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Nuclear senescence scoring

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Protocol status: Working
We use this protocol and it's working

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Abstract

Nuclear senescence scoring

Immunofluorescence

1 Stain iPSC-derived cells in monoculture and triculture systems with DAPI.

2 Perform immunolabeling of cell type-specific markers:
- MAP2 for neurons,
- IBA1 for microglia, and
- GFAP for astrocytes.

3 Conduct imaging using a Leica TCS SP8 confocal microscope with a 40×/1.4 numerical aperture oil immersion objective.

Analysis of senescence using nuclear morphology (Heckenbach et al., 2022)

4 Detection of nuclei in DAPI-stained images using a U-Net segmentation model.

5 Analysis of nuclear morphometrics, including area, convexity, and aspect ratio.

6 Resizing and conversion of nuclei to a two-color mask for consistent representation.

7 Application of an ensemble of deep neural networks trained on human fibroblasts to predict the likelihood of senescence.

Software used

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- Python 3.9.18,
- TensorFlow GPU 2.15.0,
- Keras 2.15.0,
- Numpy 1.23.5,
- Pandas 1.2.4.