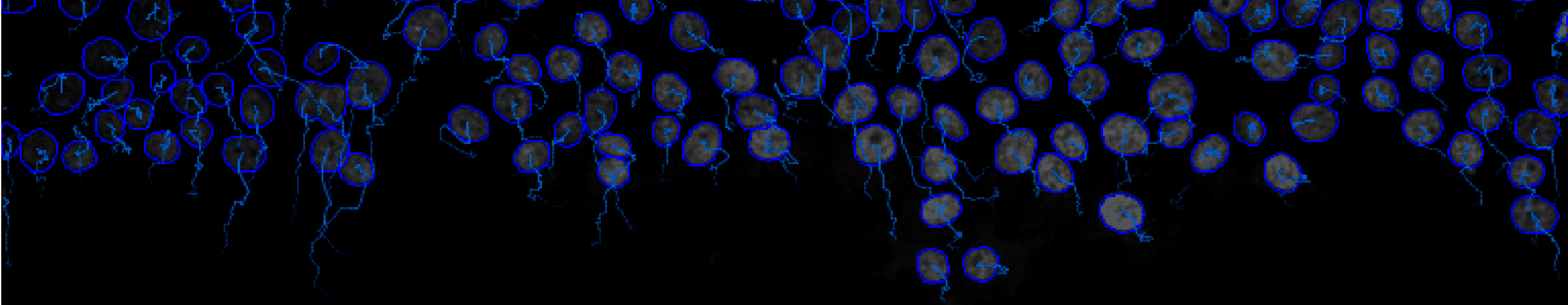


ImageJ 顯微影像分析與程式設計
物件追蹤分析

Ji-ying Huang(黃紀穎)

Understanding Tracking

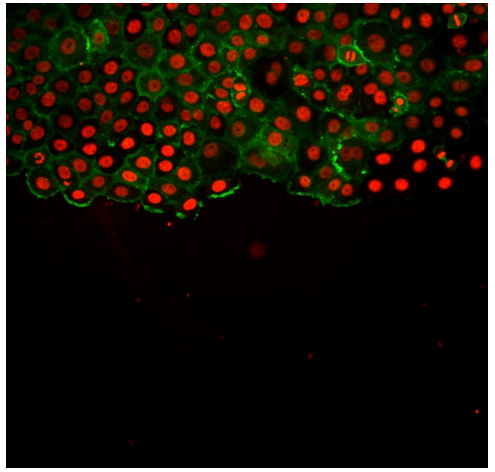
Tracking: ”following moving objects over time”

This process can be computationally intensive, especially for large datasets or complex object behaviors.

Source: Konrad Schindler, Computer Vision Lab, ETH Zuerich
http://www.vision.ee.ethz.ch/datasets/

Applications in Cell Biology

Studying cell migration, cell division, protein dynamics, and signal transduction



Cell tracking and analysis

Goals

- Learn the fundamental concepts and principles of cell tracking
- Become familiar with using FIJI/ImageJ for cell tracking plugin Trackmate
- Data processing and interpretation after cell tracking analysis

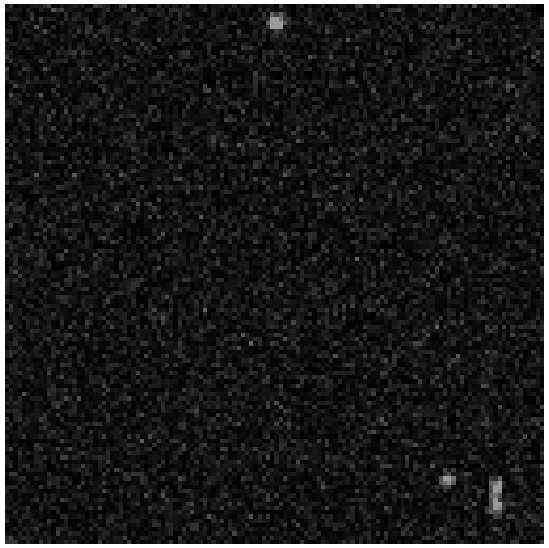
Outline

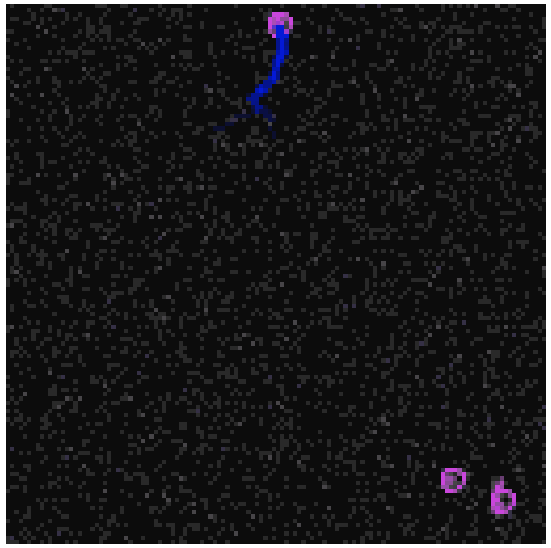
- Part1:Introduction Tracking and tools
  - What is tracking
  - Cell Tracking in Microscopy Images using FIJI/ImageJ
  - Introduction Trackmate
  - Large-scale image tacking
  - Non-particle image tracking

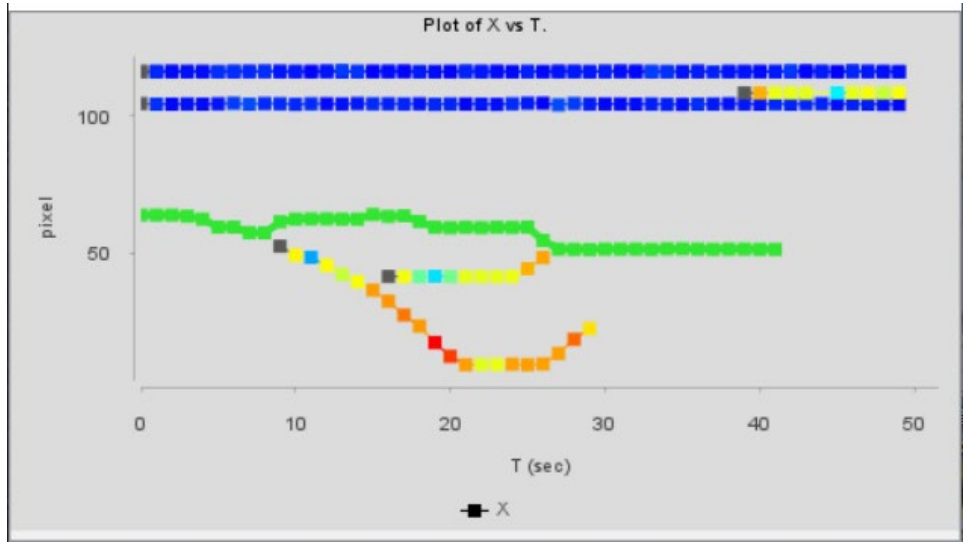
- Part2: Hand-On Tracking with Trackmate
  - Example1: DoG detector
  - Example2: Z-stack time-lapse image
  - Example3: StarDist detector

Cell tracking and analysis

From image to data







Export to CSV

Spots | Edges | Tracks

Label	Spot ID	Track ID	Quality (quality)	X (pixel)	Y (pixel)	Z (pixel)	T (sec)	Frame	R (pixel)	Visibility
ID2560	2560	0	24.06	63.964	6.043	0	1	1	2.5	1
ID2624	2624	0	25.864	51.564	86.598	0	27	27	2.5	1
ID2691	2691	0	24.754	51.448	117.476	0	40	40	2.5	1
ID2564	2564	0	25.713	59.559	19.488	0	6	6	2.5	1
ID2693	2693	0	26.76	51.486	108.431	0	37	37	2.5	1
ID2630	2630	0	24.442	54.763	82.574	0	26	26	2.5	1

Reference

Part of this document is adapted from the following material under CC BY 4.0

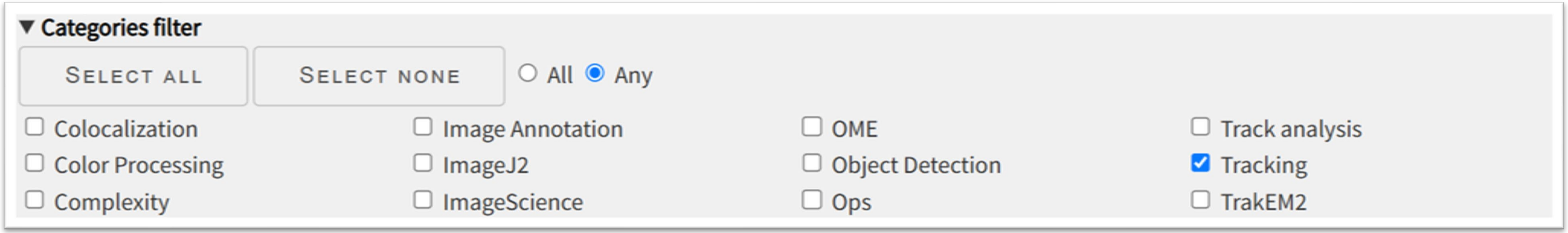
Dr. Robert Haase
“Physic of Life” (PoL), TU Dresden, Germany

https://f1000research.com/slides/11-744
https://www.youtube.com/@haesleinhuepf/videos

Dr. Jean-Yves Tinevez
Image Analysis Hub, Institut Pasteur, Paris

https://github.com/trackmate-sc/TrackMate

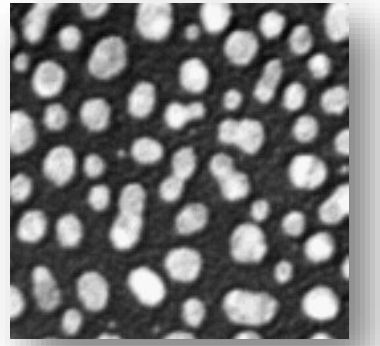
Cell Tracking in Microscopy Images

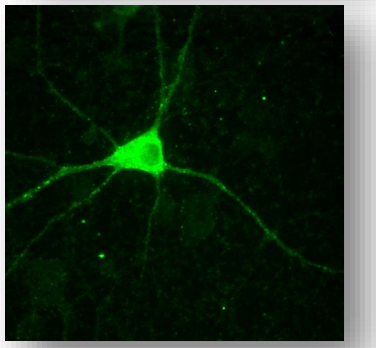


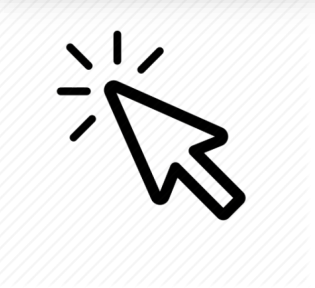
- Tissue Branch Tracker — Tracking, Tissue, Skeleton
- CiliaQ — Automation, 3D, Analysis, Particle Analysis, Segmentation, Object Detection, Colocalizatic Tracking, Cilia
- FilamentDetector — Object Detection, Tracking
- Low Light Tracking Tool — Tracking
- Getting started with MaMuT — Tracking, Tutorials
- MaMuT — Tracking
- Manual Tracking — Particle Analysis, Tracking
- Mastodon — Segmentation, Tracking
- MotiQ — Automation, Analysis, Particle Analysis, Segmentation, Object Detection, Neuron, Tracking, Skeleton
- MTrack — Tracking, Microtubules
- MTrack2 — Tracking
- MTrackJ — Tracking, ImageScience
- Particle Tracker — Particle Analysis, Tracking
- PhotoBend — Tracking
- PillarTracker — Tracking, Visualization, Analysis, Filtering
- Public data sets — Example Data, Segmentation, Tracking
- SpotTracker — Particle Analysis, Tracking
- Temporal Median — Tracking, Filtering
- ToAST — Tracking
- Close gaps in tracks by introduci — Segmentation, Tracking, Benchmark
- TrackMate Actions — Segmentation, Tracking
- Export tracking results in the cell — Segmentation, Tracking, Benchmark
- TrackMate-ExTrack — Tracking
- TrackMate DoG detector — Segmentation, Tracking
- TrackMate-Cellpose-Advanced — Segmentation, Tracking, Machine Learning
- TrackMate-Cellpose — Segmentation, Tracking, Machine Learning
- TrackMate-Ilastik — Segmentation, Tracking, Machine Learning
- TrackMate-Label-Image-Detector — Segmentation, Tracking
- TrackMate-Lacss — Segmentation, Tracking, Deep Learning, Ma Learning
- TrackMate Mask detector — Segmentation, Tracking
- TrackMate-MorphoLibJ — Segmentation, Tracking
- TrackMate-Omnipose-Advanced — Segmentation, Tracking, Machine Learning
- TrackMate-Omnipose — Segmentation, Tracking, Machine Learning
- TrackMate-StarDist — Segmentation, Tracking, Machine Learning
- TrackMate-Thresholding-Detecto — Segmentation, Tracking
- TrackMate v7 detectors — Tracking, Segmentation
- TrackMate-Weka — Segmentation, Tracking, Machine Learning
- Extending TrackMate — Segmentation, Tracking
- TrackMate-Helper — Segmentation, Tracking, Benchmark
- TrackMate Kymograph — Tracking, Kymograph
- TrackMate-Oneat — Tracking
- TrackMate — Segmentation, Tracking
- TrackMate FAQ — Tracking, Segmentation
- Scripting TrackMate — Tracking, Segmentation
- Setting keys for TrackMate detect — Tracking, Segmentation, Scripting
- Using TrackMate from MATLAB — Segmentation, Tracking
- TrackMate Accuracy — Segmentation, Tracking
- TrackMate Trackastra — Tracking, Machine Learning
- Manual editing of tracks using Tr — Segmentation, Tracking
- Manual tracking with TrackMate — Segmentation, Tracking
- Shortcuts for editing and navigat — Segmentation, Tracking
- TrackMate display settings — Tracking, Segmentation
- TrackScheme — Tracking
- Large-scale cell tracking under se — Tracking

Cell Tracking in Microscopy Images

- Particle tracking
  - Trackmate
  - Mastodon/ELEPLANT (BigDATA)
- Non-particle tracking
  - Neuron/Microtubule Tracking
  - Kymograph
- Manual tracking
  - MTrackJ
  - Manual tracking







Manual tracking

- Manually labeling target objects in an image sequence frame by frame to track their movement trajectories.
- It is a traditional and direct tracking method suitable for complex or challenging scenarios where automated tracking is difficult.
- High accuracy, High flexibility, but time-consuming and labor-intensive
- Not suitable for large-scale data sets

Trackmate: Automated Tracking for Microscopy images

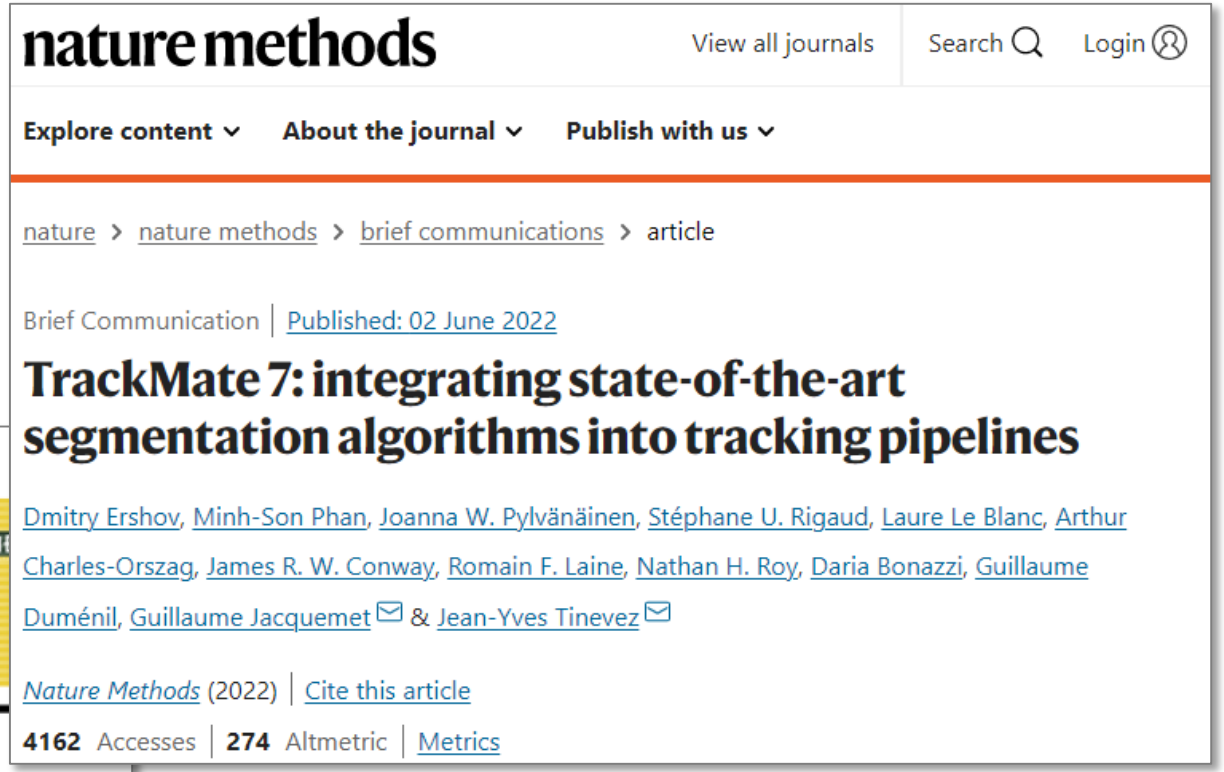
An open-source plugin for Fiji/ImageJ enabling robust tracking of particles and objects within 2D and 3D microscopy datasets.

Key Features Include:

- Simplified workflow through an interactive wizard
- Versatile: Compatible with diverse biological data, including single particles, cells, and organelles
- Multiple tracking algorithms: LAP (Linear Assignment Problem), Kalman filter, etc.
- Provides customizable analysis pipelines
- Visualization and export options for track analysis
- Extensive documentation and active community support

Citing TrackMate papers

- Please note that TrackMate is available through Fiji, and is based on several publications. If you use it successfully for your research please be so kind as to cite our work:



nature methods

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nature › nature methods › brief communications › article

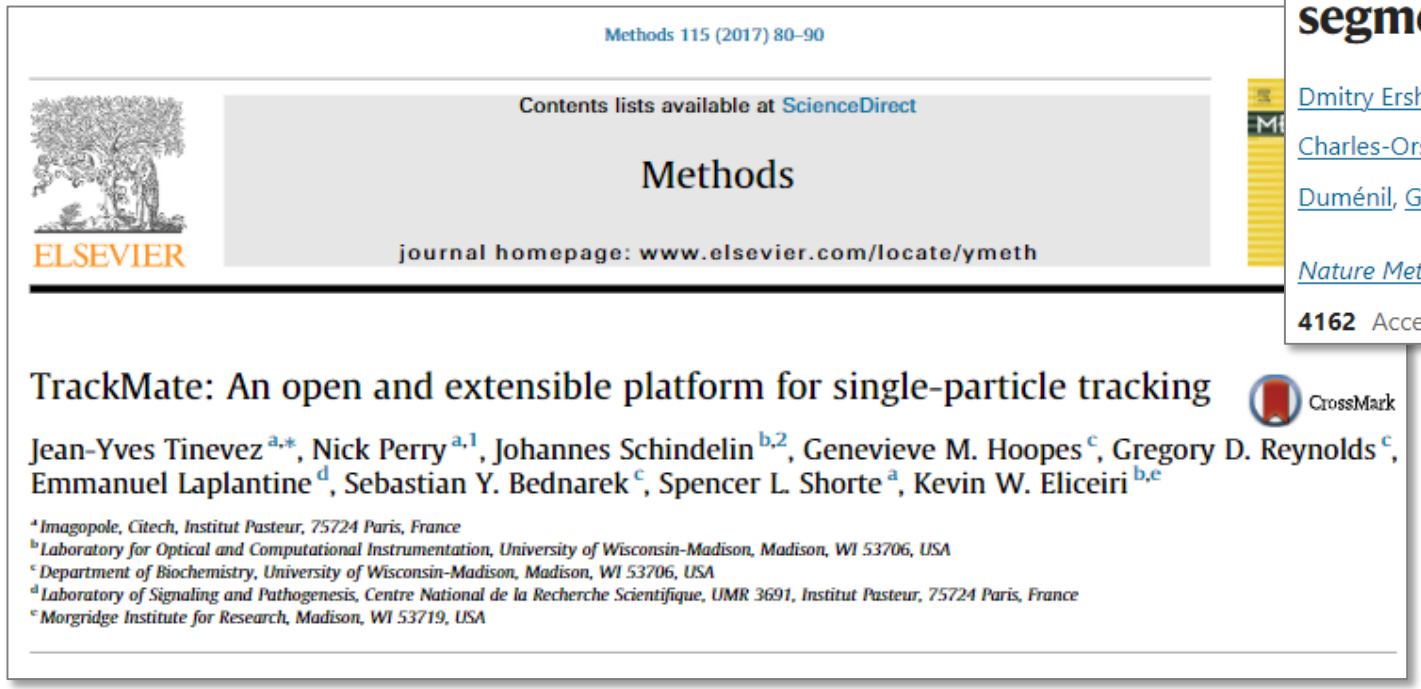
Brief Communication | Published: 02 June 2022

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

Dmitry Ershov, Minh-Son Phan, Joanna W. Pylvänäinen, Stéphane U. Rigaud, Laure Le Blanc, Arthur Charles-Orszag, James R. W. Conway, Romain F. Laine, Nathan H. Roy, Daria Bonazzi, Guillaume Duménil, Guillaume Jacquemet & Jean-Yves Tinevez

Nature Methods (2022) | Cite this article

4162 Accesses | **274** Altmetric | Metrics



Methods 115 (2017) 80–90

Contents lists available at ScienceDirect

Methods

journal homepage: www.elsevier.com/locate/ymeth

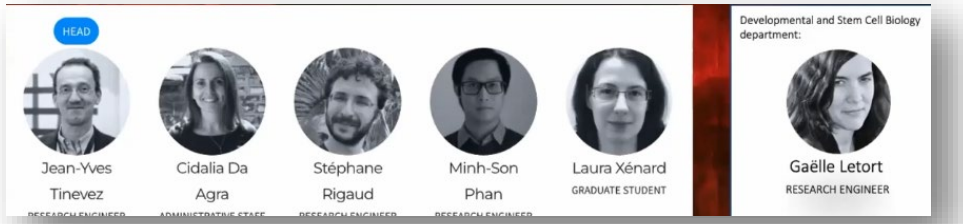
ELSEVIER

TrackMate: An open and extensible platform for single-particle tracking

CrossMark

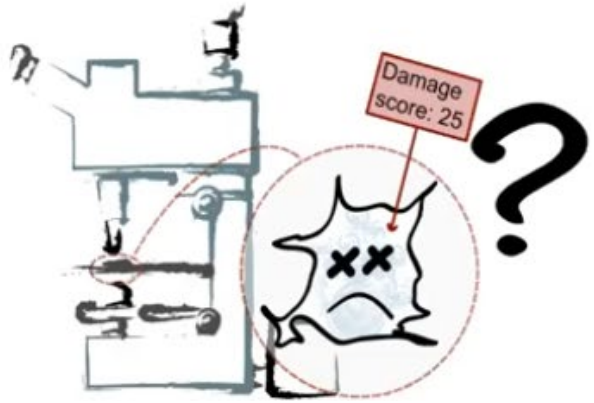
Jean-Yves Tinevez [a,*], Nick Perry [a,1], Johannes Schindelin [b,2], Genevieve M. Hoopes [c], Gregory D. Reynolds [c], Emmanuel Laplantine [d], Sebastian Y. Bednarek [c], Spencer L. Shorte [a], Kevin W. Eliceiri [b,e]

[a] Imagopole, Citech, Institut Pasteur, 75724 Paris, France
[b] Laboratory for Optical and Computational Instrumentation, University of Wisconsin-Madison, Madison, WI 53706, USA
[c] Department of Biochemistry, University of Wisconsin-Madison, Madison, WI 53706, USA
[d] Laboratory of Signaling and Pathogenesis, Centre National de la Recherche Scientifique, UMR 3691, Institut Pasteur, 75724 Paris, France
[e] Morgridge Institute for Research, Madison, WI 53719, USA

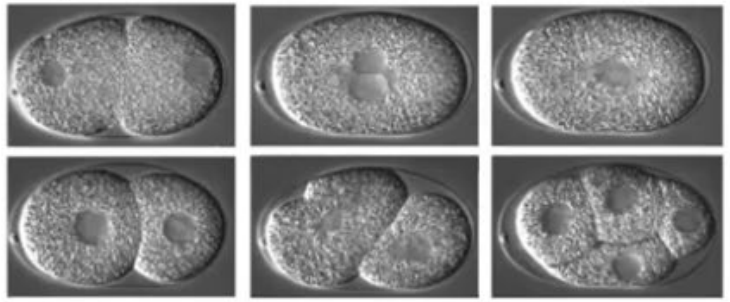


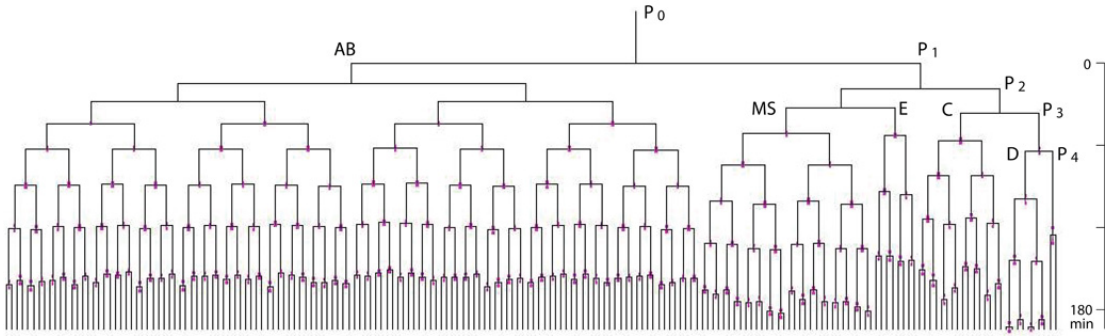
Developing TrackMate

Quantifying phototoxicity.



Using a live sample to measure something about a microscope (instead of the converse).
We picked: the *C.elegans* embryo.



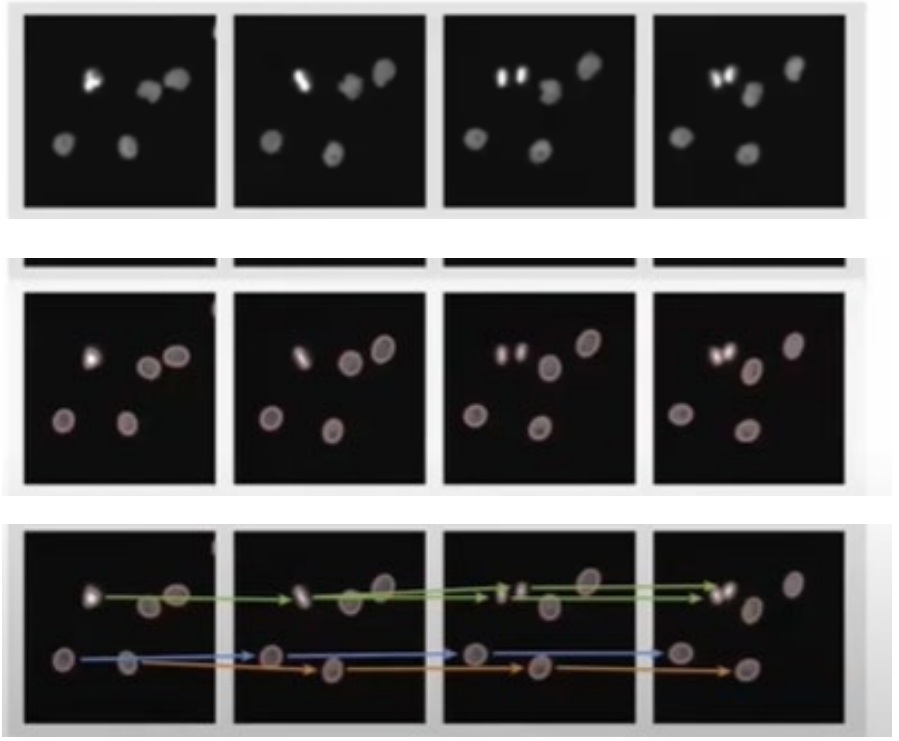


The lineage with vertical lines proportional to the average cell cycle length and the magenta bars to the standard deviation

Control of Cell Cycle Timing during C. elegans Embryogenesis Dev Biol. 2008 Mar 13;318(1):65–72. doi: 10.1016/j.ydbio.2008.02.054

Trackmate analysis pipeline

- Image enhancement
  - Filtering
  - Registration
- Cell detection and segmentation
  - Spot tracking
  - Object tracking
- Tracking Data Linking

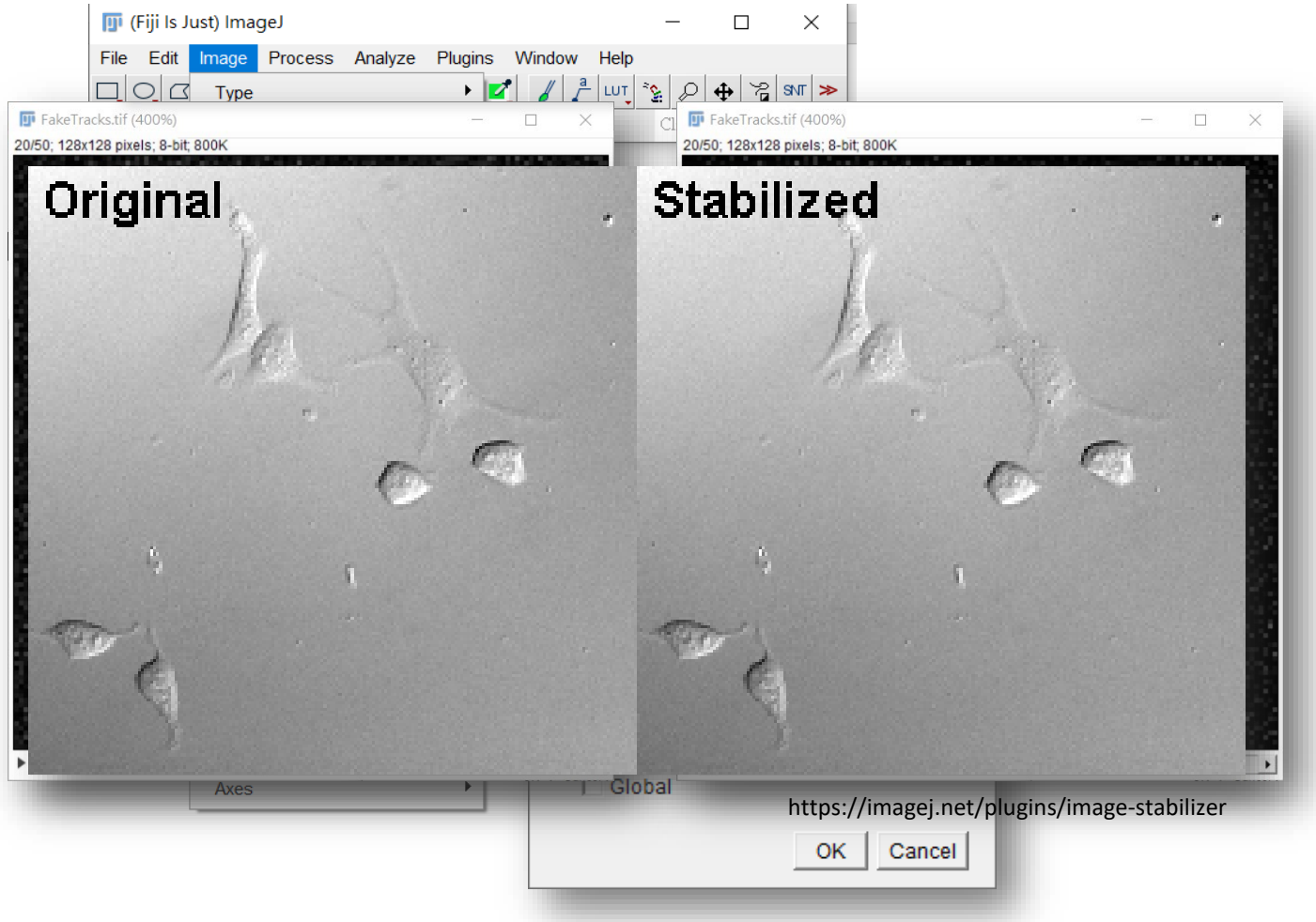


- Statistical Analysis and Visualization of Tracking Data

https://www.youtube.com/watch?v=fIE4i3G7L9Y&list=PLOwA4gPJOuz6ufHx2THW-WO7lKvUX3u-_&index=5

Image processing

- Properties
- Filtering
  - Subtract background
- Registration



Detection and Segmentation

Many cell tracking related questions can be answered without segmentation…

Objective segmentation

Semantic segmentation

Cat

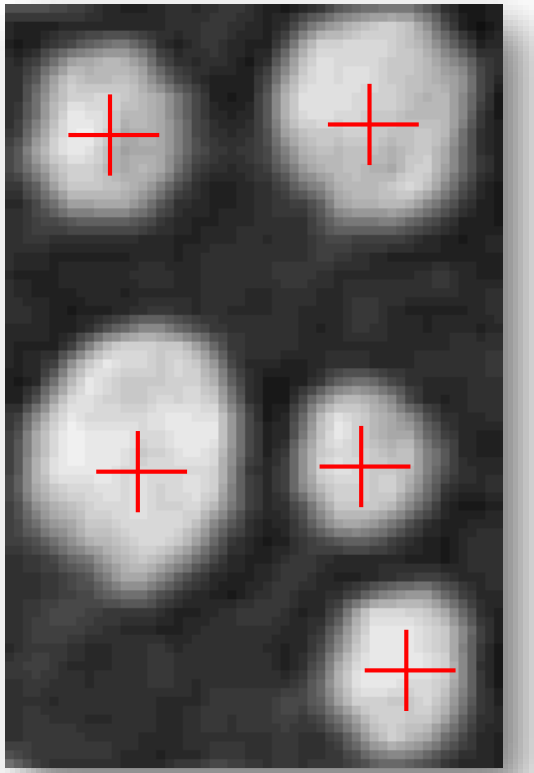
Cat

Cat

Cell identification and segmentation

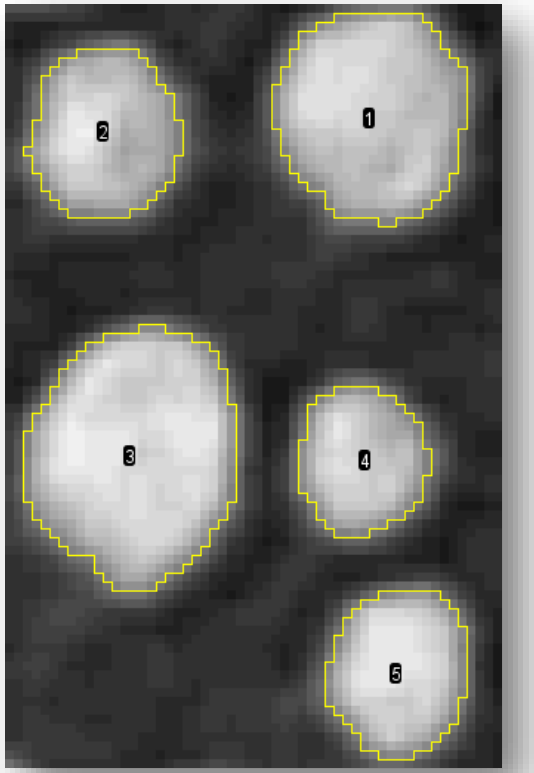
Spot tracking

Tracking the centroid of each particle’s signal



Object tracking

Tracking the entrie shape and size of the object

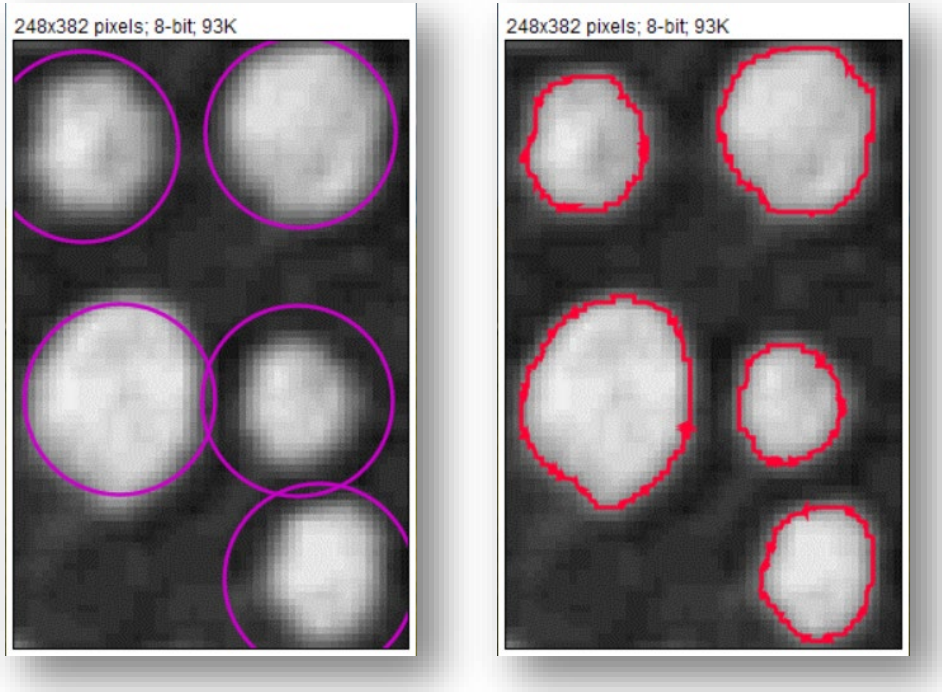


Trackmate Detectors

- Spot detectors.
  - LoG, DoG detectors
  - They can detect objects, their X, Y, Z position and a radius.
  - These spots have **NO** shape information.

- Intensity-base detectors
  - Threshold, Masks or label images
  - Get morphological descriptors and accurate intensity measurements in objects

- ML & DL Detectors
  - AI-based segmentation
  - Default & Pre-train model

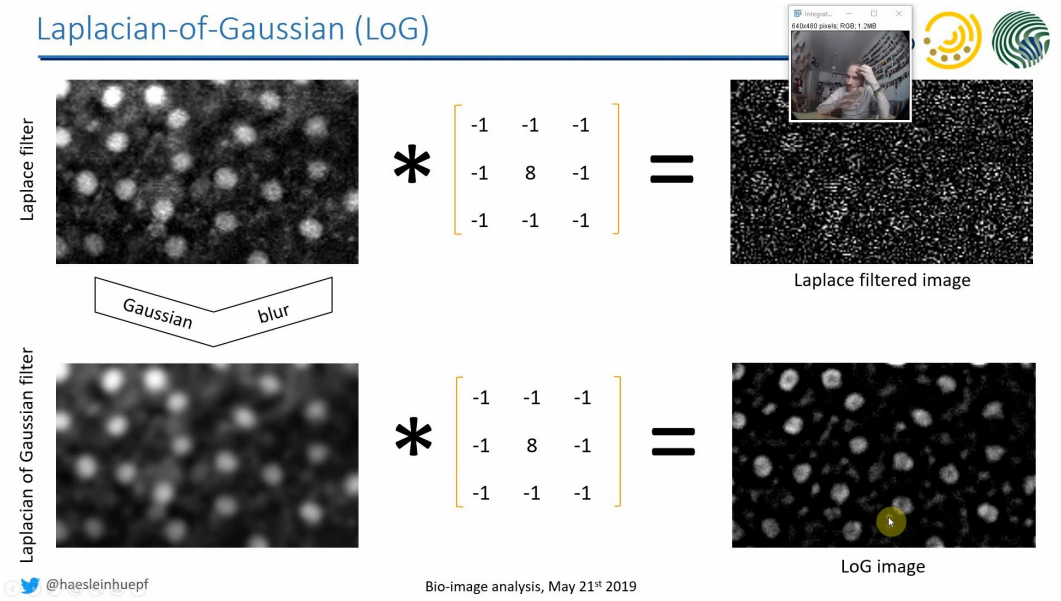


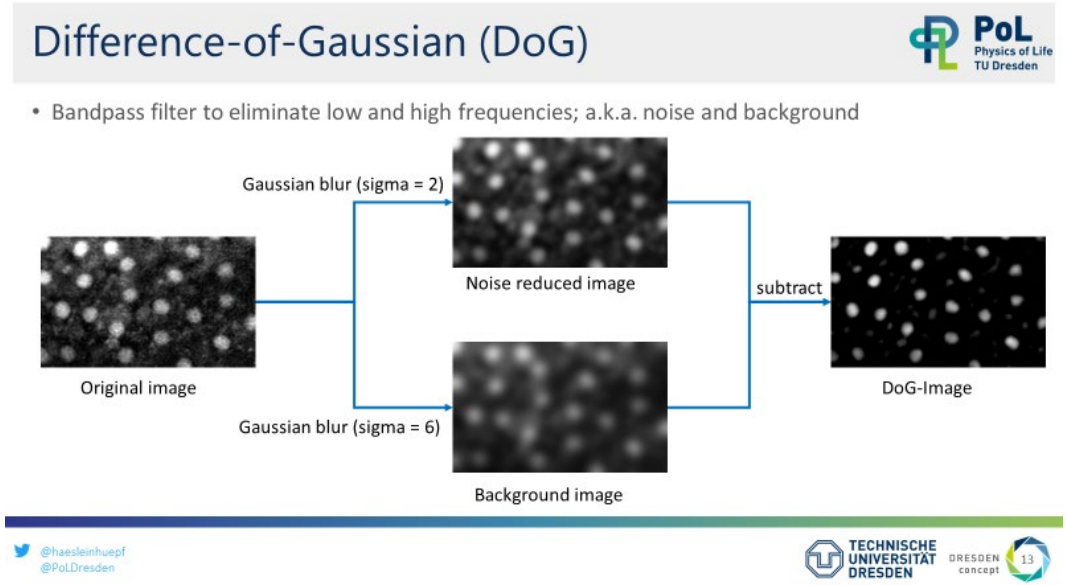
Spot Detectors

- The LoG detector
  - Laplacian of Gaussian segmentation on the image.
  - Optimal for intermediate spot sizes, between ≈5 and ≈20 pixels in diameter.

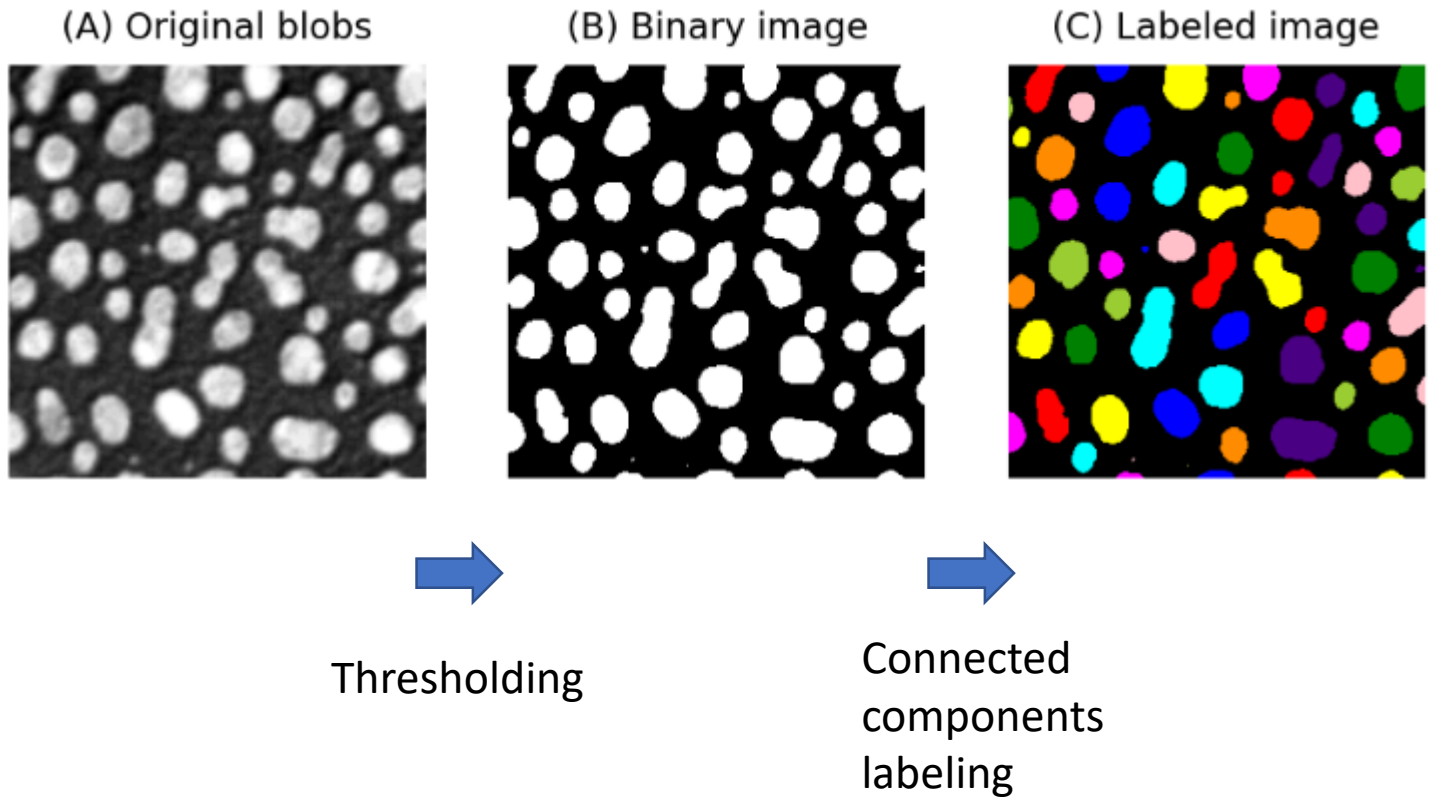
- The DoG detector
  - Difference of Gaussians approach to approximate a LoG filter by the difference of 2 Gaussians.
  - Optimal for small spot sizes, below ≈5 pixels.

- The Downsample LoG detector
  - LoG detector, but downsizes the image by an integer factor before filtering.
  - Optimal for large spot sizes, above ≈20 pixels in diameter, at the cost of localization precision.



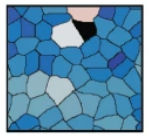


Intensity-base detectors



AI-base Detectors

- B&W masks
- Label images
- Thresholded images



MorphoLibJ morphological segmentation

Weka custom model

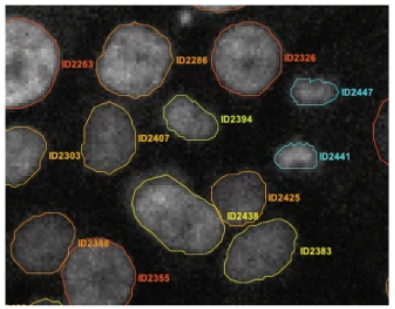
Ilastik custom pixel classification project

StarDist built-in nuclei model and custom models

Cellpose built-in models and custom models

Integrated into TrackMate UI

trackmate v7



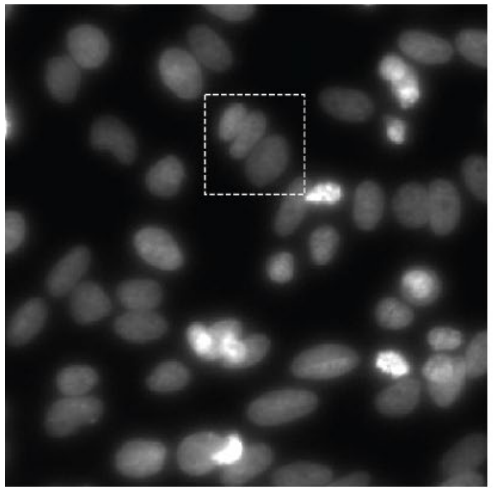
New TrackMate API:
Interoperate with external segmentation components.
Store, create and analyze object contours.

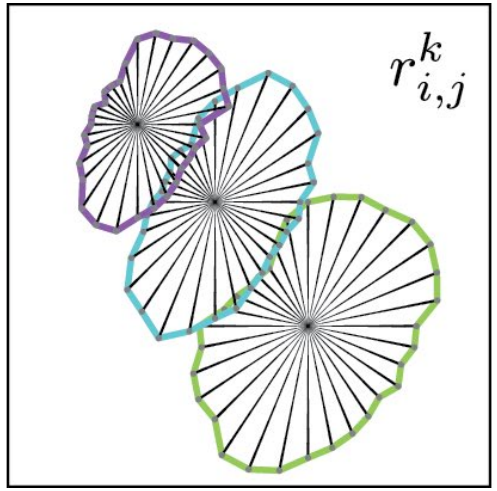
- Tracking cells
- Lineage tracing
- Changes in 2D shape over time
- Changes in intensity over time
- 2D to 3D segmentation

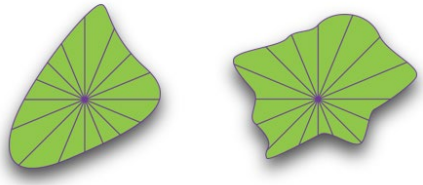
https://imagej.net/plugins/trackmate/

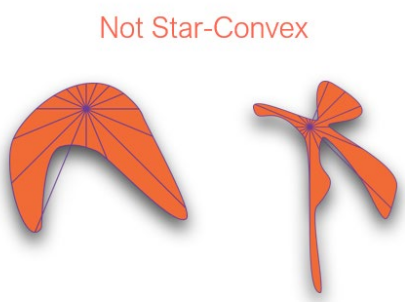
StarDist

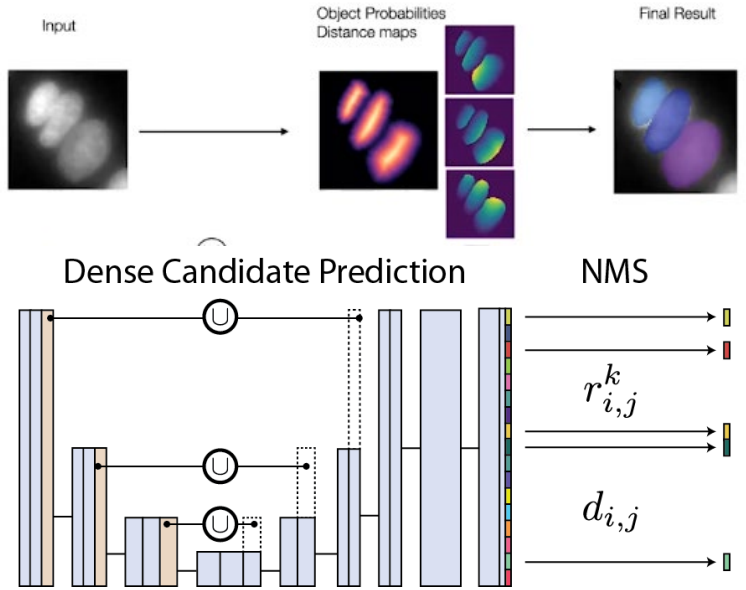
- Object Detection with Star-convex Shapes
- Utilizes deep learning algorithms for object detection segmentation in images
- It can effectively process images with overlapping or clustered cells







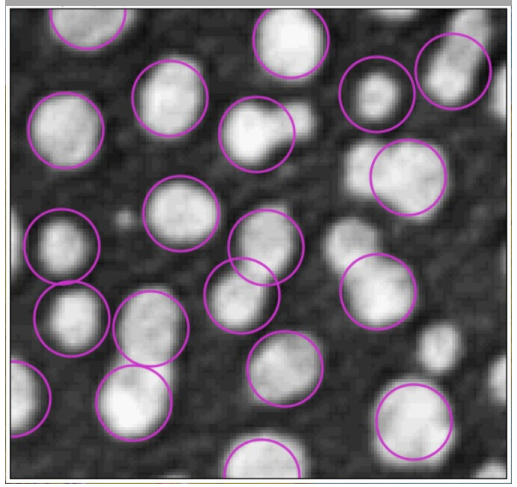




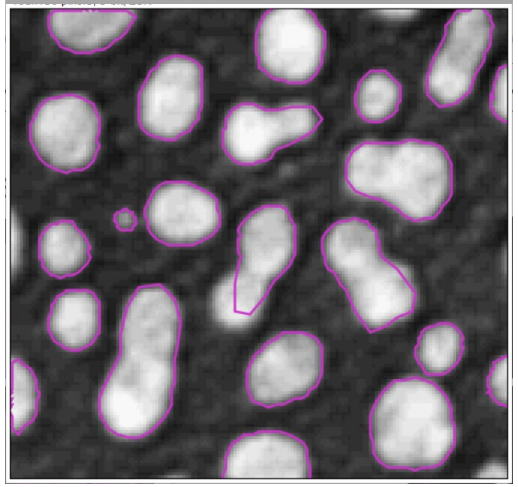
https://github.com/stardist/stardist

Trackmate object Detectors

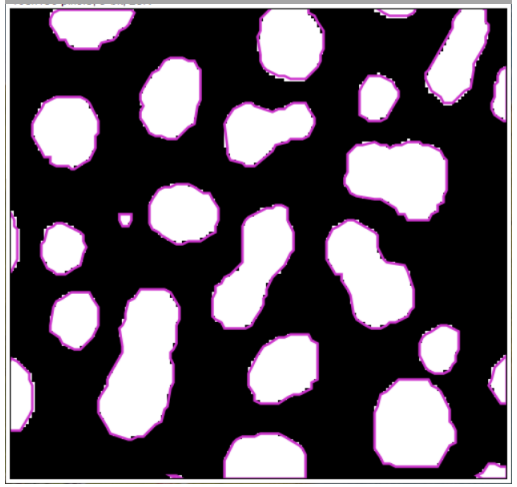
LoG detector



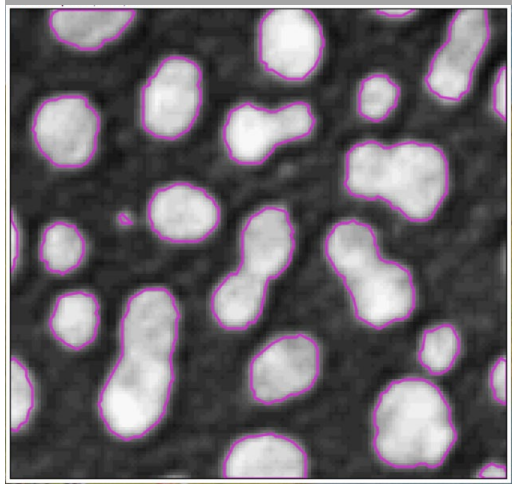
StarDist



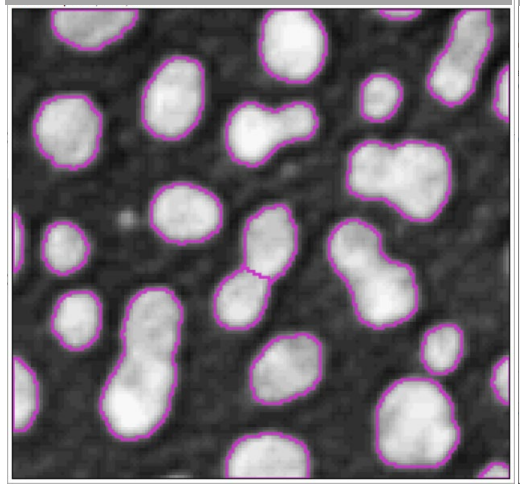
Mask detector



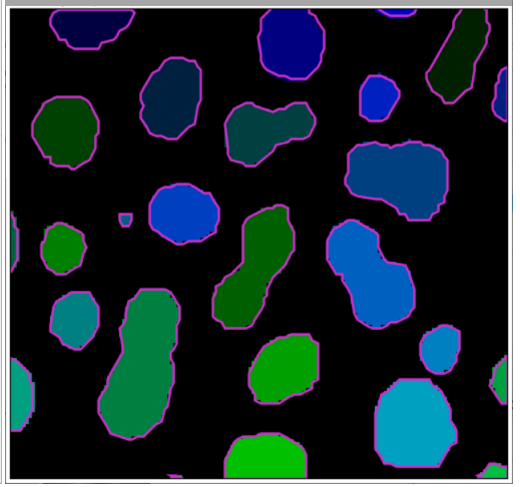
Threshold

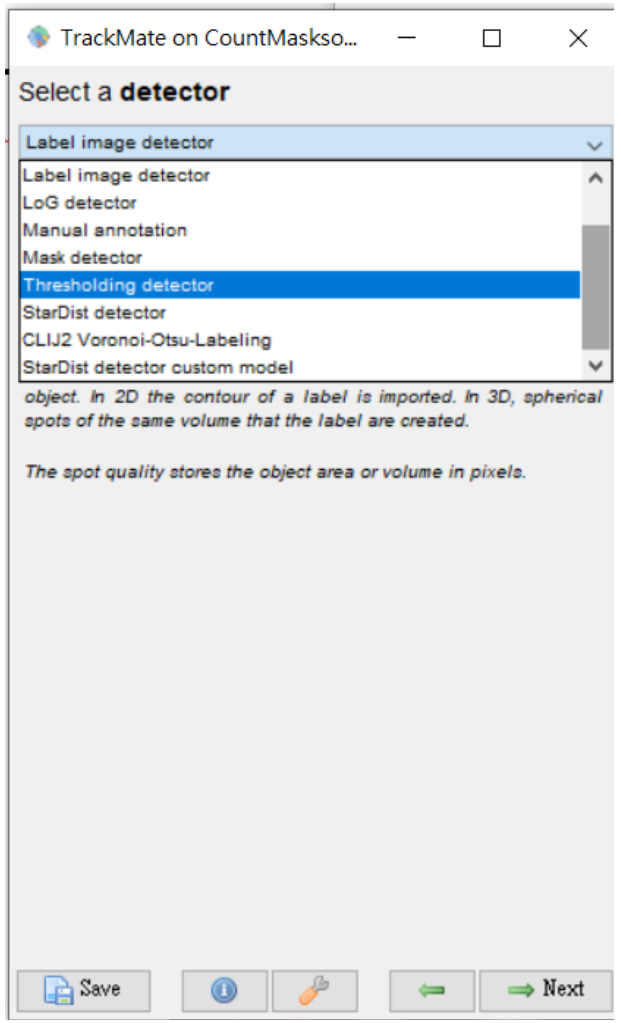


Voronoi-Otsu



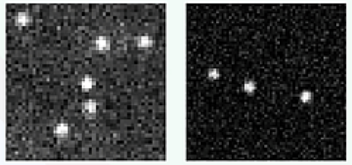
Label detector





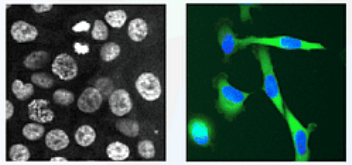
Choosing the detector in TrackMate according to your use-case.

Sub-resolved particles (shapeless because their size is smaller than the optical resolution). Objects that resemble a gaussian peak.



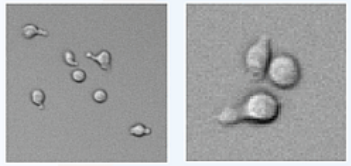
The LoG detector.
If the image is 3D and the spot size is below 8 pixels in size, the DoG detector is faster.

Densely packed nuclei images in 2D, imaged in fluorescence. Multi-channel images including a channel for nuclei. Objects that resemble a nucleus (blob-like shape, bright).



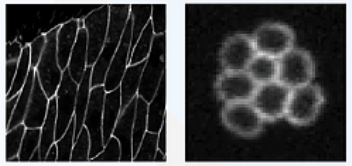
The StarDist detector with the built-in model.
For multi-channel image simply specify in what channel are the nuclei.

Cells imaged in transmitted light (bright-field, phase-contrast or DIC). Objects in 2D that are not nuclei, are dense and of complex shape.

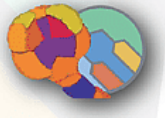


The StarDist detector with a custom model trained on the same kind of images.
First look for a suitable model *e.g.* on the BioImage Model Zoo (https://bioimage.io/). If nothing fitting can be found, trained your own model *e.g.* using ZeroCostDL4Mic (https://github.com/HenriquesLab/ZeroCostDL4Mic/wiki)

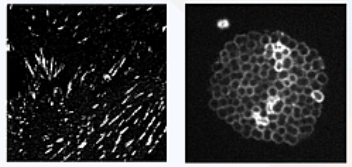
Cells stained for their membrane imaged in fluorescence.



The MorphoLibJ detector.
Preprocessing might be required to ensure the cell contours are closed and well defined. Add the result of the pre-processing as a supplemental channel in the input image.

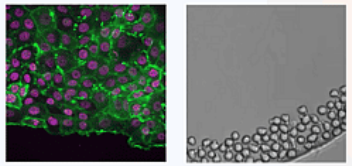


Objects of size that varies, of complex shape, or identifiable by their texture.

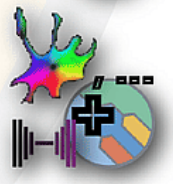


The ilastik detector or the Weka detector inputing a pixel classifier trained on the object.
The choice of one or the other is governed by performance considerations and accuracy provided by the available pixel features.

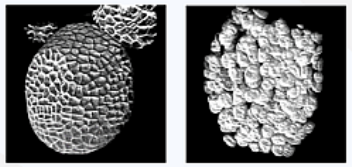
Images for which the above approaches fail.



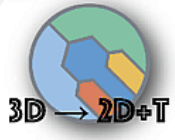
Use an external segmentation tool and input its results in TrackMate using the **Mask detector, Label-Image detector** or **Threshold detector.**
For instance, use cellpose in ZeroCostDL4Mic.



Segmenting objects in 3D images using a slice-by-slice approach.



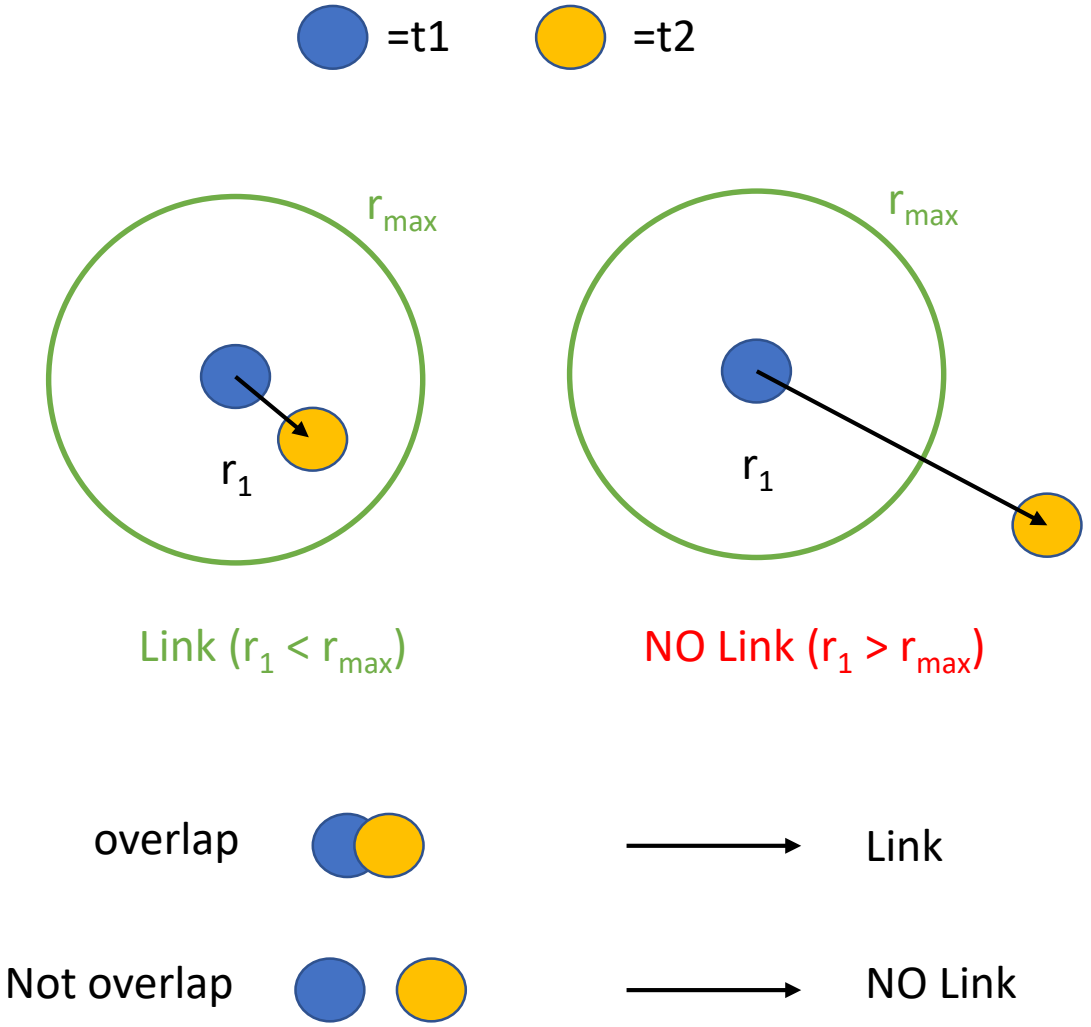
Segment the Z-stack slice by slice, using one of the approach above, tricking TrackMate into thinking the 3D stack is a 2D over time movie.
Then merge the segmentation results in Z using a tracking formulation *e.g.* with the overlap tracker. Then export the results using the *Export label image* action.



https://imagej.net/plugins/trackmate/

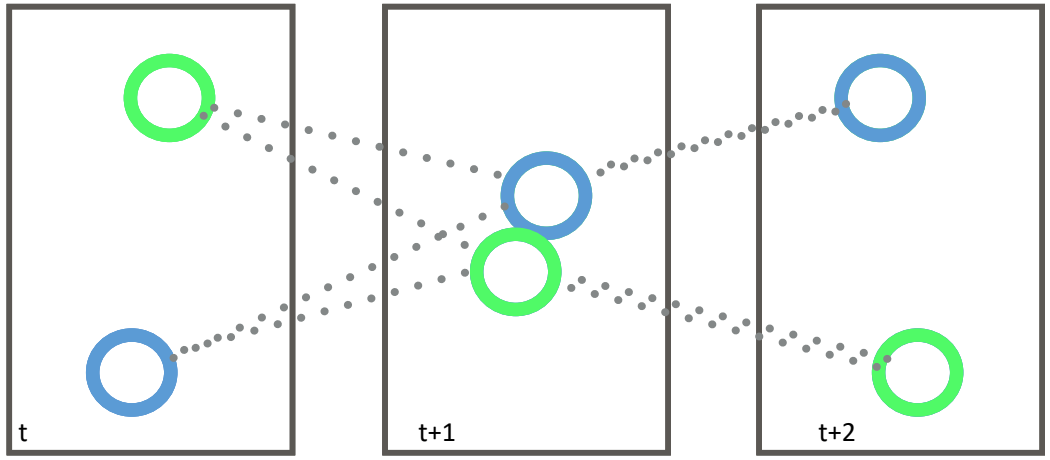
Linking

- Linking algorithms
  - LAP trackers:
    Tracking algorithms based on the Linear Assignment Problem algorithm
  - Kalman tracker:
    Tracking algorithm suitable for objects that move with a nearly constant velocity.
  - Overlap tracker:
    Tracker based on overlapping object contours, suitable for object with complex shapes and limited movements.
  - Nearest-Neighbor tracker:
    Simplest tracker.



Linking

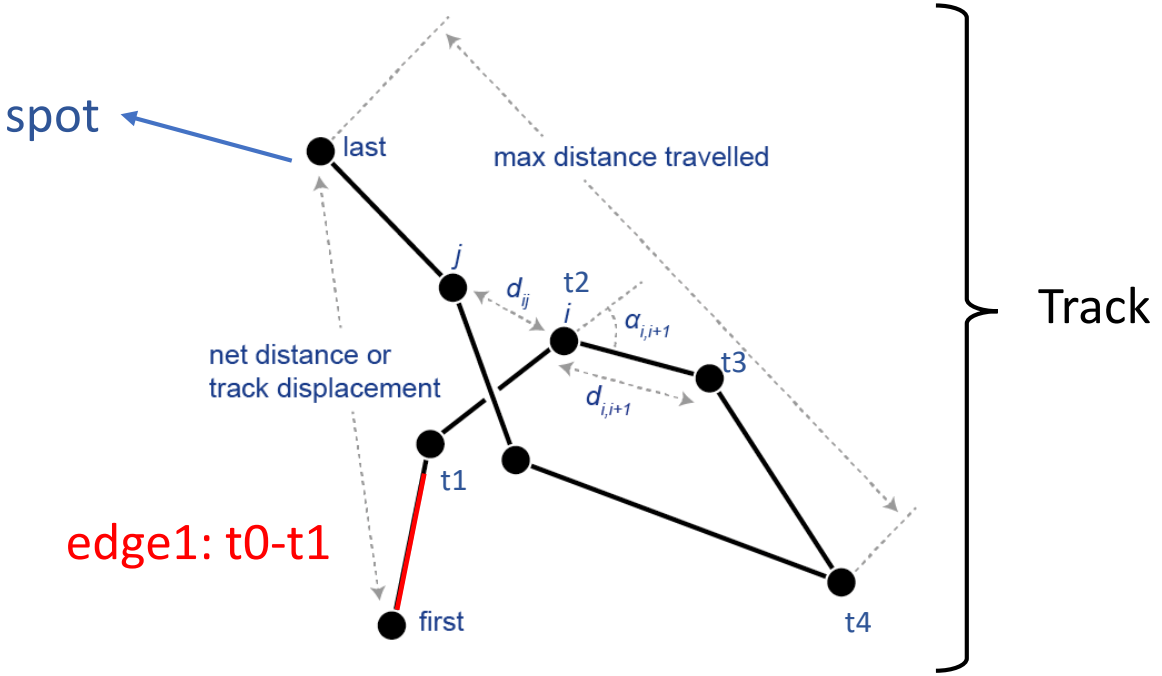
- Linking (also “matching”) is the process of connecting spots to tracks by adding edges.



- Linking is a linear assignment problem (LAP) which is solved by finding the solution with minimal costs (e.g. length of edges, or amount direction changes).

TrackMate Output

- Spots
  - XYT coordinates
  - Signal intensity
  - Morphology readouts (area, circularity)
- Edge(link)
  - Connections Between Spots
  - Distance, Velocity, and Direction of movement
- Tracks
  - Speed
  - Displacement
  - Track duration and length
  - Confinement Ratio
    Net distance/Total distance
  - Mean Straight Line Speed
    Net distance/Total track time



total distance travelled = $\sum d_{i,i+1}$

max distance travelled = Max d_{ij}

mean directional change = 1/N $\sum \alpha_{i,i+1}$

Track tables

Export to CSV

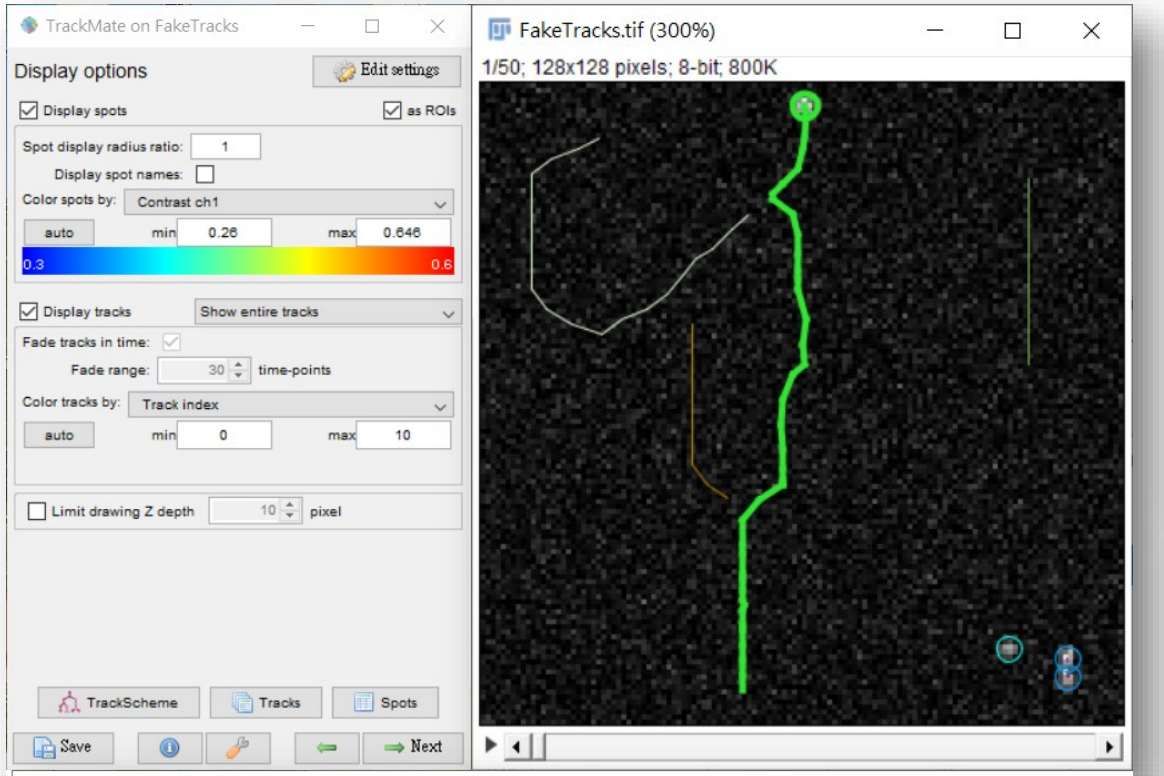
Spots | Edges | Tracks

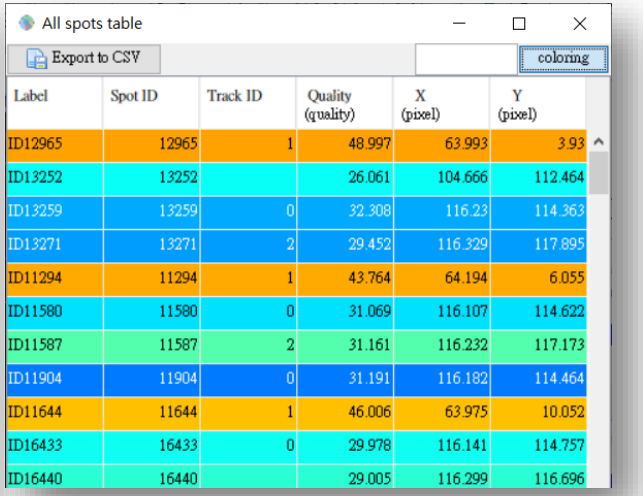
Label	Spot ID	Track ID	Quality (quality)	X (µm)	Y (µm)	Z (µm)	T (minutes)	Frame	R (µm)
ID34560	34560	0	3.473	127.545	53.789	0	3	6	5
ID39809	39809	0	1.078	132.979	49.769	0	8	16	5
ID40449	40449	0	0.737	138.896	48.618	0	19.5	39	5
ID45441	45441	0	1.257	133.344	47.383	0	25	50	5
ID38786	38786	0	1.382	133.03	49.966	0	9	18	5

https://imagej.net/plugins/trackmate/analyzers/

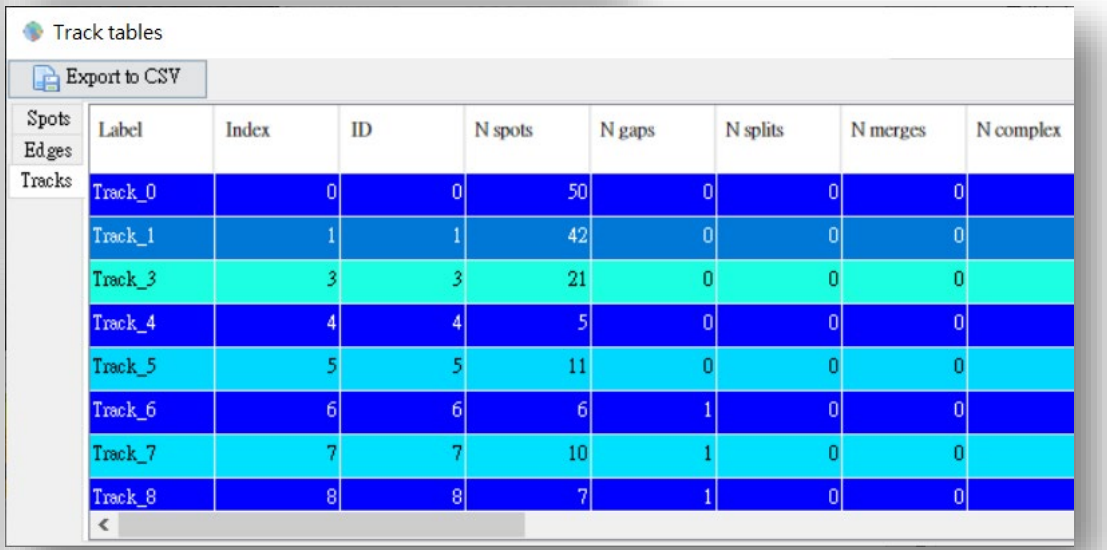
Track analysis

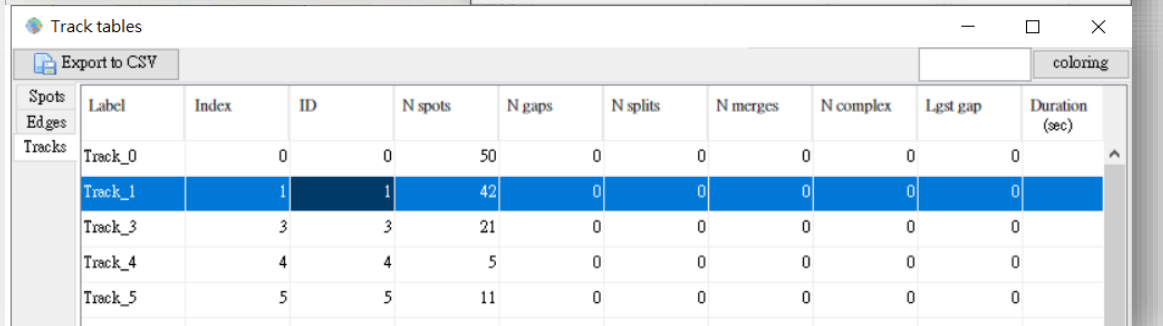
Manual setting display range and color for feature visualization





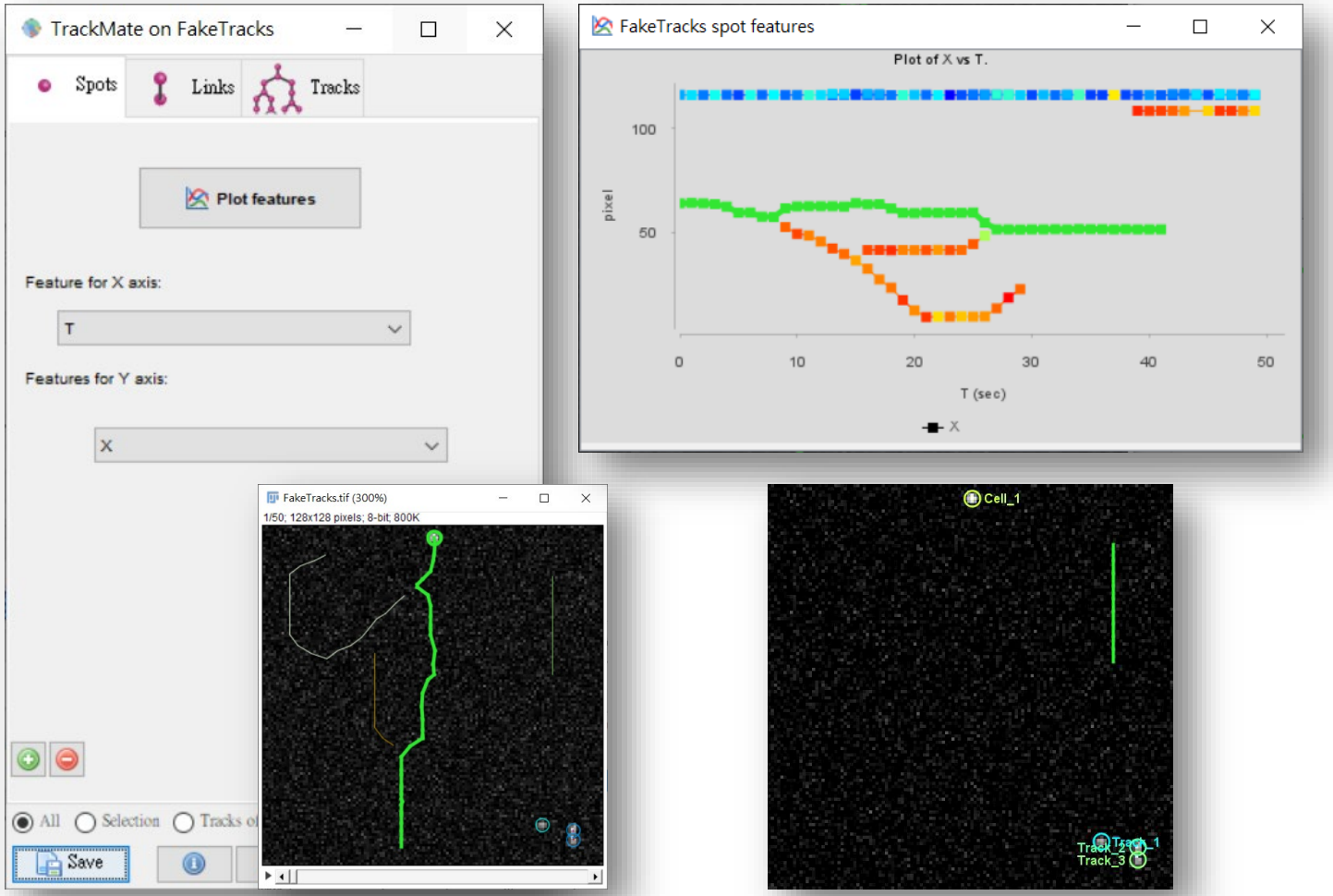
https://imagej.net/plugins/trackmate/



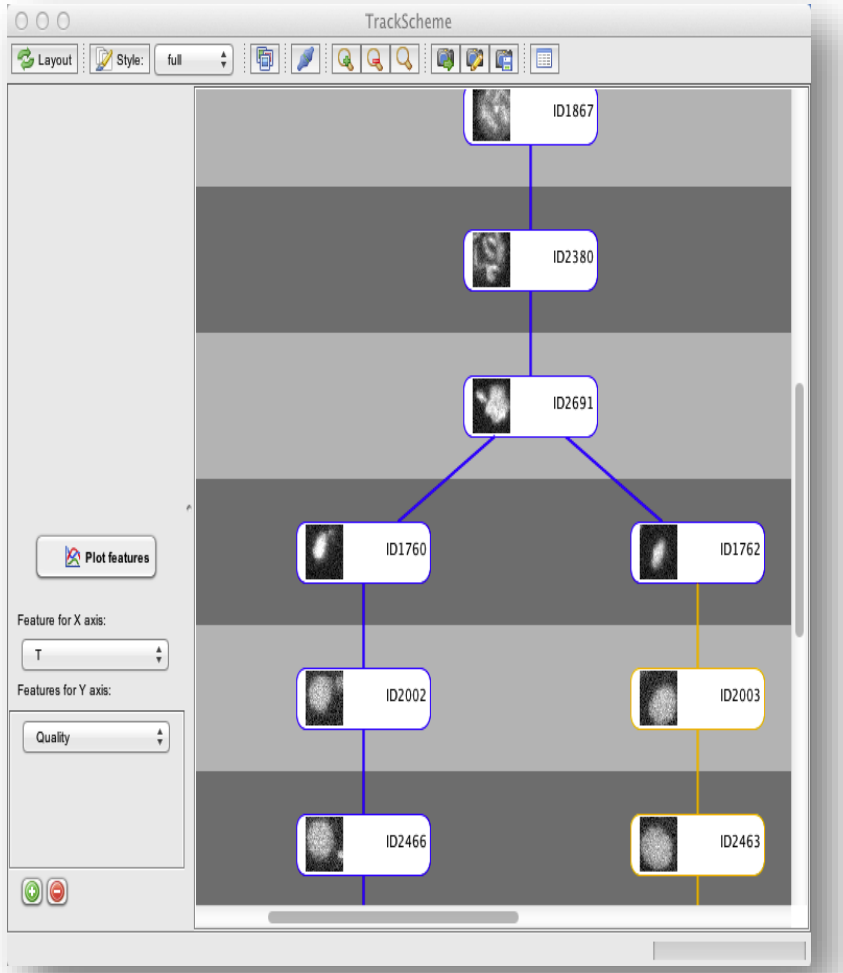


Track analysis

Manual setting display range and color for feature visualization



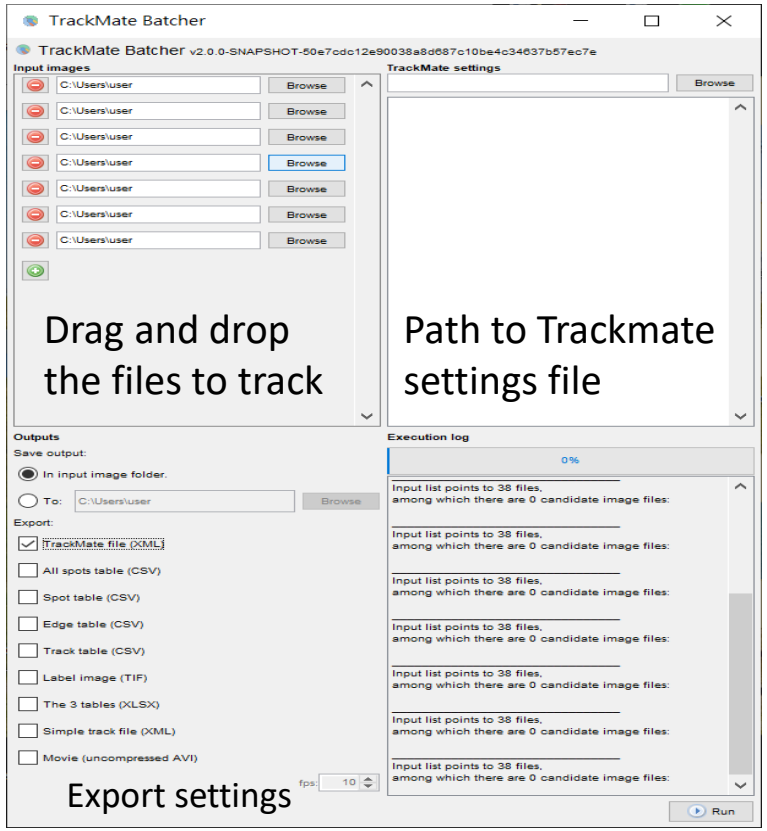
TrackScheme



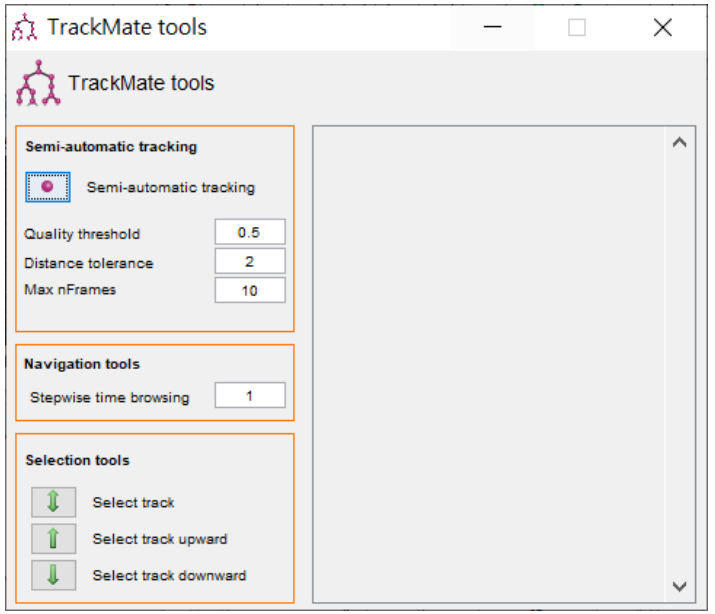
https://imagej.net/plugins/trackmate/

Extension

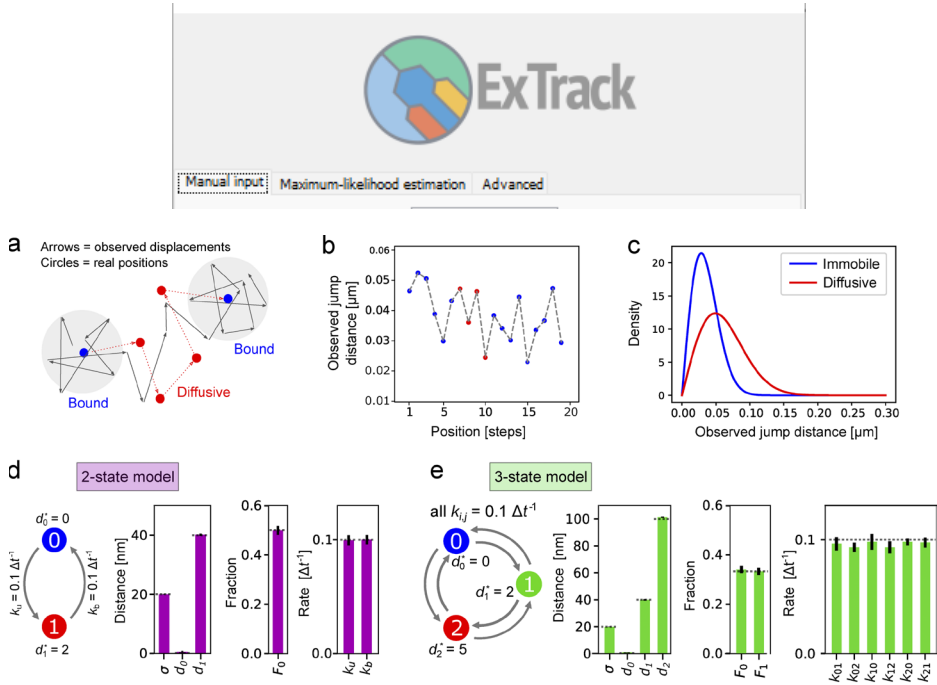
Trackmate batcher



Semi-auto tracking , auto-linking and the editing tool



Extrack
multi-state diffusion models.

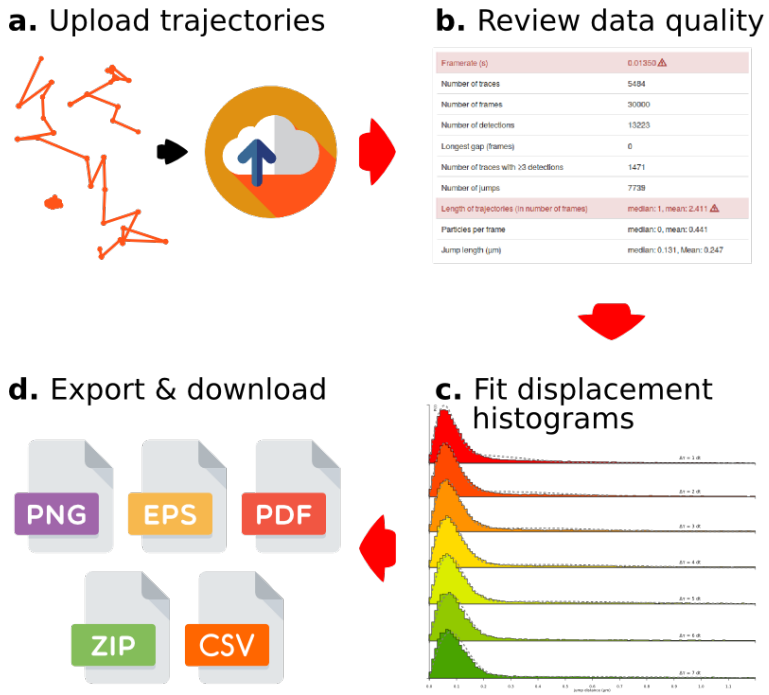


https://imagej.net/plugins/trackmate/

Exploring external platforms for advanced features

Spot-On

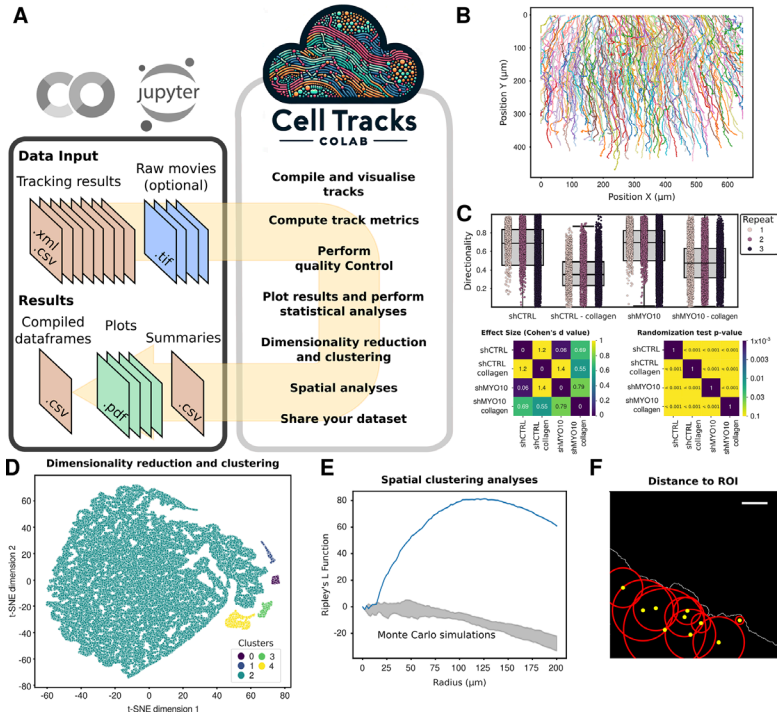
Web-interface designed for the analysis of single-molecule tracking experiments



https://spoton.berkeley.edu/SPTGUI/

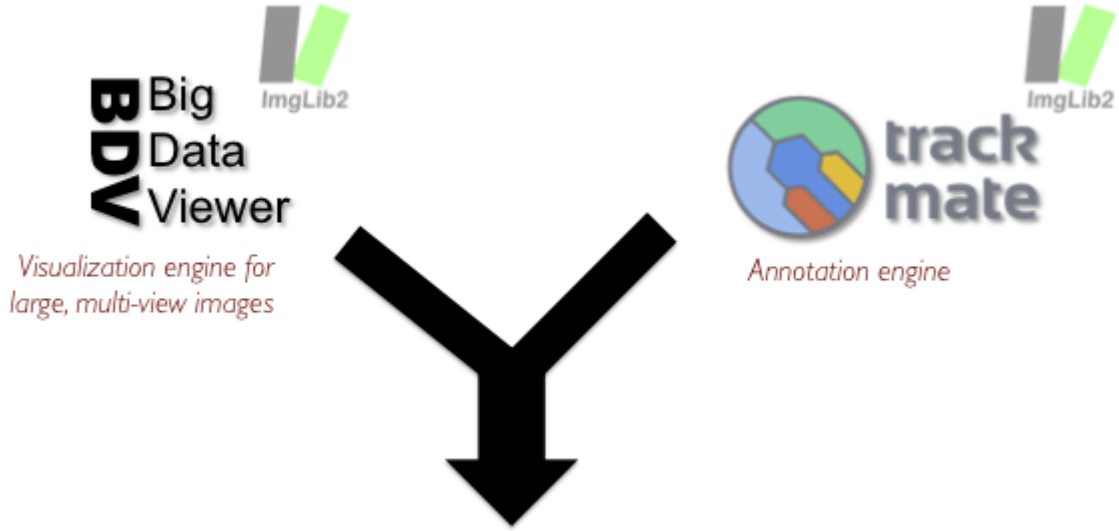
CellTracksColab

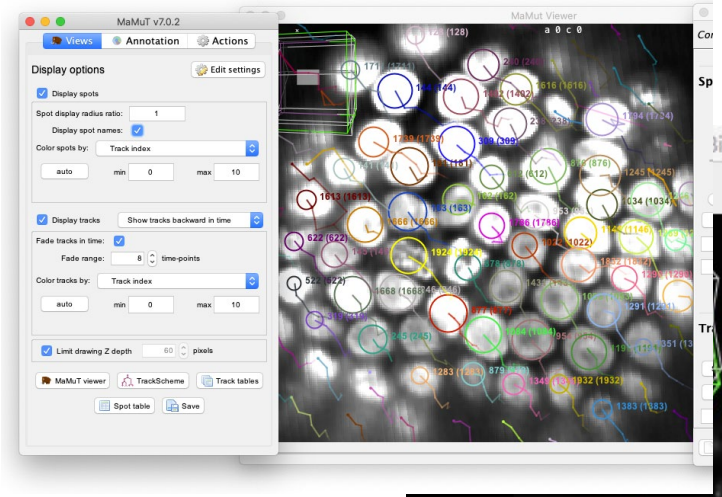
compilation, analysis, and exploration of cell tracking data from large datasets



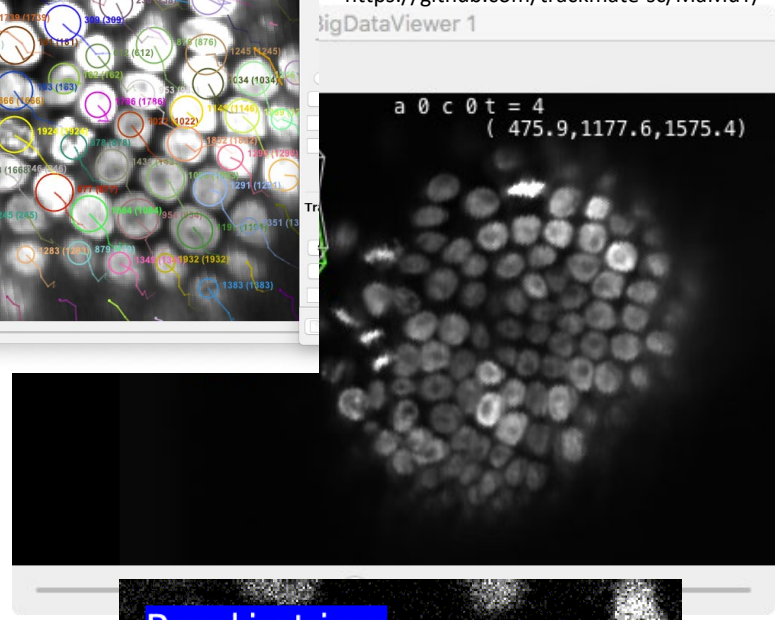
https://doi.org/10.1371/journal.pbio.3002740
https://github.com/CellMigrationLab/CellTracksColab?tab=readme-ov-file

Large-scale tracking



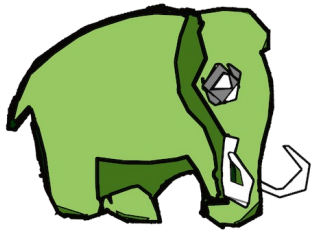


https://github.com/trackmate-sc/MaMuT/



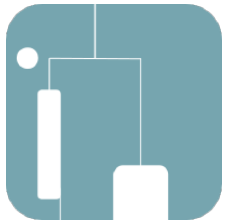
MaMuT

Semi-auto tracking



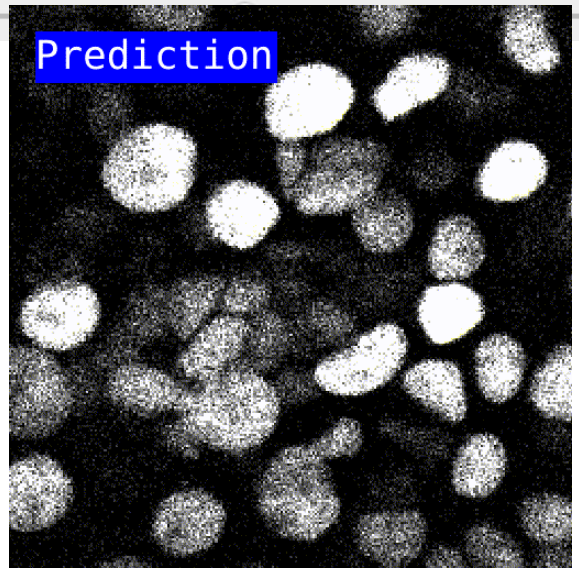
Mastodon

LoG, DoG detector



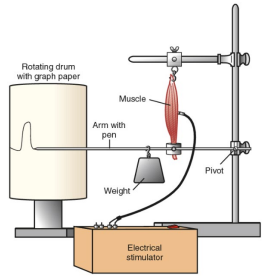
ELEPHANT

incremental and
interactive deep learning



https://elephant-track.github.io/#/v0.5/?id=overview

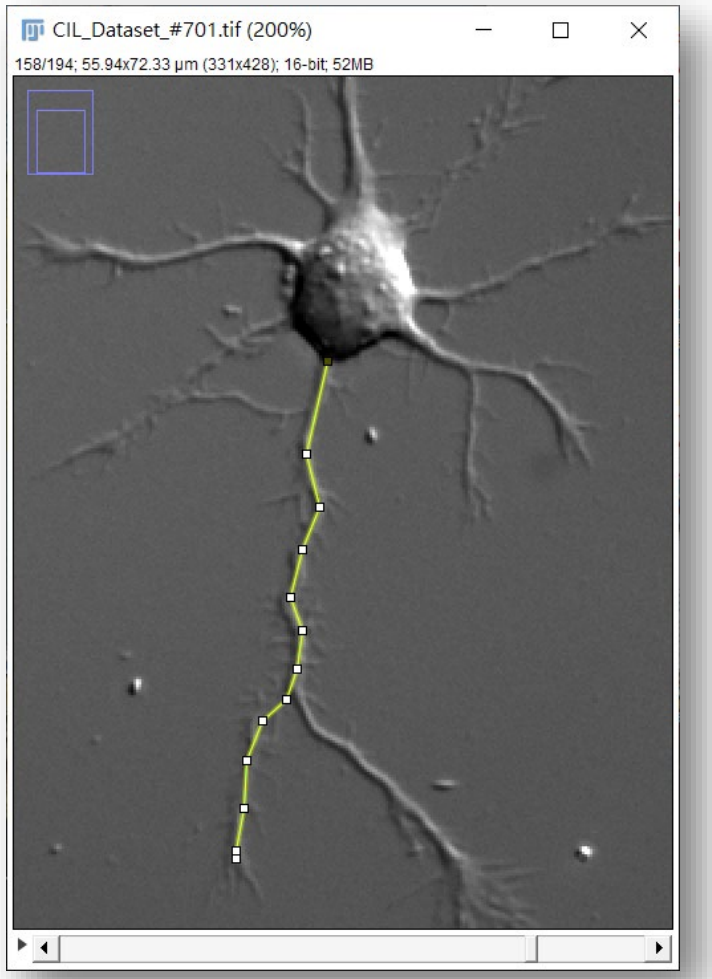
Kymograph

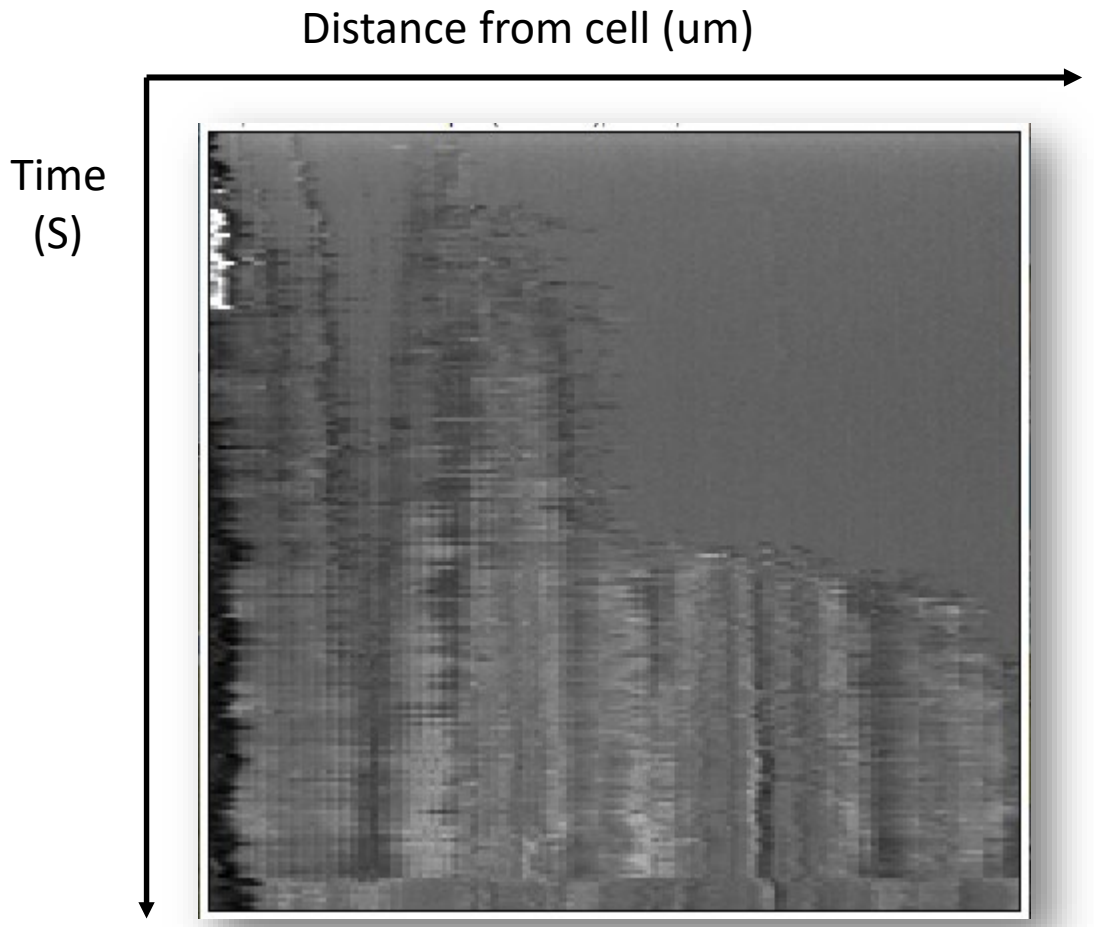


A device that graphically records changes in the mechanical activities of animal tissues“

- A graphical representation that combines spatial and temporal information from a dynamic image
- Transform a movie containing particles movement into a single picture/figure.
- Analyzing vesicles movement in cells, axons/dendrites of neurons
- By tracking the movement of lines or stripes within a kymograph, the object's movement speed and pattern can be analyzed more precisely

Kymograph


CIL_Dataset_#701.tif (200%)
158/194; 55.94x72.33 µm (331x428); 16-bit; 52MB


Distance from cell (um)
Time
(S)

Documentation and tutorials

Downloadable documents and tutorials

- The main manual for TrackMate can be found here:

TrackMate-manual.pdf - 14 MB, 150+ pages.

It contains user tutorials, technical documentation and developer documentation. It compiles in a nice polished pdf the tutorials and information you can find on this wiki, and linked below.

- With TrackMate v7 we introduced several major changes, for which we wrote an additional manual. It can found here:

TrackMate version 7 novelties.pdf - 16 MB, 70+ pages.

Again, it compiles several tutorials and developer documentation also linked below.

Online tutorials

- Getting started with TrackMate is a basic tutorial that explains how TrackMate works on an easy image. You should start here.
- Manual editing of tracks using TrackMate shows how to manually curate and edit tracking results.
- Manual tracking with TrackMate shows how to perform a fully manual annotation of tracks in a source image.
- Keyboard shortcuts for editing contains a summary of the keyboard shortcuts we use here.
- TrackMate v7 new algorithms documents the 8 new detectors introduced with version 7, and the new shape analysis framework. Each subpage contains a tutorial that explains how to use each of the new detector.

TrackMate components

TrackMate has a modular design and ships several algorithms of several types: detectors, trackers, analyzers, etc. The pages below documents individual components, or modules or TrackMate.

- TrackMate Detectors: detect objects in images.
- TrackMate Trackers: link objects to build tracks.
- TrackMate Analyzers: compute numerical values on spots, edges and tracks.
- TrackMate Actions: miscalleneous actions on tracking results.
- TrackMate Views: tracking results viewers.

Interoperability

Python

There are several Python tools contributed by the TrackMate developer community that allows