

Automated Workflow for Advanced Single Cell and Bacterium Tracking in Host-Pathogen Interactions

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Background

- Time-lapse fluorescence microscopy is a valuable tool for understanding processes of host cell defense against intracellular pathogens like *Mycobacterium tuberculosis* (Mtb)
- Quantification of signals at localized compartments around bacteria and within cells can provide deeper insight into interactions between bacteria and host-cell organelles
- Existing cell tracking tools are effective but constrained to specific images/channels and inadequate for studying intracellular dynamics
- Quantitative analysis at a single-bacterial level remains limited and dependent on manual tracking methods

Objective

Develop an automated workflow for high-throughput tracking and quantification of infected cells at a cellular and bacterial level

Results

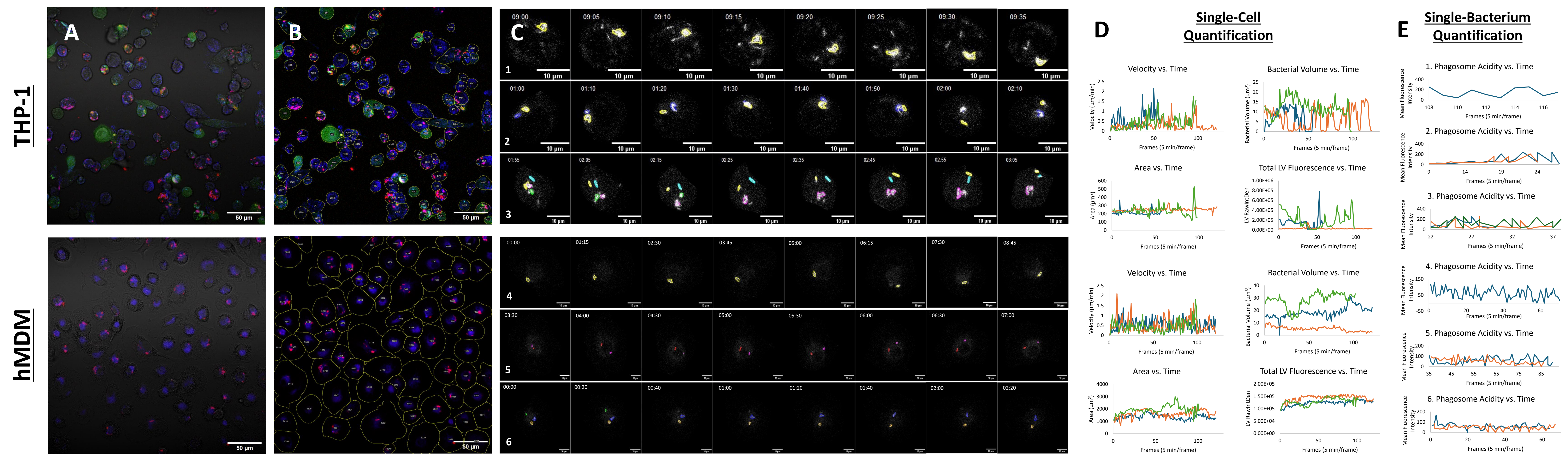
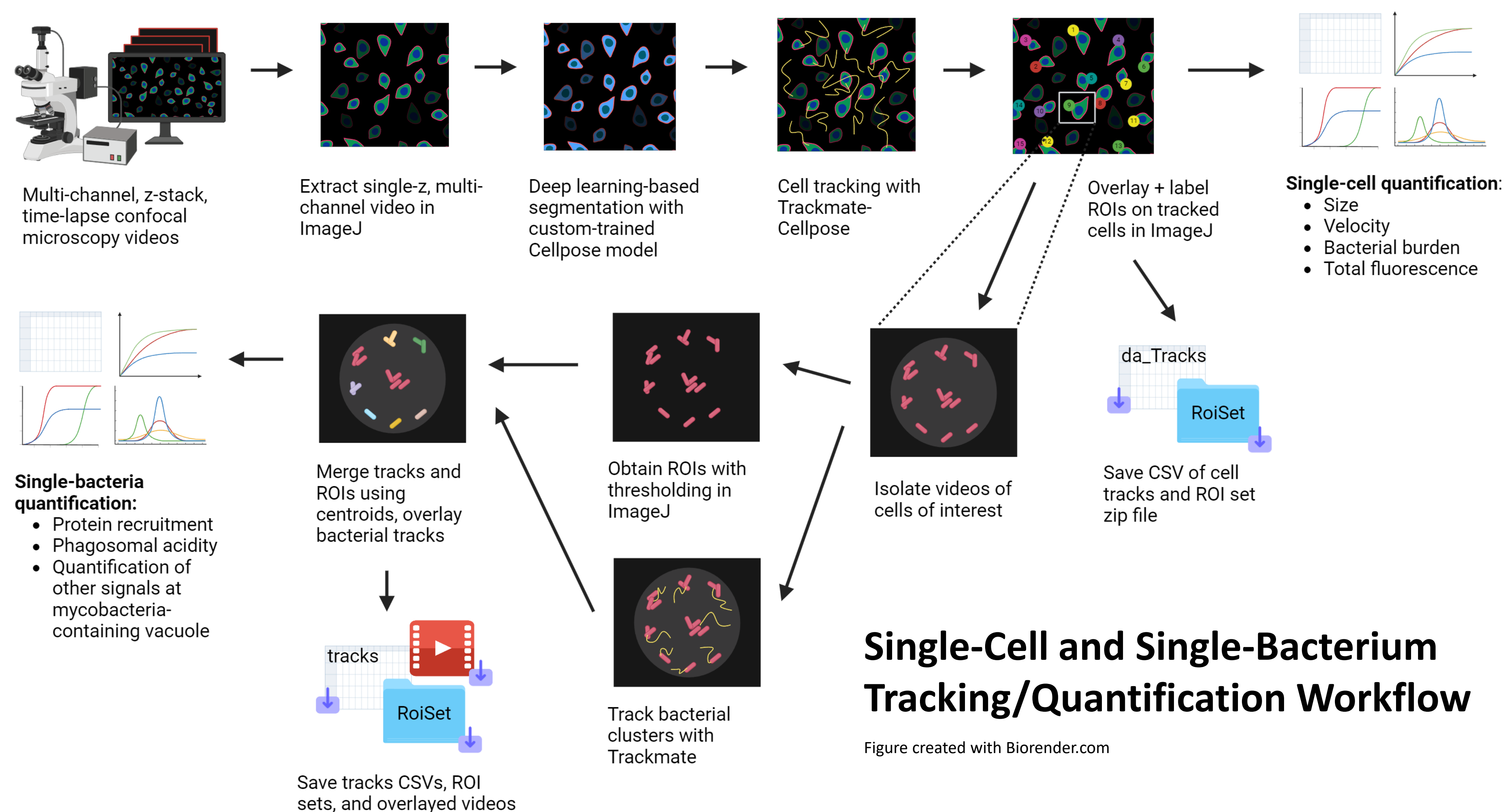


Figure 1. (A) Single slice images from confocal microscopy videos of THP-1 (top) and hMDM (bottom) cells infected with Mtb (red) and stained with Lysoview (LV) (blue). (B) Microscopy images with labeled ROIs (yellow outlines) applied on cells. (C) Snapshots from time-lapse videos (time format HH:MM) of isolated selected single cells with bacterial tracking. Consistent coloring is applied across the ROIs of each bacterium/phagosome's track. (D) Graphs of measurements taken at the cellular level (cell velocity, area, bacterial volume, and total LV fluorescence) for selected cells. Each series represents one cell. (E) Graphs of measurements taken at the bacterial level (phagosomal acidity, measured by mean fluorescence intensity of LV) for selected cells. Each series represents one ROI (phagosome).

Methods



Limitations

- Cellular tracking can be fragmented and inaccurate in videos with densely populated and/or highly mobile cells
- Bacterial tracking is dependent on adequate minimum fluorescence intensity and brightness
- Single-bacterium tracks often become fragmented in cells with high bacterial volume and high mobility
- Utilizing a higher microscopy frame rate or reducing number of slices can improve tracking accuracy and reliability

Resources

Videos: Watch live tracking videos of cells and bacteria

bioRxiv: Read the full work in the paper

Conclusion

- Developed a near-fully automated workflow for unbiased, high-throughput cell segmentation and quantitative tracking of both single cells and single bacterium/phagosomes within multi-channel, z-stack, time-lapse confocal microscopy videos
- Provide versatile toolkit for measuring relevant signal parameters at cellular and bacterial level
- Used PyImageJ library to integrate ImageJ functionality into a Python environment and combine deep learning-based segmentation from Cellpose with tracking algorithms from Trackmate and visualization within ImageJ
- Workflow facilitates exploration of virulence factors of pathogens and development of innovative therapeutic approaches
- Offers customizable methods that allow for advancement in host-pathogen interaction research in a variety of systems