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TrackMate 7: Integrating state-of-the-art segmentation algorithms into tracking pipelines.

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Abstract

TrackMate is an automated tracking software used to analyze bioimages and is distributed as a Fiji plugin. Here we introduce a new version of TrackMate. TrackMate 7 is built to address the broad spectrum of modern challenges researchers face by integrating state-of-the-art segmentation algorithms into tracking pipelines. We illustrate qualitatively and quantitatively that these new capabilities function effectively across a wide range of bio-imaging experiments.

Main text

In biosciences, object tracking is an essential image analysis technique used to quantify dynamic processes. In Life Sciences, tracking is used, for instance, to follow single particles, subcellular organelles, bacteria, cells, and whole animals. While tech developments have drastically improved image acquisition capabilities and allowed increasingly sophisticated experimental setups, they have also led to bottlenecks in downstream image analyses. Due to the sheer diversity of images, no single software can address every Life Science research tracking challenge. This has prompted the development of flexible and extensible software tracking platforms [1–5], including TrackMate, that enable biologists to build automated tracking pipelines tailored to a specific problem.

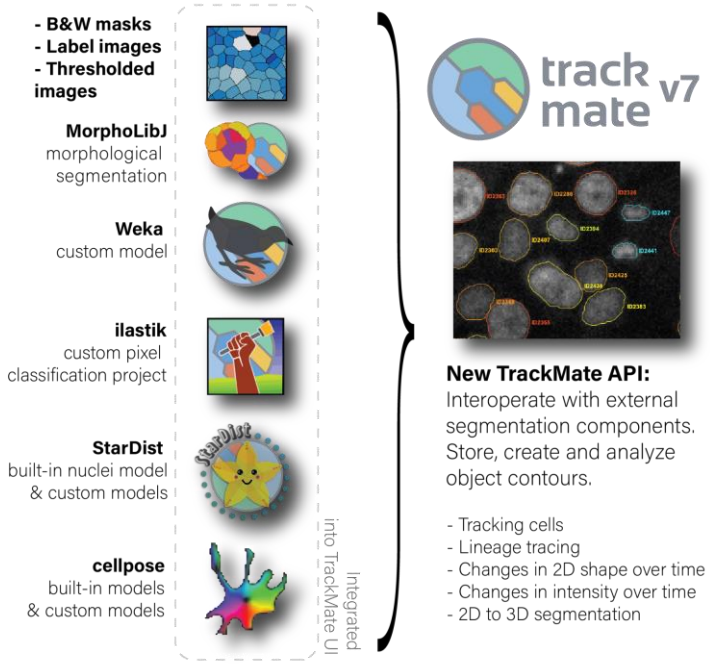

Figure 1: The new capabilities of TrackMate. TrackMate can now create, use, analyze and store object contours segmented from 2D images. These contours enable TrackMate to extract morphological features of the tracked objects over time. We also wrote a new application programming interface (API) to allow the integration of external components in TrackMate. We use this API to incorporate popular segmentation tools including ilastik, the Weka Trainable-Segmentation Fiji plugin, cellpose, StarDist, and the morphological segmentation tool MorphoLibJ within TrackMate. TrackMate can also import segmentation results as masks or label images and use them for tracking, making it tracking compatible with any segmentation algorithm.

Most tracking algorithms proceed in two steps. First, a detection algorithm detects or segments individual objects at each time point. Second, a linking algorithm links the detections to build tracks that follow each object over time. Importantly, the accurate detection of objects is crucial for the tracking process[6]. However, the low signal-to-noise ratio (SNR) typical of live-cell fluorescence microscopy often makes segmentation

challenging. Aberrant object detection then leads to missing links and the generation of tracks that end prematurely, with multiple short tracks representing an individual object over time. Objects at high density can also be challenging to segment due to overlap or close contact. Most detection algorithms will treat tightly packed objects as a single entity, resulting in breaks in tracks or single tracks linking groups of objects. Several linking strategies can partly rescue these issues, but overall all tracking algorithms tested in [6] displayed a decreasing performance with increasing object density and decreasing SNR. Modern segmentation algorithms, in particular, those based on machine learning (ML) and deep learning (DL) approaches, can address these challenges as they excel at image segmentation tasks in low SNR and high-density images[7] .

TrackMate[4], developed by us, is a user-friendly Fiji[8] plugin for tracking objects in fluorescence microscopy images. TrackMate offers automated and semi-automated tracking algorithms, together with advanced visualization and analysis tools. TrackMate is interactive and enables users to filter and curate tracking results based on defined parameters. As such, it can accommodate a wide range of tracking challenges. However, until now, TrackMate detectors were solely based on the Laplacian of Gaussian (LoG) filter. The LoG filter is efficient against sub-resolved particles[9] or other blob-like objects but performs poorly for textured objects, objects with complex shapes, and other imaging modalities than fluorescence. These detectors are also limited to measuring the object's position and not their shape.

Here we introduce a new version of TrackMate (TrackMate 7) rewritten to improve performance, usability, and versatility, all of which present several advantages over other available tracking tools (Table S1). In particular, we developed a new API that enables developers to integrate segmentation tools as TrackMate detectors. As examples, we provide detectors based on ilastik[10], Weka[11], cellpose[12], MorphoLibJ[13], and StarDist[14]. While the training of custom ML and DL models must be performed with external tools (using *e.g.* ilastik or the ZeroCostDL4Mic platform for StarDist and cellpose), popular built-in models are now fully integrated into TrackMate with a user-friendly interface and scripting capabilities. TrackMate can also import segmentation results as mask or label images for tracking, making it possible to perform tracking with any segmentation algorithm. Importantly, as TrackMate now detects object contours in every frame, we reconfigured the TrackMate data model to store, display and analyze 2D morphological features of the tracked objects over time (Figure 1). The new detectors work for 2D and 3D images when possible, but the analysis of object contours is currently limited to 2D images.

These new features widely increase the breadth of TrackMate applications and capabilities (Figure 2, Movie 1-11, Supplementary Figure 1-4, Supplementary manual, and tutorials) and its tracking performance (Table S2). For instance, the StarDist integration offers efficient and versatile nuclei detection in fluorescence images via the built-in model (from image set BBBC038v1 in[15]). Our integration also provides an interface to use custom StarDist models. To illustrate this, we used custom StarDist models trained with the ZeroCostDL4Mic platform[16] to track fluorescently labeled nuclei of collectively migrating breast cancer cells, or rapidly migrating T cells from brightfield images (Figure 2a-b and Movie 1-2). Before this integration, fully automated tracking of label-free cells was not possible in TrackMate.

As TrackMate supports multi-dimensional images, users can now track objects using one channel, while measuring the changing intensities of the tracked objects in separate channels over time. As an example, we tracked the nuclei of breast cancer cells expressing a kinase translocation reporter, following changes in ERK activity in single cells as they migrated (Figure 2c, Supplementary Figure 1, and Movie 3).

To further showcase the versatility of TrackMate, we used a Weka model (trained using the Weka Fiji plugin) together with the new overlap tracker (linking algorithm based on object overlap between consecutive frames) to follow focal adhesions in endothelial cells (Supplementary Figure 2 and Movie 4). We also used

an ilastik pixel classifier (trained using ilastik) to follow *Neisseria meningitidis* growth and correlate lineage information to single bacteria morphological measurements (Figure 2d and Movie 5). To showcase that TrackMate can now import segmentation results directly, then follow the imported objects, we tracked migrating cancer cells (fluorescent images and brightfield images), and hematopoietic stem cells ([17], brightfield images) previously segmented using cellpose[12] (Figure 2e, Supplementary Figure 3 and Movie 6-8).

TrackMate's new detectors can also be used to perform 3D segmentation. Indeed, by swapping the Z dimension of the source image with time, TrackMate can link 2D segmentation results across Z planes and generate a 3D segmentation result. This new feature makes the segmentation of 3D objects accessible, flexible, and possible without programming knowledge (Figure 2f, Supplementary Figure 4, and Movie 9-11).

TrackMate v7 currently offers a choice of 10 segmentation detectors (plus the integration of custom models for some of them) and five particle-linking algorithms for tracking the detected objects. To facilitate choosing an optimal combination for a specific dataset, we developed an additional module, the TrackMate helper (Supplementary manual and Supplementary Figure 5). This module is a user-friendly application that performs parameter sweeps over any combination of detectors and particle-linking algorithms. Using the ground-truth provided by the user, TrackMate helper computes the Cell-Tracking-Challenge (CTC) metrics[19] for each parameter combination and reports the optimal one for each of the CTC metrics (Table S2). In a nutshell, TrackMate helper allows the optimization of the tracking parameters for a whole dataset systematically.

Altogether, TrackMate now enables powerful segmentation approaches for tracking purposes directly in Fiji within a user interface already familiar to many. We envision that by enabling scientists to resolve complex tracking problems more efficiently, this new version of TrackMate will accelerate discoveries in Life Sciences. We expect that TrackMate will continue to evolve in the years to come. In particular, as core libraries handling 3D objects are further developed in Fiji, the analysis of 3D object contours and shapes during tracking could become an invaluable addition. TrackMate was also built as a software platform to be extended by others independently and is documented as such. As DL-based segmentation and tracking algorithms are being developed, we hope contributors will consider TrackMate as a platform to accelerate the dissemination of their work towards researchers in the Life Sciences and beyond[18,19].

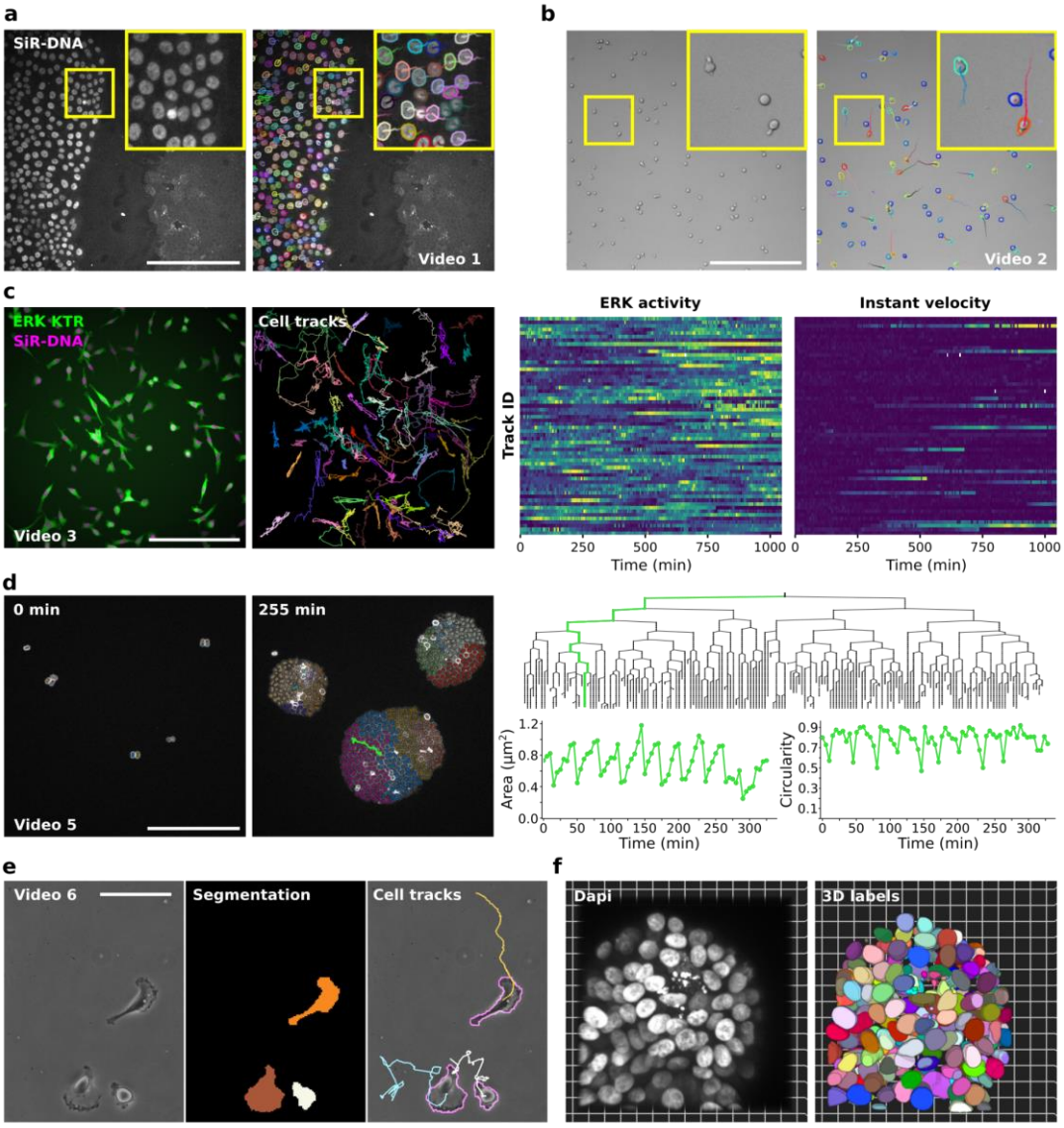

Figure 2: TrackMate can be used to track objects from a wide variety of bio-imaging experiments.
a. Migration of MCF10DCIS.com cells, labeled with SiR-DNA, recorded using a spinning disk confocal microscope and automatically tracked using a custom StarDist model loaded in TrackMate (see also Movie 1). Detected cells and their local tracks (colors indicate track ID) are displayed. Scale bar = 250 µm.
b. The migration of activated T cells plated on ICAM-1 was recorded using a brightfield microscope and automatically tracked using a custom StarDist model loaded in TrackMate (see also Movie 2). Detected cells (colors indicate the mean track speed; blue slow-moving cells, red fast-moving cells) and their local tracks (colors indicate track ID) are displayed. Scale bar = 250 µm.
c. MDA-MB-231 cells stably expressing an ERK activity reporter (ERK-KTR-Clover) and labeled using SiR-DNA were recorded live using a wide-field fluorescence microscope over 17 hours. Cell nuclei were automatically tracked over time using a StarDist model available in TrackMate (see Movie 3). For each tracked cell, the average intensity of the ERK reporter was measured in their nucleus over time (directly in TrackMate). Changes in ERK activity and in instant velocity are displayed as heatmaps (blue high, yellow low).
d. The growth of *Neisseria meningitidis* expressing PilQ-mCherry was recorded using a spinning-disk confocal microscope. An ilastik pixel classifier, trained to segment individual bacteria, was loaded into TrackMate to follow bacteria growth. Representative fields of view and the lineage tree of the bacteria highlighted in green are displayed (see Movie 5). Changes in area and circularity of a bacterium over the tracking period are also highlighted (green track).

Cell division events translate in sharp decreases in area, followed by a quasi-linear increase. The circularity roughly plateaus during cell growth then decreases before cell division. Scale bar = 25 µm.
e. Glioblastoma cells migrating on a polyacrylamide gel were automatically segmented using a custom cellpose model trained in the ZeroCostDL4Mic platform. The resulting label images were automatically tracked using TrackMate (see Supplementary Figure 1 and Movie 6). Example raw and label images, as well as cell tracks, are displayed.
f. MCF10DCIS.com 3D spheroids were stained for Dapi and imaged using a spinning disk confocal microscope. Across the Z volume, nuclei were detected at each Z plane using StarDist and tracked (all performed in TrackMate). Tracked nuclei were then exported as a label image to create 3D labels (see Movie 9).

Data availability statement

The version of TrackMate described here is available in the Fiji software[8] by simply updating it. TrackMate is documented on the ImageJ wiki: https://imagej.net/plugins/trackmate/ and the documentation for the new features can be accessed from https://imagej.net/plugins/trackmate/trackmate-v7-detectors. We also provide 14 test datasets that are made available via a dedicated Zenodo collection (https://zenodo.org/communities/trackmate/).

Author contributions

GJ and JYT conceived the project; JYT wrote source code; GJ, JWP, NHR, and LLB performed the image acquisition of the test and example data; GJ, JWP, RFL, JYT, MSP, DE, and SUR tested the code; JRWC, DB, GD and ACO provided critical reagents; GJ, JWP, JYT, MSP, DE, SUR, and JYT wrote the documentation and tutorials.; GJ and JYT wrote the manuscript with input from all co-authors.

Declaration of Interests

The authors declare no competing interests.

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