimental point spread function (PSF) was generated to perform subsequent image deconvolution of the transduced bovine chondrocyte images. Utilizing Fiji, an estimate of the full width at half maximum (FWHM) yielded a value of ~1.4 microns for the lateral PSF and ~2.0 microns for the axial PSF. Images from the transduced bovine chondrocytes had a greater fluorescent intensity when compared to the control gels, under identical laser power and exposure time, indicating successful gene delivery and detectable sensitivity of the imaging system.

Discussion: Acquisition of fluorescent bead images allowed for the development of an accurate PSF, which will be the basis for future image deconvolution with the SPIM system. Our results demonstrate that the SPIM system achieves an in-plane resolution better than 5 microns, allowing for high-resolution spatial imaging. Comparison of fluorescent intensity between transduced and non-transduced bovine chondrocytes increases confidence in the SPIM's ability to detect subtle, dynamic calcium-burst intensity changes across time series imaging data in future studies. The SPIM system offers considerable potential for studies of chondrocyte mechanotransduction, enabling correlation of mechanical stimuli with intracellular signaling events in both healthy and OA chondrocytes.

Significance/Clinical Relevance: Live cell imaging of agarose-embedded chondrocytes subjected to mechanical stimulation with the SPIM system offers a powerful approach in understanding chondrocyte mechanotransduction. Insights gained from the SPIM system will be relevant in informing strategies for prevention or treatment of OA.

References: **1.** S.H Chang+ 19', PMID: 30926814. **2.** Guilak F. 11', PMID: 22265263. **3.** Sage D.+ 17', PMID: 28057586. **4.** "Calibration." OpenSPIM. Accessed August 20, 2025. https://openspim.org/Operation.

Acknowledgements: We are grateful for funding from NSF (CMMI 1554708 and 2140127) and NIH (NIAMS R01AR073964 and R01AR081489).

Images and Tables:

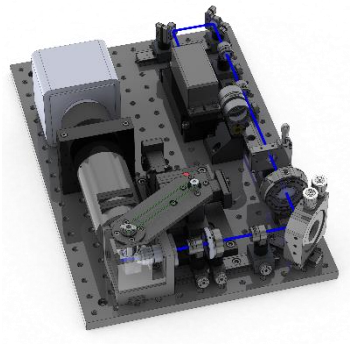

Figure 1: Rendering of the OpenSPIM system[4], compression motor placed on the 4D motor axis.

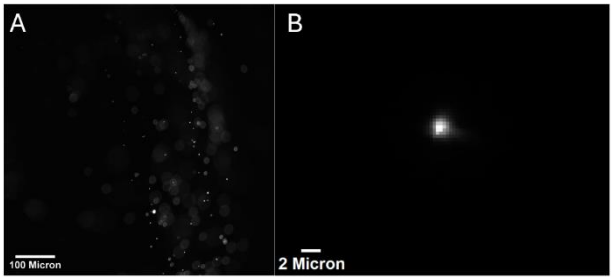

Figure 2: **A)** Full view image of 0.5 micron fluorescent beads in agarose gel **B)** Single fluorescent 0.5 micron bead, PSF example

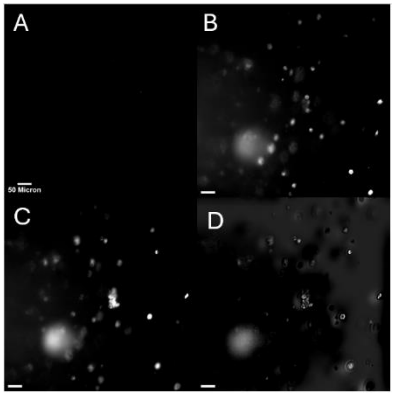

Figure 3: **A)** non-transduced cells in agarose gel **B)** transduced cells in agarose gel **C)** raw transduced cells **D)** deconvolved transduced cells