

Biophotonic Lysosomal Profiling of Cellular Senescence Using LysoTracker Fluorescence Imaging and Automated Single-Cell Analysis

Javier Estrada Toro, University at Albany, State University of New York, Albany, NY, USA

Abstract

Cellular senescence is increasingly recognized as a contributor to aging-related tissue dysfunction and has been implicated across neurodegenerative disease contexts through inflammatory signaling, altered proteostasis, and reduced regenerative capacity. Despite this relevance, senescence is often evaluated using endpoint assays such as SA- β -Gal staining, p16/p21-associated markers, and growth arrest, which can be labor-intensive and difficult to quantify reproducibly across imaging sessions. Because senescent cells also undergo marked remodeling of the lysosomal compartment, fluorescence probes that report acidic vesicular organelles offer an opportunity to turn a commonly observed phenotype into a quantitative, scalable imaging readout. LysoTracker is widely used for visualizing acidic organelles, but it is frequently interpreted qualitatively. Here, we describe a fluorescence microscopy workflow designed to standardize LysoTracker-based lysosomal profiling into a single-cell, quantitative metric that can be paired with orthogonal senescence markers and extended to data-driven analysis.

The assay combines LysoTracker with DAPI for nuclear reference and segmentation and incorporates SA- β -Gal as an optional benchmark marker. Human IMR-90 fibroblasts are used as a model system, with planned evaluation across passage-driven replicative senescence and comparison to stress-induced paradigms relevant to cellular damage, including DNA damage (etoposide) and oxidative stress (hydrogen peroxide). Imaging is performed under fixed acquisition settings (objective, exposure, and gain) to support cross-plate and cross-day comparability. Images are processed with an automated analysis pipeline that segments nuclei from DAPI, defines per-cell regions, and extracts LysoTracker-derived features including mean and integrated intensity, lysosomal area fraction, puncta size statistics, and texture descriptors that quantify spatial organization. Outputs are summarized as per-cell feature distributions and per-well aggregate scores to enable systematic comparisons across conditions and timepoints.

By shifting from endpoint-only, binary calls toward quantitative single-cell feature distributions, this workflow is designed to capture heterogeneity within wells and support development of a graded senescence score. This framework can be adapted for repeated measurements over time in live-cell imaging, enabling longitudinal assessment of stress responses and drug perturbations rather than a single terminal readout. These measurements can be scaled to multi-condition screening formats and are compatible with machine learning models that learn image-derived signatures of senescence-related states, creating a practical bridge between optical phenotyping and predictive analytics.