Integrating Morphology and Transcriptomics: Live-Cell Profiling of Senescent Fibroblasts with Laser-Particle Barcoding

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Introduction

Background: Cellular senescence is a state of stable cell cycle arrest that plays a dual role in aging and disease. Senescent fibroblasts exhibit characteristic morphological and transcriptomic changes, including enlarged cell size, altered cytoskeleton, and lysosomal expansion. Traditional approaches, such as RNA sequencing, provide detailed gene expression profiles but are costly, destructive, and cannot track live-cell dynamics over time. Live-cell imaging, on the other hand, captures morphological heterogeneity but does not directly reveal transcriptional states.

Project Aims: To bridge that gap by integrating live-cell imaging with morphological profiling and transcriptomic data**.** Using a modified Cell Painting protocol, we stain key cellular structures including nuclei, mitochondria, membranes, and lysosomes, and perform high-resolution confocal imaging. CellProfiler is applied to extract hundreds of morphological features, which will be paired with single-cell RNA transcriptomics and analyzed using machine learning.

Goal: Develop a predictive tool that can infer gene expression states from live-cell morphology, enabling cost-effective, longitudinal studies of aging and drug response.

Methods and Results

Workflow for Live-Cell Morphological Profiling of Senescent Fibroblasts

A. Cell Culture
1. Human fibroblasts grown in 8-well plates
2. Serial passaging to induce replicative senescence

B. Live-Cell Staining (Modified Cell Painting)
1. 4 fluorescent dyes for cell structures
2. Dye concentrations optimized

C. Confocal Microscopy
1. Multichannel grayscale imaging
2. Z-stacks flattened to 2D projections

D. Image Processing (FIJI/ImageJ)
1. Channel pseudo-coloring
2. Exported Files

E. Cell Segmentation & Features (CellProfiler)
1. segment nuclei and cytoplasm
2. 50–200+ morphological features

F. Laser Particle Tagging (Future)
1. LPs used to tag individual cells

G. RNA-Seq & Machine Learning (Future)
1. Morphology–transcriptome correlation via ML clustering
2. Build predictive model for transcriptomic states

Figure 1: (A) Human dermal fibroblasts were cultured in 100mm-well plates and serially passaged to induce replicative senescence then transferred to an 8-well plate. **(B)** Live-cell staining was performed using a modified Cell Painting protocol with 4 fluorescent dyes optimized for low toxicity to visualize nuclei, membranes, lysosomes, and mitochondria. **(C)** Confocal microscopy was used to capture high-resolution multichannel grayscale images and Z-stacks, which were flattened into 2D projections. **(D)** Image preprocessing, including pseudo coloring and channel alignment, was performed in FIJI/ImageJ to generate files compatible with CellProfiler. **(E)** CellProfiler was used for automated cell segmentation and extraction of 50–200+ morphological features (e.g., cell area, eccentricity, texture). **(F)** Future work includes integrating laser particle barcoding for longitudinal single-cell tracking. **(G)** Single-cell RNA-seq data will be paired with morphological features to build predictive models of transcriptomic states using machine learning.

Optimization of Live-Cell Staining and Seeding Density for Morphological Profiling

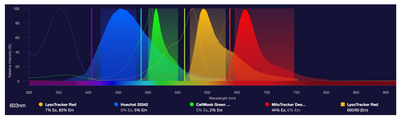
A.

Channel	Stain Name	Stain Color	Cell Structure
C1	**Hoechst 33342**	Blue	nuclear
C2	**LysoTracker**	Orange/Yellow	lysosomes
C3	**CellMask**	Green	membrane
C4	**MitoTracker**	Red	mitochondria
C5/C6	Transmitted Detector	Brightfield	not shown

B.

Name	Stock (mod)	Volume	Stain solut	Final conc	Ex. Em.
MitoTracker	0.5 mM	2 µL	1000 nM	500 nM	640 690/80
CellMask	1000X	2 µL	2X	1X	488 525/50
LysoTracker	1000X	2 µL	2X	1X	561 605/50
Hoechst 33342	500 mg/L	2 µL	2 µg/mL	1 µg/mL	405 445/70

C.



D.

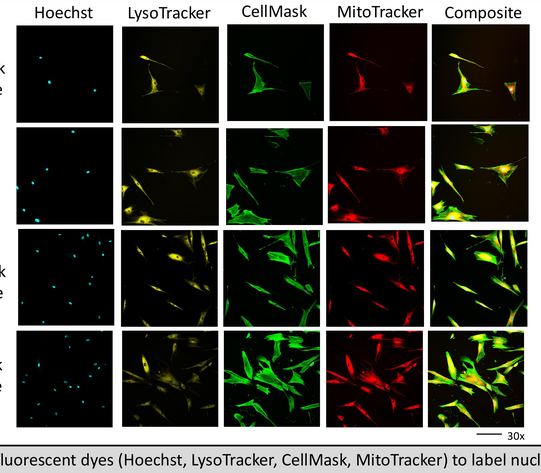
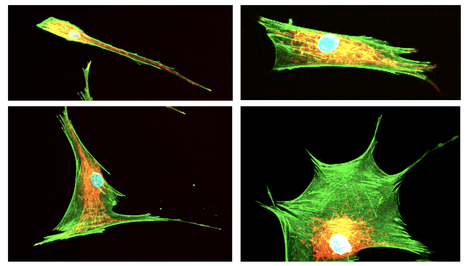


Figure 2: (A) Overview of the modified Cell Painting protocol using four live-cell fluorescent dyes (Hoechst, LysoTracker, CellMask, MitoTracker) to label nuclei, lysosomes, plasma membrane, and mitochondria. (B) Optimized staining concentrations and excitation/emission parameters to minimize spectral overlap and toxicity. (C) Emission spectra of the selected dyes illustrating compatibility for multichannel imaging. (D) Confocal imaging of human fibroblasts seeded at 2.5k, 5k, 7.5k, and 10k cells/well to determine optimal cell density for segmentation and morphological feature extraction.

Morphological Heterogeneity and Whole-Well Imaging for High-Content Analysis

A.



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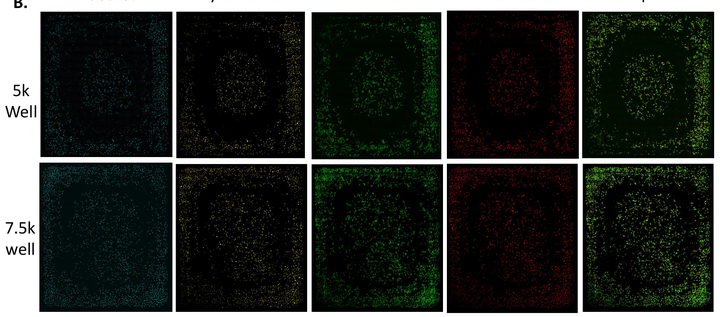
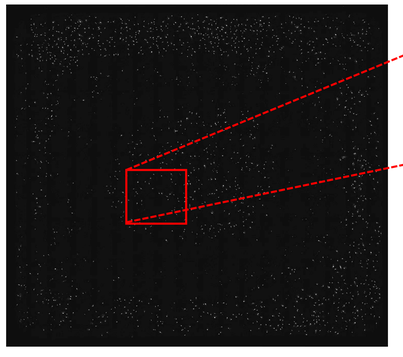


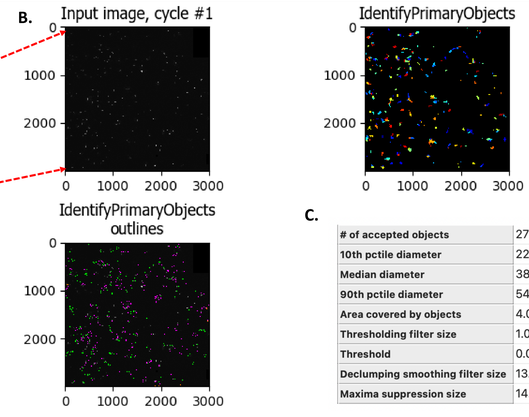
Figure 3: (A) High-resolution composite confocal images of fibroblasts at passage 15 (P15) reveal pronounced morphological heterogeneity, including enlarged, flattened senescent-like cells, elongated spindle-shaped cells, and variations in cytoskeletal organization. These morphological differences reflect the diverse cellular states within the population and highlight the need for single-cell analysis. **(B)** To enable high-content analysis, entire wells were imaged by acquiring and stitching 288 individual tiles at 30× magnification for each fluorescent channel (Hoechst for nuclei, LysoTracker for lysosomes, CellMask for membranes, and MitoTracker for mitochondria) using the FIJI stitching plugin. The resulting composite images provide a complete well view, capturing thousands of cells with precise structural detail. These stitched images are optimized for downstream computational segmentation and feature extraction with CellProfiler, allowing the quantification of hundreds of parameters, such as cell area, shape, texture, and intensity distribution. This pipeline sets the foundation for correlating morphological profiles with transcriptomic states in future experiments.

CellProfiler-Based Nuclear Segmentation and Morphological Parameter Extraction

A.



B.



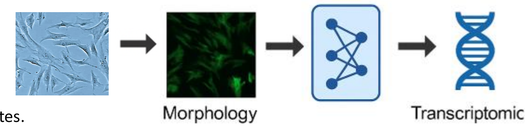
C.

# of accepted objects	277
10th pctile diameter	22.5 pixels
Median diameter	38.8 pixels
90th pctile diameter	54.5 pixels
Area covered by objects	4.0 %
Thresholding filter size	1.0
Threshold	0.0392
Declumping smoothing filter size	13.4
Maxima suppression size	14.0

Figure 4: (A) Confocal image of the 5k-cell density well showing Hoechst-stained nuclei, used as the basis for segmentation analysis. A random field of view was selected from the stitched well image to illustrate the segmentation process. **(B)** CellProfiler workflow applied to this region, with nuclei segmented as individual primary objects and color-coded to represent distinct cells. **(C)** Example of the quantitative output, including nuclear size, area, and shape descriptors (e.g., 10th percentile diameter, median diameter, and 90th percentile diameter). These parameters are part of a larger set of 200+ morphological features—including area, eccentricity, texture, intensity, and granularity—that will be extracted from the complete stitched dataset. This high-dimensional feature set will form the foundation for building a comprehensive morphological profile of the senescent fibroblast population, enabling downstream machine learning models to correlate morphology with transcriptomic data.

Conclusion

- Live-cell imaging and modified Cell Painting enable non-destructive morphological profiling.
- Morphological heterogeneity reflects underlying cellular states linked to aging.
- Optimized staining and confocal imaging allow reliable extraction of structural features.
- CellProfiler segmentation provides quantitative parameters for morphology-based analysis.
- Integration of laser particle tracking will enable longitudinal single-cell studies.
- Machine learning and UMAP visualization will enhance the ability to cluster and interpret cell states.
- Pairing morphology data with single-cell RNA transcriptomics reveals gene-expression correlations.
- This approach aims to create a predictive tool for inferring transcriptomic states from live-cell images.



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References

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