

# Deep Learning for Real-Time Stain-Free Assessment of Multipotent Mesenchymal Stromal Cells Senescence

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## ABSTRACT

Live assessment of cellular senescence is a crucial, mostly unsolved task in cellular biology and medicine. Current methods require cell sampling, impeding real-time monitoring and subsequent use. Particular attention is directed towards monitoring senescence in multipotent mesenchymal stromal cells (MSCs), given their key role in tissue maintenance. Senescent cells not only alter the properties of the entire population but also influence neighboring cells due to paracrine signaling. Methods enabling non-invasive, single-cell (which is important due to cell heterogeneity) observation of MSCs are important for studying aging, which is critical for advancing research in cellular biology and medicine. In our study, we developed a deep learning-based tool for classifying MSCs as senescent or non-senescent based on phase-contrast microscopy images of live cells, allowing for real-time assessment of senescence and quality control of both the entire population and individual cells. The dataset included images obtained through a combination of techniques, such as phase-contrast microscopy, staining for the aging marker beta-galactosidase activity, and fluorescent staining with the nuclear dye DAPI. Automated cell segmentation using Cellpose 2.0 yielded masks for each image, which were then used for classification into senescent and non-senescent classes based on staining presence. The dataset was split into training and testing sets while maintaining a 1:1 ratio of senescent and non-senescent cells. The EfficientNet-B6 model was chosen for classification due to achieving high metrics for both classes. Resulting two-stage approach with combined image segmentation and classification techniques demonstrates significant efficiency thus providing a novel tool for studying cellular senescence.

**Keywords:** stem cell senescence, deep learning, real-time stain-free monitoring of cellular senescence, multipotent mesenchymal stromal cells