AutoMorphoTrack: An Automated Python Package for Organelle Morphology, Motility, and Colocalization Analysis in Live-Cell Imaging

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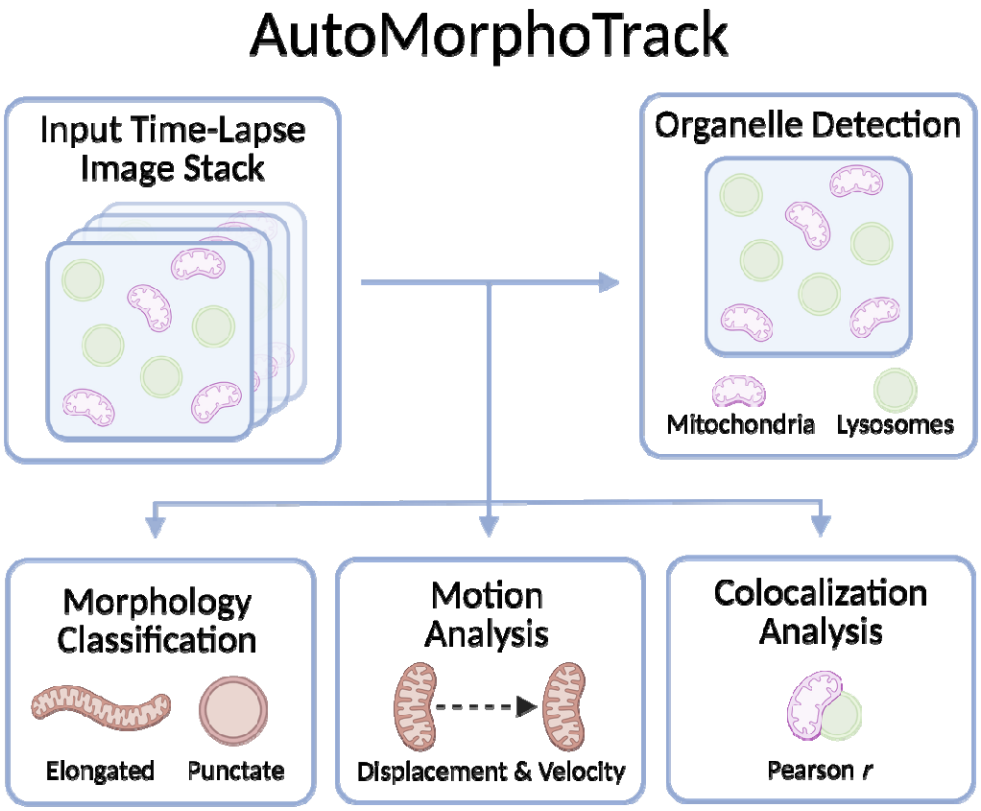
Graphical Abstract



Abstract

Understanding the dynamics and interactions of intracellular organelles is critical for understanding the mechanisms of cellular homeostasis and disease. Here, we present AutoMorphoTrack, an open-source Python package for automated detection (optional

thresholding), morphology classification, motility tracking, and colocalization analysis of mitochondria and lysosomes in live-cell imaging data. The package provides a pipeline capable of processing multichannel time-lapse image stacks, generating organelle-specific segmentations, organelle-specific count, tracking organelle motility, quantifying morphology parameters, and performing frame-by-frame colocalization analysis. We demonstrate the utility of AutoMorphoTrack using a representative dataset, illustrating each analysis step and the associated outputs. This package offers a flexible and scalable tool for organelle dynamics studies, facilitating reproducible and quantitative analysis for the broader cell biology community.

Introduction

Mitochondria and lysosomes play essential roles in cellular health, mediating energy production, degradation, and quality control of cellular components.[1,2] Both organelles have been heavily implicated in neurodegenerative diseases as disruption of their function and interaction has been shown to be a precursor for cellular dysfunction and apoptosis.[3] Quantitative analysis of organelle morphology, dynamics, and colocalization in live cells is critical to understanding these processes in cellular models of neurodegenerative diseases. However, such analyses are often labor-intensive, requiring multiple tools and manual organelle counting, manual segmentation, and further intervention.

To address these challenges, we developed AutoMorphoTrack, an automated Python package designed for the analysis of organelle morphology, motility, and colocalization from multichannel time-lapse microscopy datasets. The package utilizes image processing, object detection, and tracking algorithms while providing outputs suitable for both exploratory and hypothesis-driven analyses. Lastly, although this package was developed specifically for lysosomal and mitochondrial dynamics and morphology in mind, it can be easily adapted for use with any cellular structures or fluorescent proteins.

Methods

Organelle Morphology and Tracking Analysis using AutoMorphoTrack

To analyze lysosomal and mitochondrial morphology, dynamics, and colocalization, we developed and utilized the AutoMorphoTrack Python package. This package provides a pipeline for the automated processing of multichannel microscopy image stacks, integrating organelle detection, morphology classification, movement tracking, and colocalization analysis. The entire workflow is fully automated, requiring user-defined input files and customizable parameters.

The full package utilizes Python v3.13.5 and is available at https://zenodo.org/records/15678799.

Package Architecture

AutoMorphoTrack is structured into modular components with specific analytical steps:

1. Core Module (core.py)

This module handles image loading, output directory setup, and pipeline coordination. It sequentially runs organelle detection, mitochondrial morphology classification, lysosomal counting, and visualization. It prepares multichannel .tif image stacks and ensures outputs are saved for each analysis step.

2. Detection Module (detection.py)

This module detects organelles using adaptive thresholding followed by contour extraction. Detected structures are filtered by area to remove background noise and non-specific signals. The detection parameters can be adjusted to optimize for different imaging conditions or biological models.

3. Morphology Module (morphology.py)

Mitochondrial morphology is classified into elongated or punctate structures using area and eccentricity descriptors. Thresholds are customizable but can also default to optimized presets. By default, mitochondria are classified as:

- **Elongated:** area ≥ 0.2 μm^2, eccentricity ≥ 0.85
- **Punctate:** area ≥ 0.03 μm^2, eccentricity ≤ 0.7

4. Lysosome Counting Module (Lyso_count.py)

This module identifies and counts individual lysosomes per frame from binary masks. It also generates annotated images showing lysosome labels and count overlays for quality control. A minimum area filter helps exclude artifacts. Visualization is focused on Frame 0, with optional export.

5. Tracking Module (tracking.py)

Tracks mitochondria and lysosomes across time-lapse frames using centroid-based motion tracking. Outputs include displacement and velocity calculations per organelle, as well as visualizations of trajectory overlays. Designed for dynamic studies of organelle motility.

6. Visualization Module (visualization.py)

Generates visual overlays for each stage of the analysis, including raw organelle detection, labeled mitochondrial morphology, lysosome counts, and movement trajectories. Visuals are optimized for interpretability and publication-quality rendering, with customizable styling.

AutoMorphoTrack Workflow Summary

1. *Organelle Detection*
   The pipeline begins by detecting mitochondria and lysosomes from a multichannel image stack using adaptive thresholding. This step isolates the structures of interest and filters out background noise.
2. *Mitochondrial Morphology Classification*
   Detected mitochondrial structures are analyzed based on shape features (area and eccentricity) and classified into:
   - Elongated
   - Punctate

   A labeled image is then generated to visualize these classifications.

3. *Lysosome Counting*
   The binary lysosomal mask is processed to identify and count discrete lysosomes per frame. An annotated image of Frame 0 displays count labels directly over each lysosome.
4. *Organelle Tracking*
   Mitochondria and lysosomes can be tracked across time-lapse frames to compute displacement and velocity metrics. This step supports dynamic motility analysis.
5. *Visualization and Output*
   The pipeline saves:
   - Detected organelle overlays
   - Morphology classification maps
   - Lysosomal count visualizations

   All outputs are stored in the specified output directory.

Implementation and Usage Example

The package is fully implemented in Python and is distributed as an installable package, accompanied by an example Jupyter notebook (Example_Usage.ipynb). The notebook guides users through the complete pipeline, from loading image stacks to generating final figures.

A typical analysis begins by loading a multichannel .tif stack containing mitochondria and lysosome channels. Detected organelles can be overlaid onto raw images for verification, and mitochondria are classified into elongated or punctate categories. Organelle trajectories are visualized across frames, and colocalization overlays highlight areas of mitochondria-lysosome proximity. Quantitative outputs, including lysosome counts, mitochondrial morphology distribution, and organelle movement metrics (specifically displacement and velocity), are generated and exported as .csv files for further analysis.

Sample images

Live images of dopaminergic neurons were used as sample images for developing and testing this package. Dopaminergic neuronal samples were obtained following the plating of neural progenitor cells (STEMCELL, 200-0620) on glass-bottomed plates (MatTek, P35G-1.5-14-C), followed by their differentiation and maturation into dopaminergic neurons over the course of 4 weeks (STEMCELL, 100-0038 and 100-0041). On the day of imaging, neurons were stained with Mitotracker and Lysotracker (Invitrogen, L7526 and M7510) for 30 min before imaging them on Nikon C2 Confocal Microscope.

Results

Automated Organelle Detection and Morphology Classification using AutoMorphoTrack

To validate the capabilities of the AutoMorphoTrack package, we applied the pipeline to a two-channel time-lapse microscopy dataset containing lysosomes and mitochondria (Figure 1A). Channel 0 depicted lysosomes as discrete punctate structures (Figure 1B), while channel 1 visualized mitochondria as an interconnected tubular network (Figure 1C). These raw inputs served as the foundation for the automated analyses described below.

Organelle Detection through Adaptive Thresholding

Organelle detection commenced with adaptive thresholding applied separately to lysosome and mitochondria channels. Following thresholding, the detection step using AutoMorphoTrack accurately identified lysosomes as punctate objects (Figure 2A) and mitochondria as elongated structures (Figure 2B), with red outlines marking the detected organelles on Frame 0. Alternative visualization approaches were applied to improve the clarity and validation of detection outputs. Here, individual organelles were represented using unique color-coded masks, facilitating distinction among closely positioned structures (Figure 3A and B).

Mitochondrial Morphology Classification

Following detection, mitochondria were classified based on area and eccentricity descriptors into elongated (E) or punctate (P) morphologies. In Frame 0, the classification results were overlaid onto the raw images, with elongated mitochondria labeled in red and punctate structures in cyan (Figure 4). This morphological classification enabled quantitative assessment of mitochondrial diversity across the dataset.

Dynamic changes in mitochondrial morphology were monitored across the entire time-lapse sequence. The counts of elongated and punctate mitochondria were tracked over 40 frames, revealing temporal fluctuations in mitochondrial populations (Figure 5).

Lysosomal Counting and Organelle Tracking

Lysosomal counting was performed by annotating each detected lysosome in Frame 0 with a unique identifier (Figure 6), enabling precise enumeration and tracking of individual organelles across frames.

Organelle movement trajectories were visualized using both dot and line trace overlays. Cumulative organelle tracks over the entire stack were overlaid on Frame 0, illustrating the distinct movement patterns of mitochondria (red) and lysosomes (green) (Figure 7A and B). Alternatively, continuous line traces can be used to visualize individual organelle trajectories across frames, providing insights into the directionality and displacement of lysosomes (Figure 8A) and mitochondria (Figure 8B). These visualizations demonstrated the capability of AutoMorphoTrack to track organelle movement with high precision while simultaneously calculating metrics such as displacement and velocity.

Quantitative analysis of organelle motility showed dynamic variability in both displacement (Figure 9A) and velocity (Figure 9B) of mitochondria and lysosomes over 40 frames, reflecting their distinct motility behaviors.

Colocalization Analysis of Mitochondria and Lysosomes

Colocalization between mitochondria and lysosomes was assessed using pixel overlap analysis. In Frame 0, regions of overlap were visualized in cyan, highlighting areas of potential mitochondria-lysosome interactions (Figure 10). Quantification of colocalization across all frames using Pearson correlation coefficient yielded a relatively stable value around 0.2 (Figure 11), suggesting a consistent but very small degree of interaction throughout the imaging period. Both the visual overlays and frame-wise correlation metrics were automatically generated by the package, supporting quantitative evaluation of organelle interactions.

Package Summary

Below is a summary of the package's functions (See Table 1 for a full list of capabilities):

- *__init__.py:* Initializes the package and provides a namespace for importing key modules like core, detection, morphology, tracking, visualization, and Lyso_count.
- *core.py:* The central orchestrator that runs the full analysis pipeline. It coordinates detection, morphology classification, lysosome counting, and visualization steps, and optionally includes organelle tracking.
- *detection.py:* Implements adaptive thresholding to detect lysosomes and mitochondria. Applies filtering to exclude background and small artifacts.
- *morphology.py:* Analyzes mitochondrial structures and classifies them as "elongated" or "punctate" based on user-defined or default area and eccentricity criteria.
- *Lyso_count.py:* Identifies and counts individual lysosomes per frame. Includes functionality to visualize lysosomal counts and label them on the first frame.

- *tracking.py:* Tracks the movement of mitochondria and lysosomes across time-lapse frames, calculating displacement and velocity metrics. Includes support for trajectory visualization.
- *visualization.py:* Handles generation of all figures including overlays, morphology labels, lysosome counts, and track paths. Designed for both QC and publication-ready outputs.
- *test_basic.py:* A basic test script to check that the core functions of the package are operating correctly on sample input.
- *setup.py:* Standard Python setup script to install the package using pip.
- *README.md:* Provides a description of the package, setup instructions, usage overview, and key features.

Discussion

In this study, we present AutoMorphoTrack, an automated, Python-based, image analysis package designed to quantify lysosomal and mitochondrial morphology, dynamics, and colocalization from time-lapse microscopy stacks. The package includes adaptive thresholding, morphology classification, organelle tracking, and colocalization analysis, with built-in visualization tools that facilitate quality control and the generation of figures.

Through applying AutoMorphoTrack to a representative two-channel time-lapse dataset, we demonstrated its ability to detect lysosomes and mitochondria (Figures 1–3), accurately classify mitochondrial morphology (Figures 4, 5), and enable precise, unbiased lysosomal counting (Figure 6). Importantly, the dynamic tracking capabilities of the package provided detailed visualizations of organelle motility (Figures 7 and 8), and quantification of displacement and velocity over time (Figure 9). The visualization of organelle tracks both as overlays and as line traces revealed distinct movement patterns of mitochondria and lysosomes, underlining the package's capacity to capture organelle-specific dynamics within complex cellular movements. Furthermore, AutoMorphoTrack includes integrated colocalization analysis, enabling both qualitative visualization of overlapping regions (Figure 10) and frame-wise quantification using Pearson correlation (Figure 11). The modest colocalization coefficient (~0.2) suggests a consistent interaction between mitochondria and lysosomes. The package's ability to combine morphological, dynamic, and colocalization analyses into a unified pipeline facilitates comprehensive assessments of organelle behavior under basal or experimental conditions.

Compared to traditional manual or semi-automated workflows, AutoMorphoTrack offers several advantages, including automation of the pipeline, unbiased and reproducible quantification, and generation of useful data outputs. By providing detailed visualizations at each step, the package also improves transparency and interpretability of results, supporting both experimental validation and presentation needs.

While the current implementation is optimized for lysosomes and mitochondria, the modular architecture of AutoMorphoTrack allows easy adaptation to other organelles or vesicular systems by adjusting detection and classification parameters. Future developments may further

extend its capabilities to integrate machine learning-based segmentation and classification, as well as advanced trajectory modeling.

Conclusion

AutoMorphoTrack provides a versatile, fully automated, and user-friendly platform for analyzing lysosomal and mitochondrial morphology, dynamics, and interactions in time-lapse microscopy data. Its comprehensive workflow, from detection to tracking and colocalization analysis, enables researchers to gain deeper insights into organelle behavior with minimal manual intervention, other than initial image selection. The package's modular design, coupled with its visualization outputs, makes it an invaluable tool for cell biology, neurobiology, and systems biology applications focused on organelle dynamics and inter-organelle interactions.

Contributions

Software Development: AB; Writing – Original Draft: AB and JGS; Writing – Review & Editing: AB, JGS, and XC; Supervision: XC; Funding Acquisition: XC. All authors have read and approved the final version of the manuscript.

Data Sharing Statement

All software, resources, and code used in this study can be found at https://doi.org/10.5281/zenodo.15692734.

Declaration of interests

AB, JGS, and XC declare no conflicts of interest.

Acknowledgements

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Tables and Figures

Table 1: Summary of AutoMorphoTrack's features and capabilities

Feature	Description	Module	Output
Image Input Handling	Loads multi-frame, multi-channel .tif image stacks	core.py, notebook	image_stack variable
Organelle Detection	Adaptive thresholding and mask generation for lysosomes and mitochondria	detection.py	mito_masks, lyso_masks
Morphology Classification	Labels mitochondria as **elongated** or **punctate** using area & eccentricity	morphology.py	morphology_labels
Lysosome Counting	Counts and labels individual lysosomes per frame	Lyso_count.py	lyso_counts, lyso_labeled
Tracking (optional)	Computes displacement and velocity of organelles across frames	tracking.py	mito_tracks, lyso_tracks
Visualization - Detection	Overlays binary masks onto original images	visualization.py	detection_overlay.png
Visualization - Morphology	Shows labeled mitochondria as E (elongated) or P (punctate)	visualization.py	mitochondria_morphology.png
Visualization - Lysosome Count	Displays numbered lysosomes on first frame, reports total count	Lyso_count.py	lysosome_count_frame0.png
Colocalization	Correlates lysosome and mitochondria overlap spatially	core.py + visualization.py	Pearson correlation
Full Pipeline Execution	Runs end-to-end analysis and saves outputs to directory	core.py	All visualizations + result dictionary

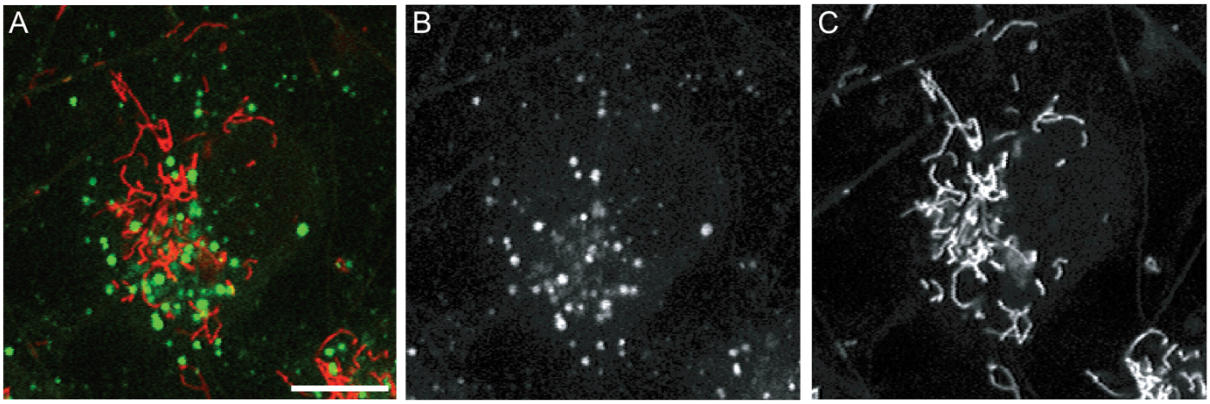

Figure 1. Original input data from a two-channel time-lapse microscopy stack.
(A) RGB image representing the original fluorescent image stack used. Scale bar = 10 µm. (B) Channel 0 represents lysosomes, visualized as discrete punctate structures. (C) Channel 1 represents mitochondria, displayed as an interconnected tubular network. These raw channels serve as the starting point for the AutoMorphoTrack analysis pipeline.

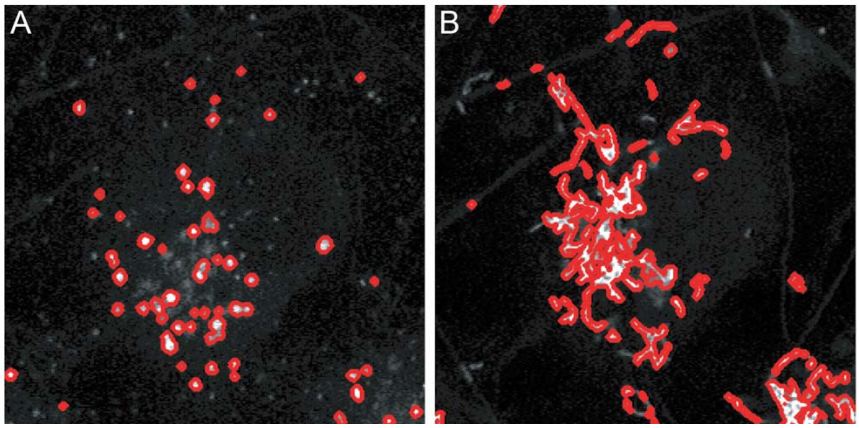

Figure 2. Organelle detection using AutoMorphoTrack.
(A) Detected lysosomes in channel 0 (Frame 0) outlined in red. (B) Detected mitochondria in channel 1 (Frame 0) outlined in red, highlighting the segmented tubular network.

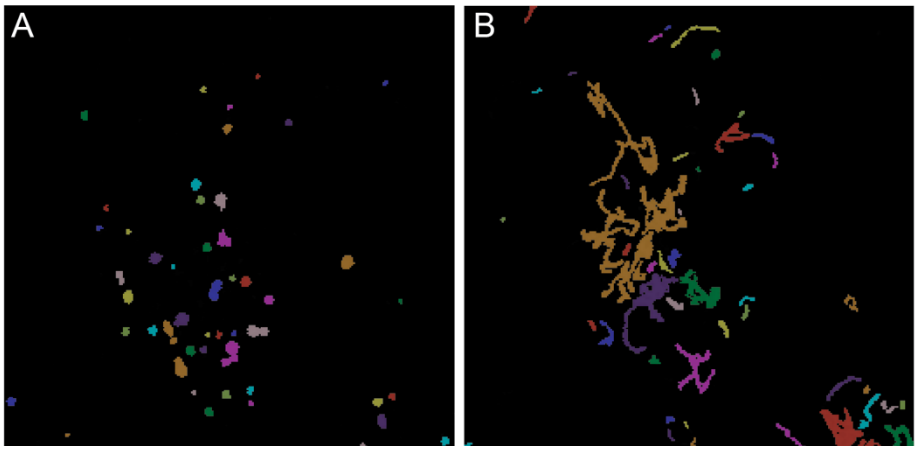

Figure 3. Alternative visualization of organelle detection using object overlays.
(A) Lysosome detection overlay in channel 0 (Frame 0), where each detected lysosome is represented with a unique color-coded mask. (B) Mitochondria detection overlay in channel 1 (Frame 0), with segmented mitochondria shown as color-coded objects.

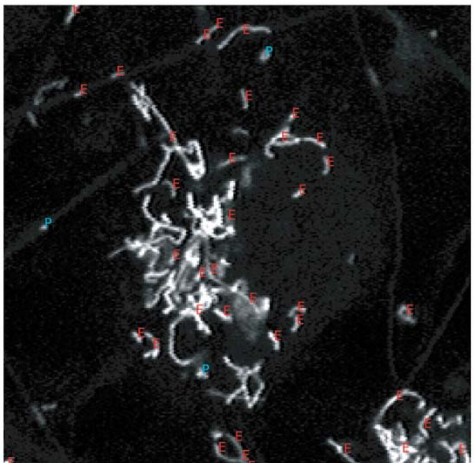

Figure 4. Mitochondrial morphology classification in Frame 0.
Mitochondria are classified as elongated ('E', red) or punctate ('P', cyan) based on area and eccentricity thresholds.

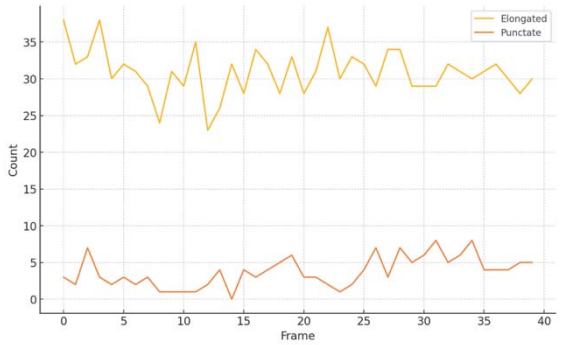

Figure 5. Mitochondrial morphology dynamics across 40 frames.
Plot showing the counts of elongated and punctate mitochondria over time, demonstrating fluctuations in mitochondrial populations.

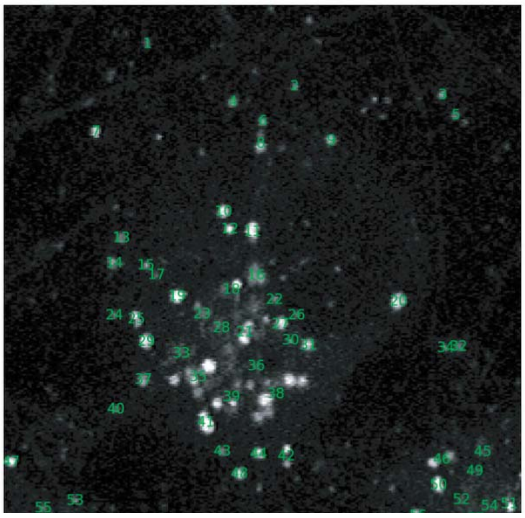

Figure 6. Lysosomal counting in Frame 0.
Each detected lysosome is annotated with a unique identifier in green, facilitating quantification and tracking across frames.

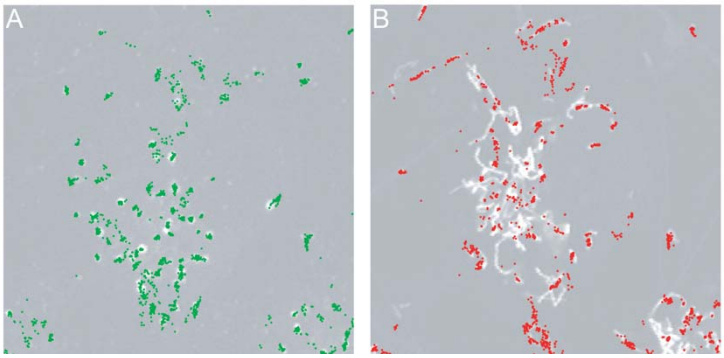

Figure 7. Organelle tracking visualizations (dot traces).
(A) Cumulative mitochondrial tracks over all frames, overlaid on Frame 0, shown in red. (B) Cumulative lysosomal tracks over all frames, overlaid on Frame 0, shown in green.

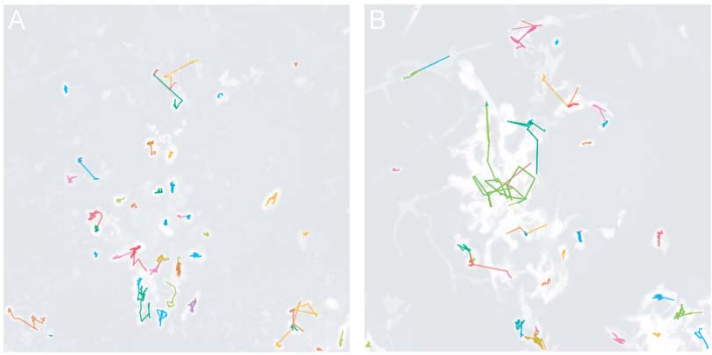

Figure 8. Organelle tracking visualizations (line traces).
(A) Lysosomal trajectories shown as continuous color-coded line traces, illustrating movement paths over time. (B) Mitochondrial trajectories visualized as color-coded line traces, highlighting movement paths and motility complexity.

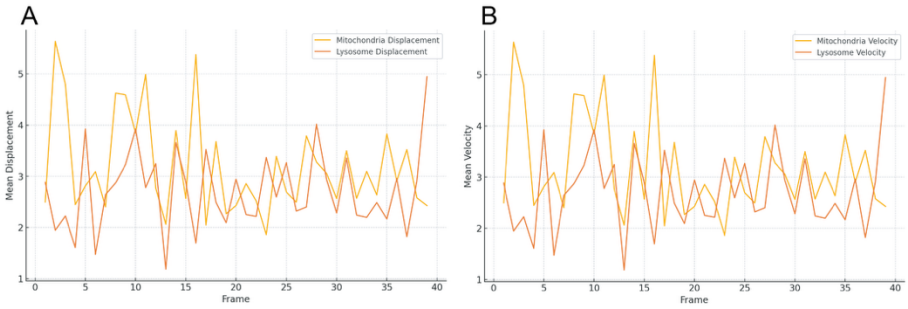

Figure 9. Organelle displacement and velocity across 40 frames.
(A) Plot of mean displacement of mitochondria and lysosomes, demonstrating variability in organelle motility. (B) Plot of mean velocity of mitochondria and lysosomes, providing insights into their relative movement dynamics.

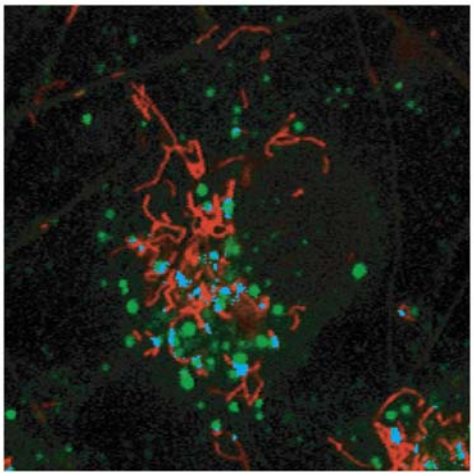

Figure 10. Colocalization overlay of mitochondria and lysosomes in Frame 0.
Mitochondria are shown in red, lysosomes in green, and colocalized regions in cyan, indicating areas of potential interaction.

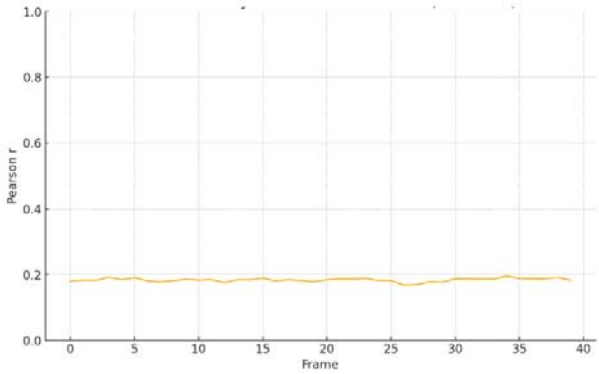

Figure 11. Pearson correlation coefficient of mitochondria-lysosome colocalization across frames.
Graph depicting the Pearson correlation coefficient per frame, indicating a stable but modest degree of colocalization throughout the time-lapse sequence.