

## DATA DRIVEN AND CELL SPECIFIC DETERMINATION OF NUCLEI-ASSOCIATED ACTIN STRUCTURE

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In the specialized field of cellular mechanosignaling, the intricate relationship between the cytoskeleton and nucleus is crucial for determining the behavior and fate of mesenchymal stem cells (MSCs). Existing analytical methods for filamentous actin fibers (F-actin) suffer from issues of accuracy and reproducibility. To address this, our research introduces a significant advancement in image analysis by utilizing two deep-learning segmentation Convolutional Neural Networks (CNNs) based on U-Net architecture. This algorithm precisely quantifies F-actin and offers reproducible data from 3D confocal microscopy images. Our CNNs automatically extract the 3D shape of both the nucleus and individual actin fibers, thereby enabling the geometric reconstruction of these critical cellular components. We applied this methodology to MSCs exposed to low-intensity vibration, and hypothesized that mechanical stimuli will induce perinuclear actin remodeling.

Confocal Z-stack images of MSCs were captured using a 40x oil lens and a Zeiss LSM 900 microscope. Nucleus and F-actin structures were stained with Hoechst 33342 and Phalloidin, respectively. Nucleus-based images were employed to mask and isolate actin fibers within the nuclear vicinity.

A specialized deep-learning segmentation model was engineered within the PyTorch framework. For model training, a selective 2% of nuclei and F-actin images were annotated. Specifically, three layers

from each of six representative nuclei, among approximately 500 cross-sectional layers, were meticulously selected; a similar approach was employed for actin. The model underwent 200 epochs of training. Post-training, the deep-learning model was employed to process all collected cross-sectional images of F-actin and nuclei. A subsequent analytical step involved the scrutiny of intersecting segmented fibers between consecutive layers for geometrically accurate actin fiber reconstruction.

We analyzed 102 control and 110 vibration-treated mesenchymal stem cells (MSCs) by utilizing our deep-learning-based reconstruction algorithm on confocal Z-stack images. Our data revealed an 8% decrease in nuclear height from 5.73  $\mu\text{m}$  to 5.28  $\mu\text{m}$  ( $p < 0.001$ ) upon application of low-intensity vibration. In contrast, the two groups observed no significant difference in nuclear volume. Accompanying these changes at the nuclear level, the following changes in the F-actin cytoskeleton were observed upon LIV application. The total number of actin filaments per cell increased from 117 to 150. The average volume of individual actin fibers rose from 0.40  $\mu\text{m}^3$  to 0.43  $\mu\text{m}^3$ . Notably, the volume of fibers at the apical nuclear surface increased from 0.35  $\mu\text{m}^3$  to 0.46  $\mu\text{m}^3$ .

While the average length of actin fibers in the apical nuclear surface remained largely unchanged due to the addition of smaller fibers in LIV groups, the LIV group had a larger number of fibers that were larger than 15 $\mu\text{m}$ .