

Bioimage Analysis for Quantitative Microscopy

30.9-4.10.2024

Trainers:

Hanna Grobe

Elnaz Fazeli

Joanna Pylvänäinen

Sujan Ghimire

Stéphane Rigaud

*open to all interested

Locations:

Auditorium Biokemi,
Biocity 2nd floor

Auditorium Biologi,
Biocity 3rd floor

Putous Auditorium, Joki
conference center

	Mon	Tue	Wed	Thu	Fri
9-10.00	Intro + Fiji Basics Hanna Grobe	DL lecture Joanna Pylvänäinen	QuPath Stéphane Rigaud	TrackMate + CellTracks Colab lecture Joanna Pylvänäinen	
10.00-10:15					Presentation: TBI image data team Pasi Kankaanpää
10-15-10.30	<i>break</i>	<i>break</i>	<i>break</i>	<i>break</i>	Project presentations
10.30-11.45	Fiji Basics Hanna Grobe	DL demo annotation and training Sujan Ghimire	QuPath Stéphane Rigaud	Cell tracking with TrackMate Joanna Pylvänäinen	
12-13	<i>Lunch (at own cost)</i>	<i>Lunch (at own cost)</i>	<i>Lunch (at own cost)</i>	<i>Lunch (at own cost)</i>	<i>Lunch (at own cost)</i>
13.00-13.15	Presentation: Euro-Biolmaging Jiri Funda, Susanne Va	Science talk *: Deep Learning in Microscopy Guillaume Jacquemet	Science talk *: Deep Learning in Histopathology Pekka Ruusuvuori	Keynote talk *: How to not lie with charts - better data visualisations for life sciences Helena Jambor	Science talk *: Next-generation file formats, version control, and publishing your data Junel Solis
13.15-14	Fiji Macros Elnaz Fazeli		DL demo quality control Joanna Pylvänäinen	Track Analysis using CellTracksColab Hanna Grobe	Work with your own data
14.14-15.15			QuPath Stéphane Rigaud		
15.15-15.30	<i>break</i>	<i>break</i>	<i>break</i>	<i>break</i>	<i>break</i>
15.30-16.45	Fiji Macros Elnaz Fazeli	DL demo apply to own data Joanna Pylvänäinen	QuPath Stéphane Rigaud	GPU accelerated Fiji image processing Stéphane Rigaud	Work with your own data Goodbye and farewell
17.00-21.00				Course dinner in Mauno	

ANALYSIS OF CELL BEHAVIOR USING TRACKMATE AND CELLTRACKSCOLAB

What, where, how?

Can I also use it?

Joanna Pylvänäinen, joanna.pylvanainen@abo.fi

Image analysis course, Turku, Finland

Materials modified from Guillaume Jacquemet and Robert Haase

Contents

- Objects
- What are tracks and how to link them
- How to analyze
- What is TrackMate

Tools developed at Cell migration lab



track
mate +



Cell Tracks
— COLAB —

An open-source plugin for Fiji/ImageJ designed
for tracking particles and objects

Platform tailored to simplify the exploration
and analysis of cell tracking data.



What is TrackMate?

An open-source plugin for Fiji/ImageJ designed for tracking particles and objects in 2D (and 3D) microscopy data.

- **Key Features:**

- User-friendly interface with a step-by-step wizard
- Flexible: Can handle a variety of biological data (single particles, cells, organelles)
- Multiple tracking algorithms: LAP (Linear Assignment Problem), Kalman filter, etc.
- Customizable analysis workflows
- Visualization and export options for track analysis
- Extensive documentation and active community support.



Cell Tracks
— COLAB —

What is CellTracksColab?

Cloud-based tool for track analysis.

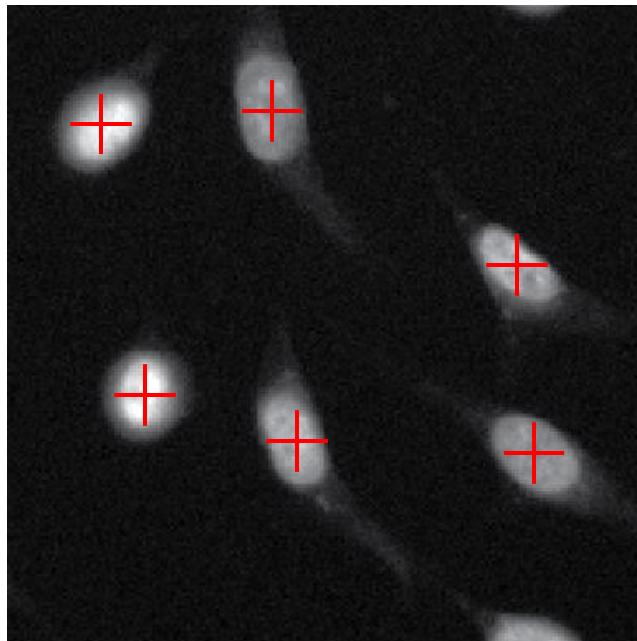
- **Key Features:**

- **Cloud-based:** No installation required; runs on Google Colab notebooks
- **Accessible:** Suitable for users without advanced computing infrastructure
- **Export and Visualize:** Outputs track data, graphs, and visualizations in real-time.

What is Particle and Object Tracking?

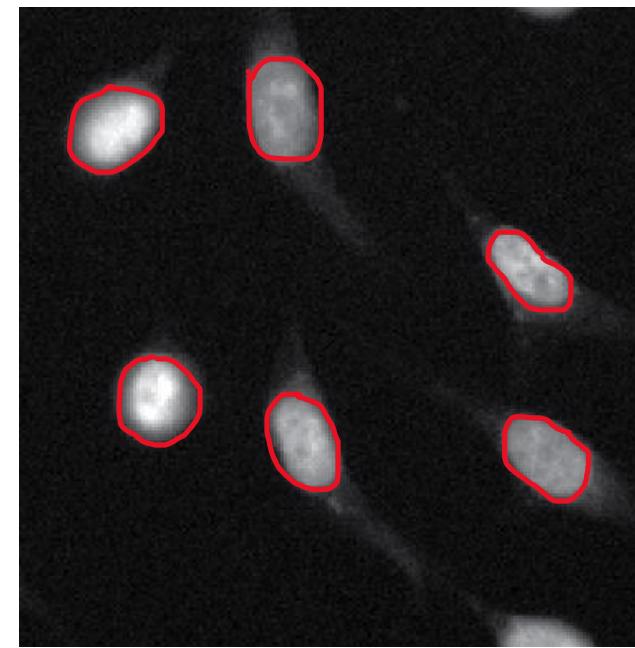
Particle tracking

Tracking the centroid of each particle's signal



Object tracking

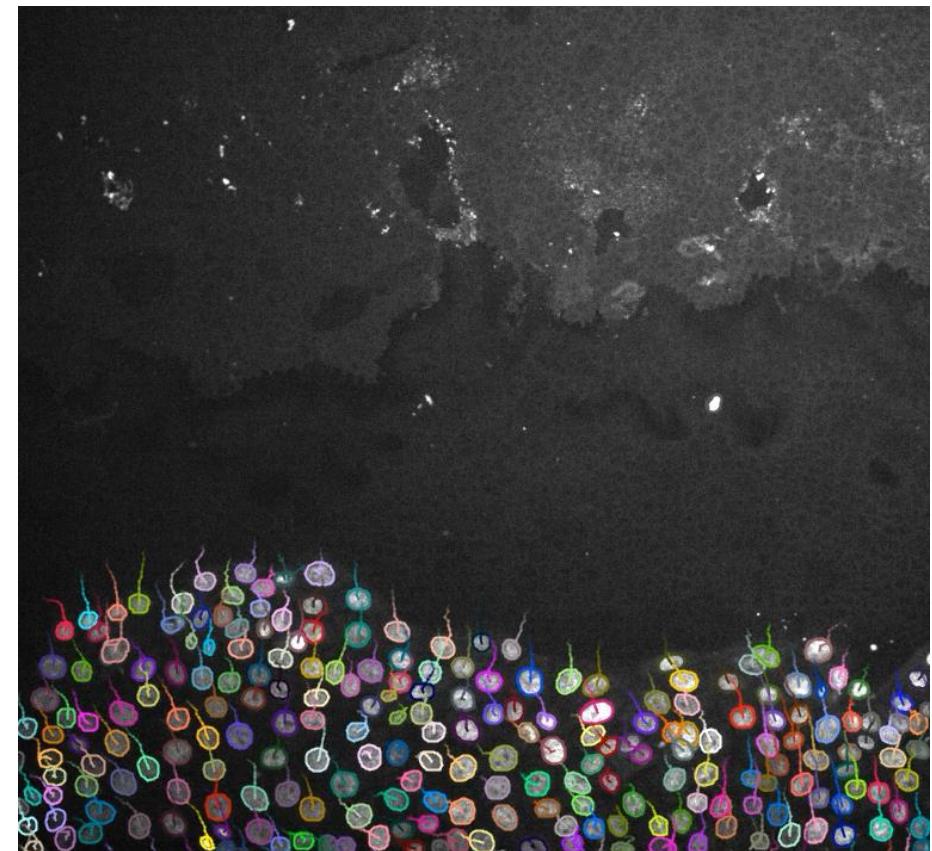
Tracking the entire shape and size of the object



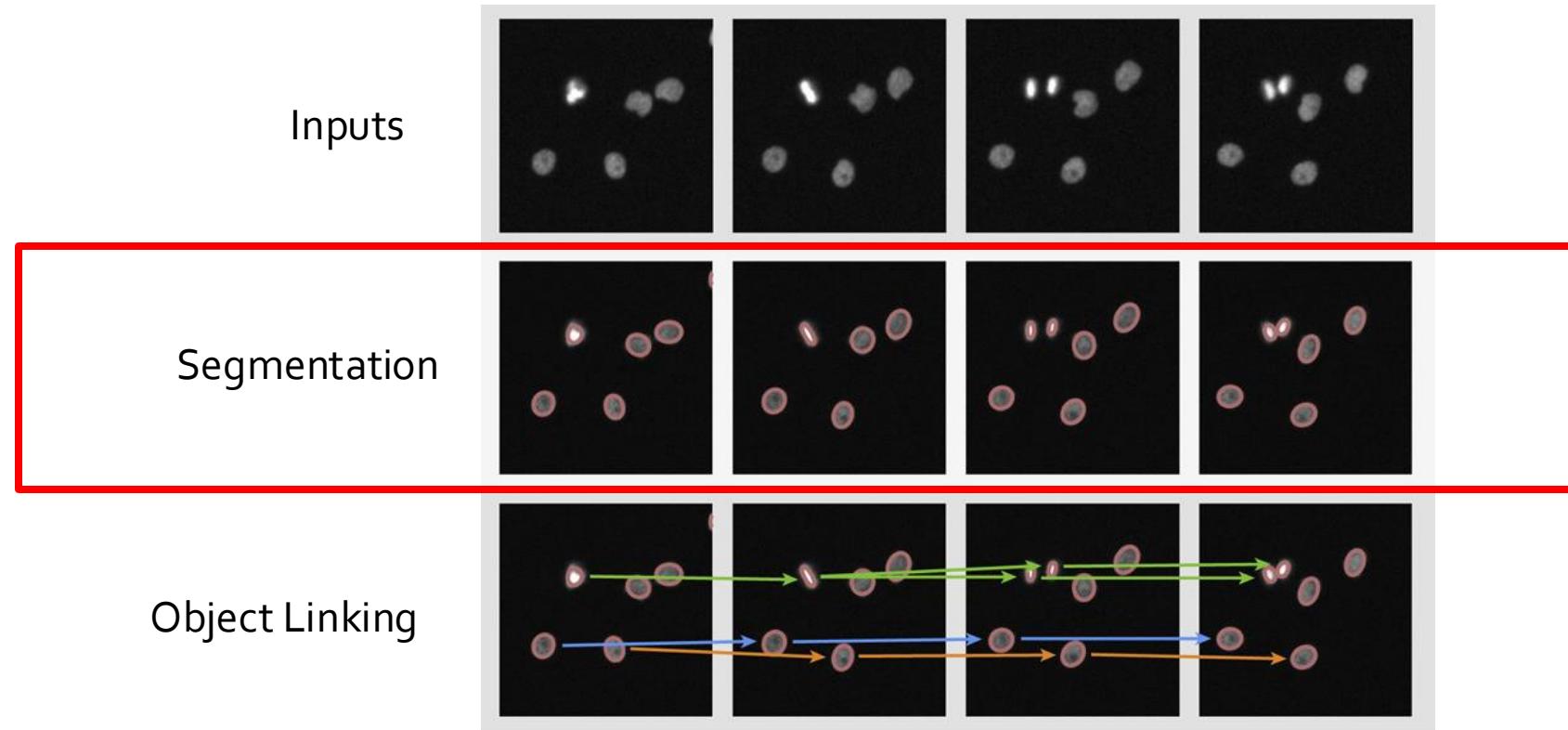
Source: Konrad Schindler, Computer Vision Lab, ETH Zuerich
<http://www.vision.ee.ethz.ch/datasets/>

Applications in Cell Biology

- **Particle Tracking:**
 - Monitoring molecular motors
 - Tracking cell division over time
 - Tracking individual proteins in live cells
- **Object Tracking:**
 - Cell motility and migration assays
 - Tracking cell division over time
 - Following organelle movement or shape changes

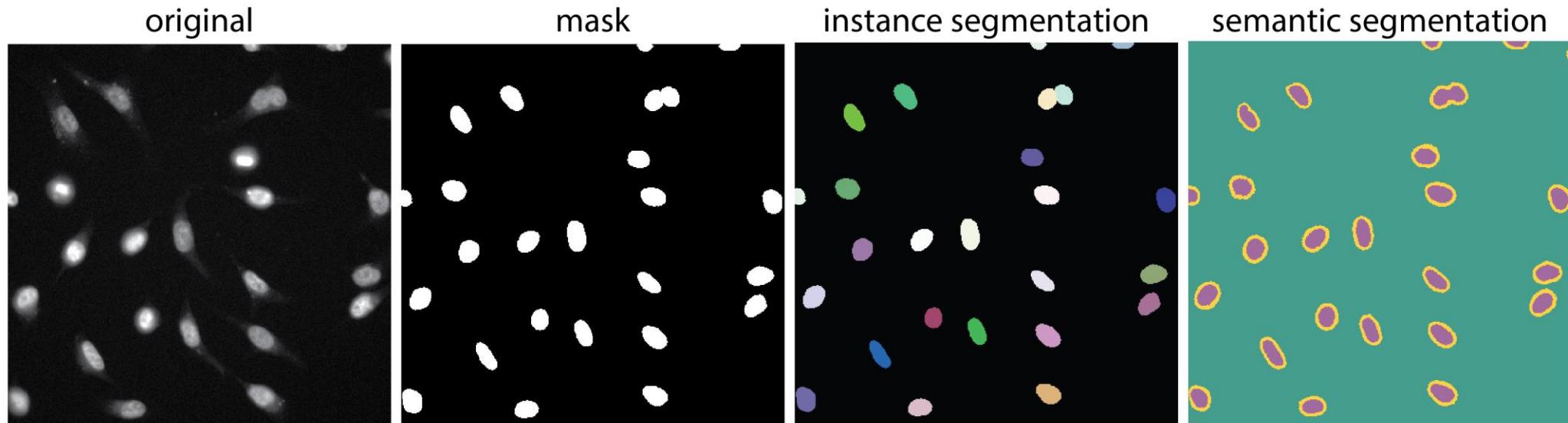


Tracking principle in TrackMate



Modified from Tian et al. (Cell Reports, 2010)

Segmentation



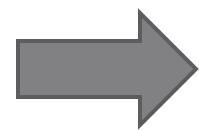
- Intensity-based segmentation
- Feature-based segmentation
- Using a pixel classifier (WEKA, ilastik, ect)
- Using Deep-Learning (Unet, cellpose, StarDist, SplineDist, EmbedSeg, ect)

Segmentation

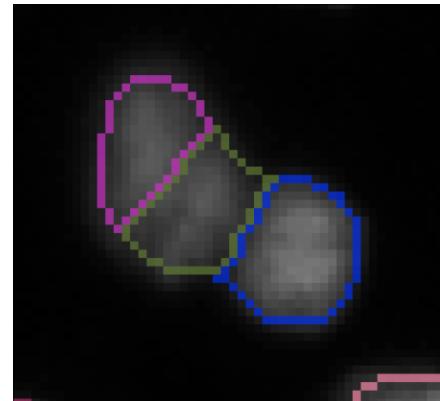
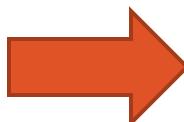
In the tracking context, object differentiation is important



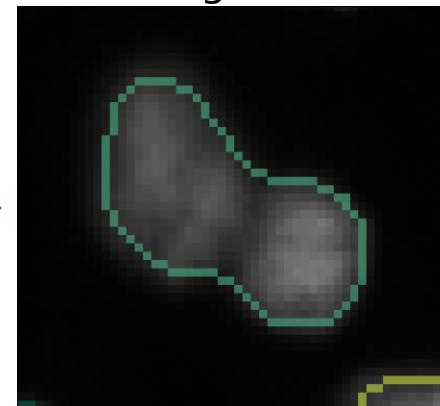
Raw image (nuclei)



Instance segmentation

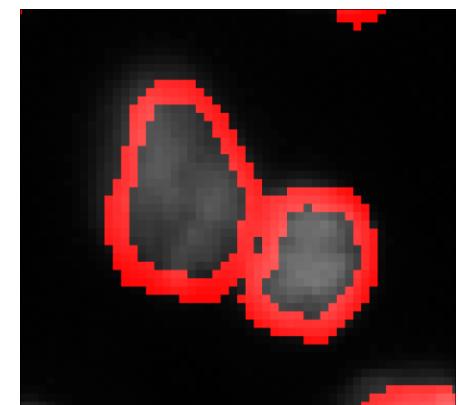


False objects
(over-segmentation)



Missed objects
(under-segmentation)

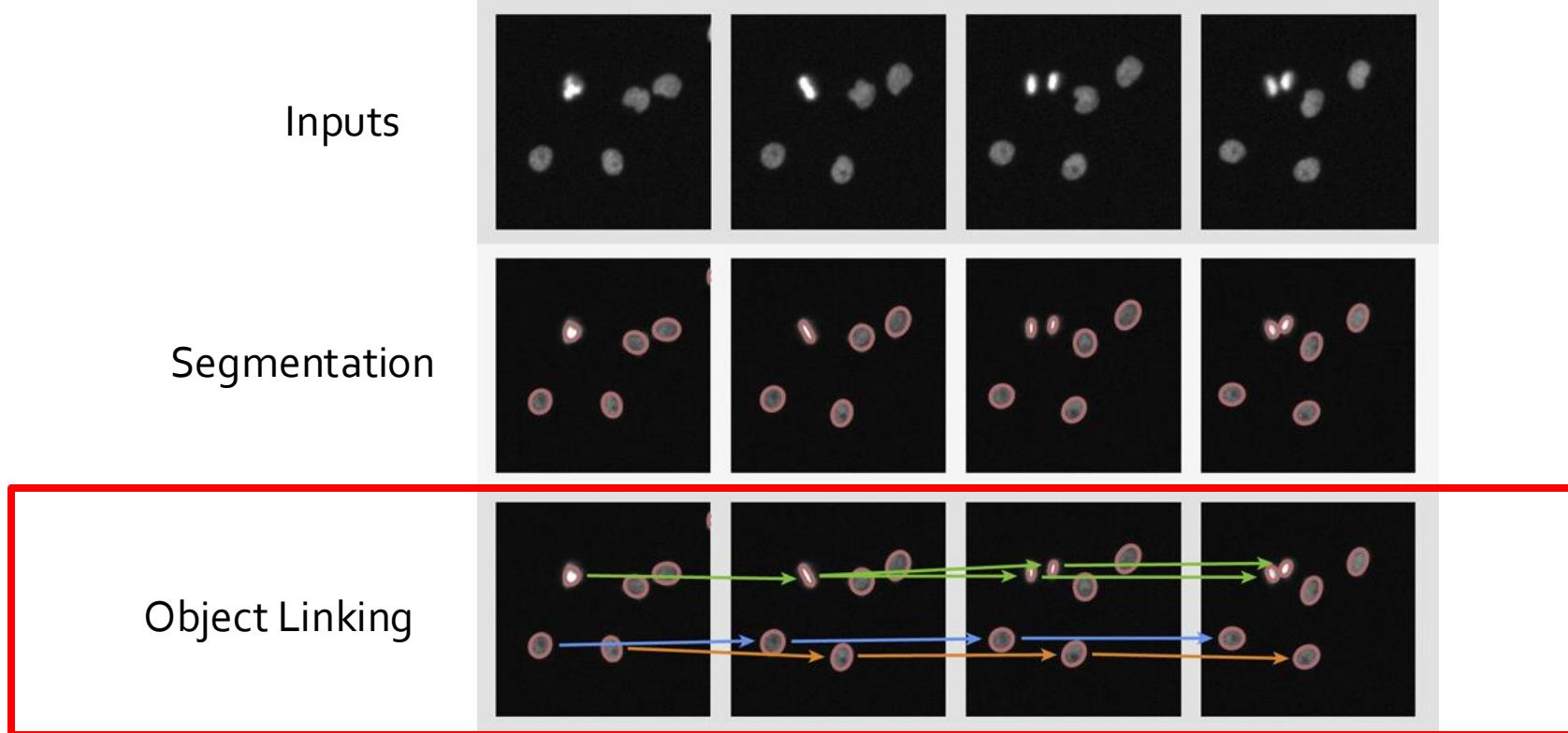
Outline precision can be of secondary interest



"Ground truth"
(manual annotation)

Image data source: Jones et al, Proc. ICCV Workshop on Computer Vision for Biomedical Image Applications, 2005 / Broad Bioimage Benchmark Collection [[Ljosa et al., Nature Methods, 2012](#),
<https://bbbc.broadinstitute.org/BBBC007>]

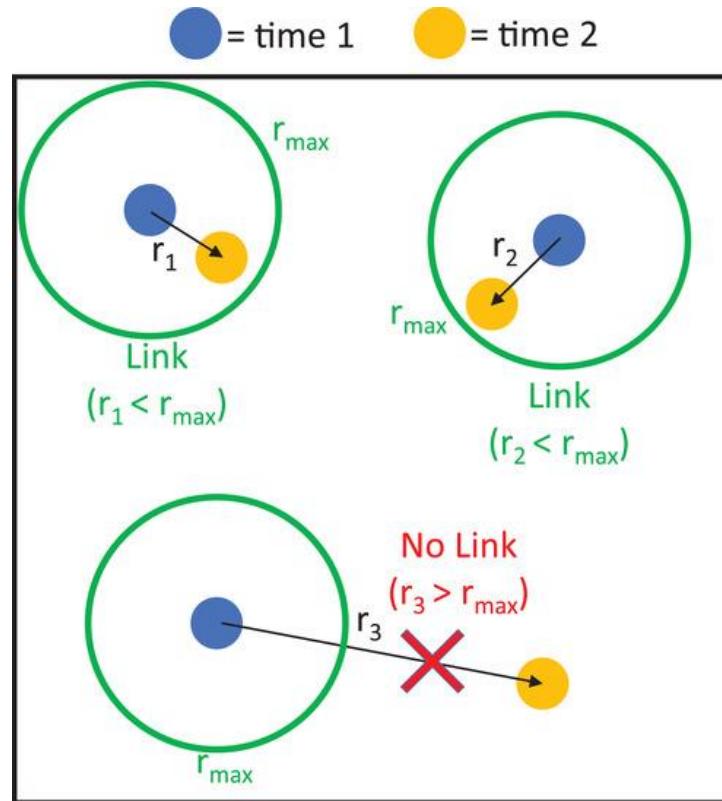
Tracking principle in TrackMate



- **Trackers** are algorithms or tools used to follow the movement of objects over time in a series of images or videos.

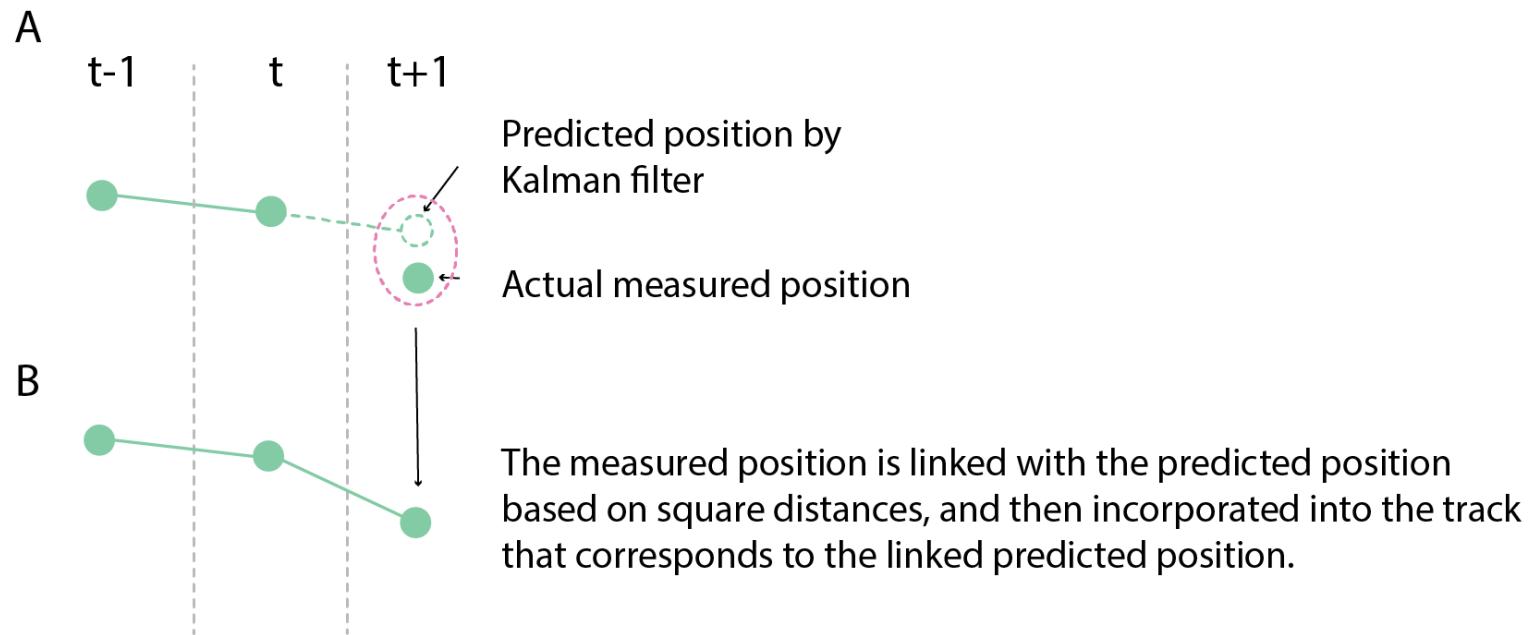
Tracking algorithm example - LAP tracker

Object linking using a search radius (LAP tracker)



Tracking algorithm example – Kalman tracker

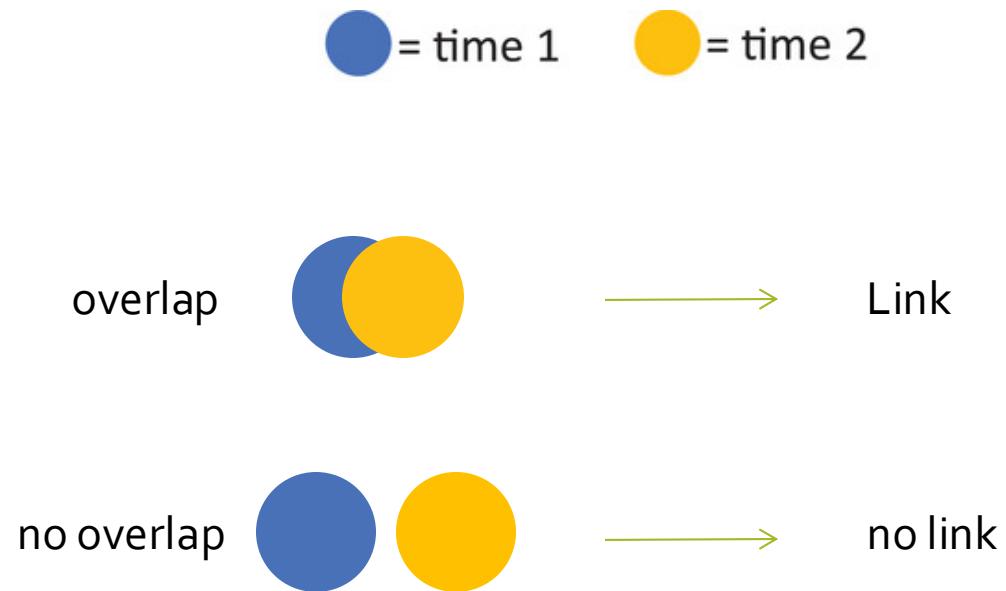
- The **Kalman tracker** is a mathematical tool used to estimate the position of moving objects over time, even when the measurements are noisy or incomplete.



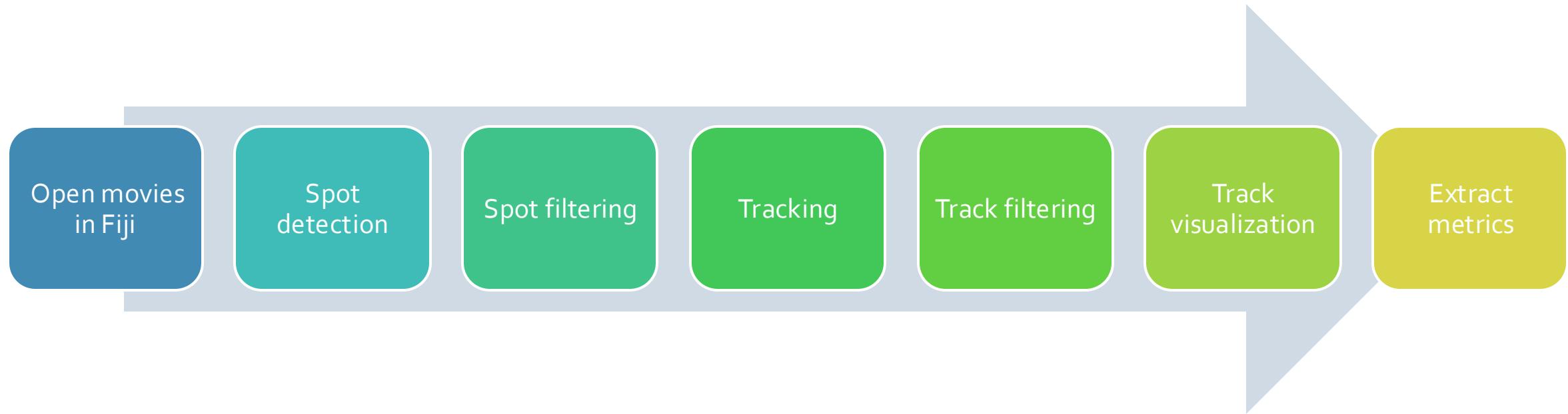
Tracking algorithm example - overlap tracker

Object linking using an overlap (The overlap tracker)

The Overlap tracker is well suited for large objects that move by less than their diameter.

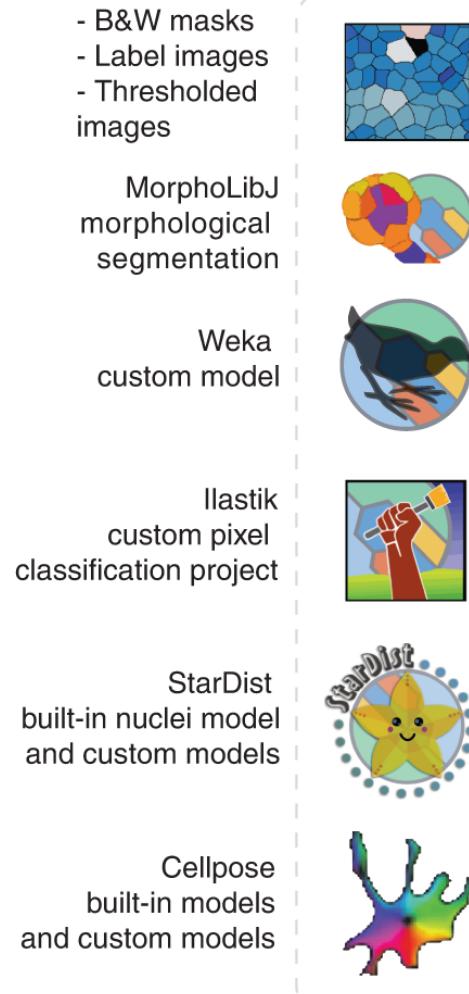


TrackMate Workflow

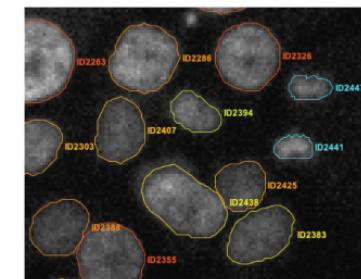


- **Pros:**
 - Easy to integrate with ImageJ
 - Suitable for both beginners and advanced users
 - High accuracy with robust algorithms

TrackMate overview



Integrated
into TrackMate UI

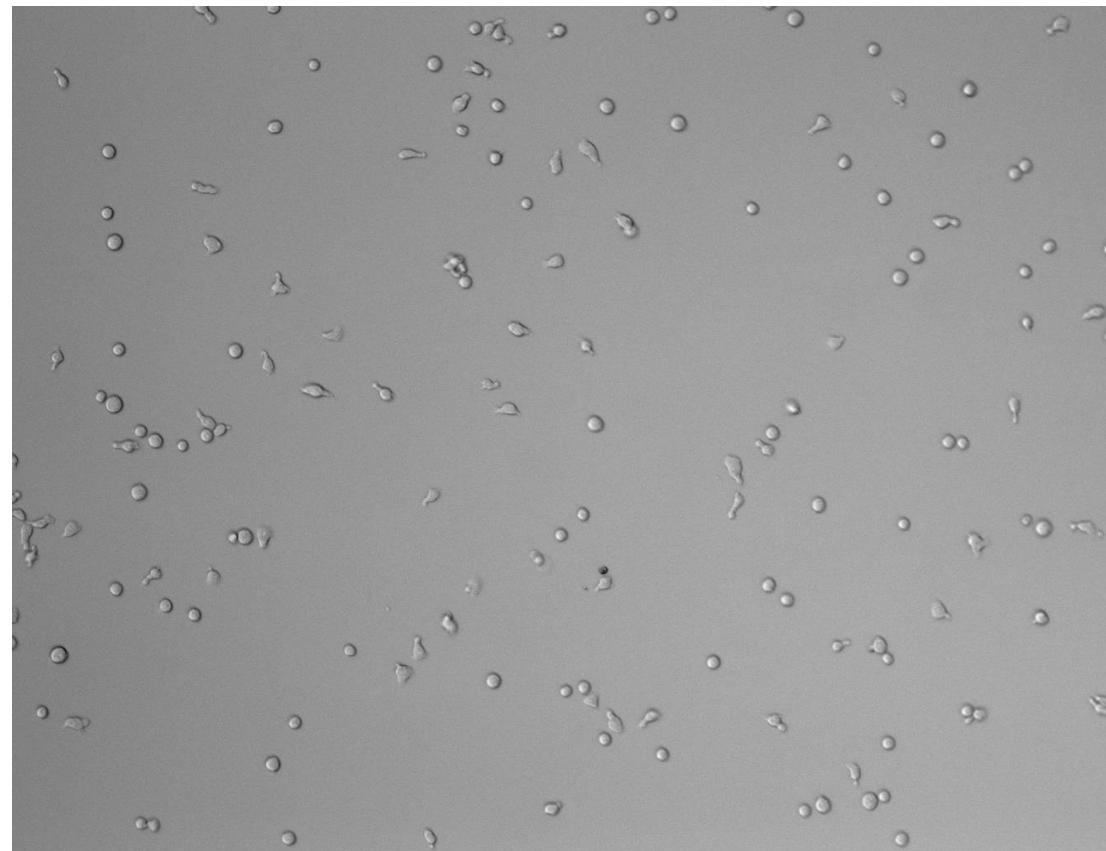
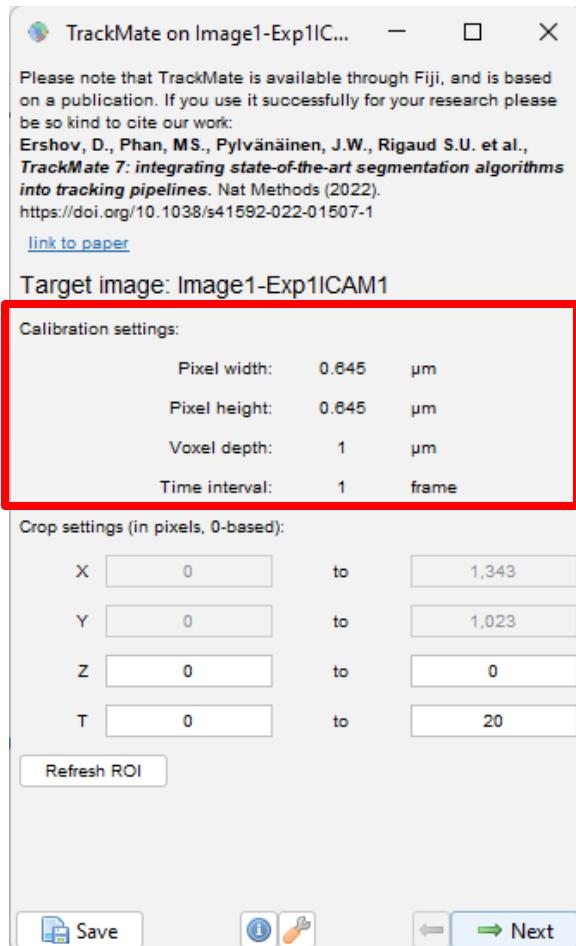


New TrackMate API:
Interoperate with external
segmentation components.
Store, create and analyze
object contours.

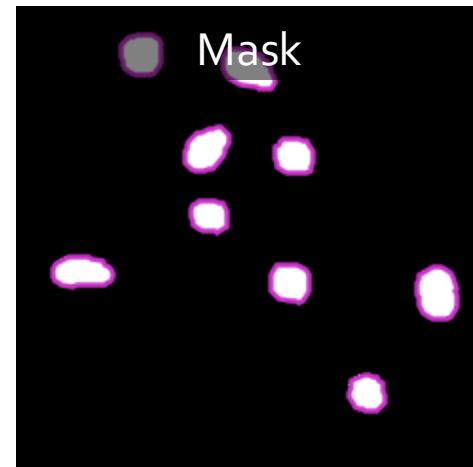
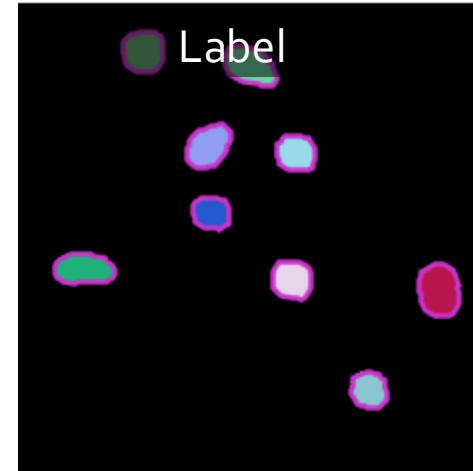
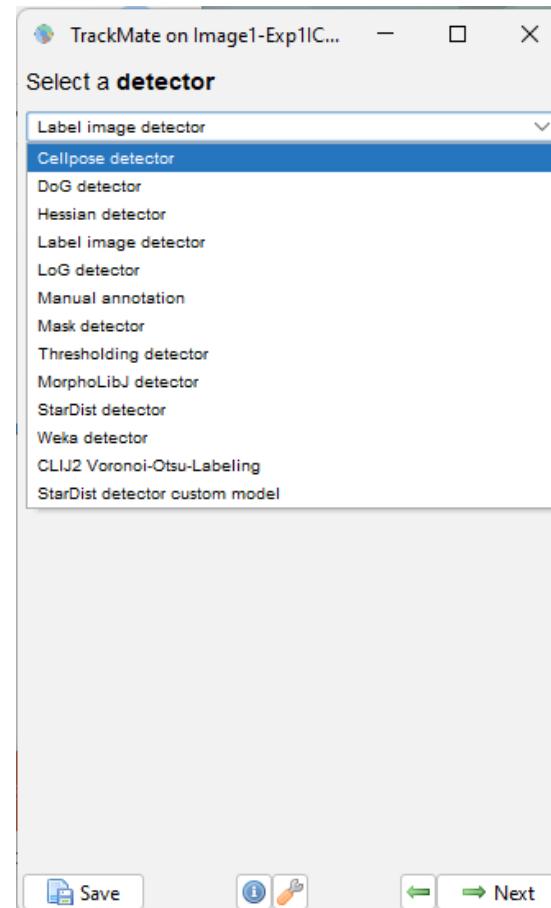
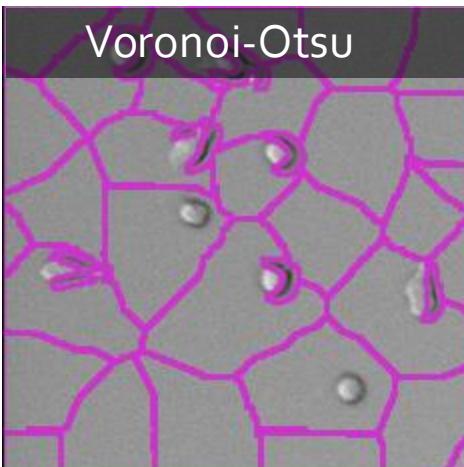
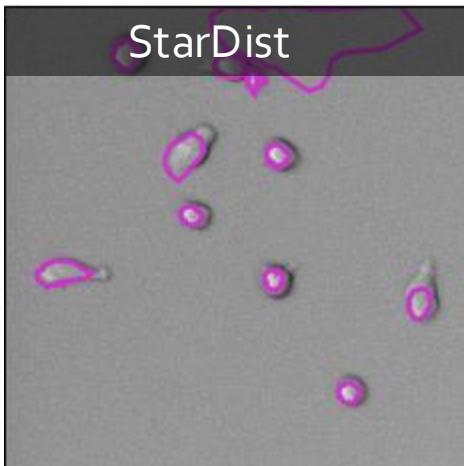
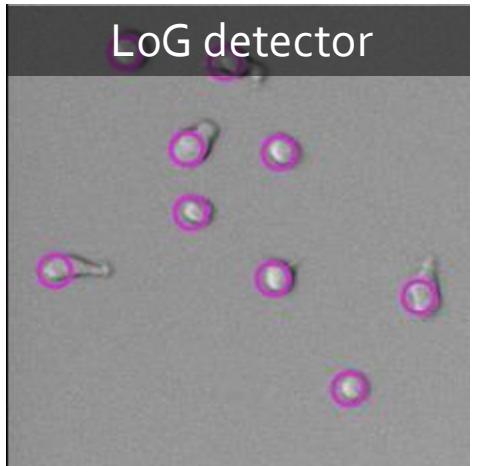
- Tracking cells
- Lineage tracing
- Changes in 2D shape over time
- Changes in intensity over time
- 2D to 3D segmentation

Open image + TrackMate

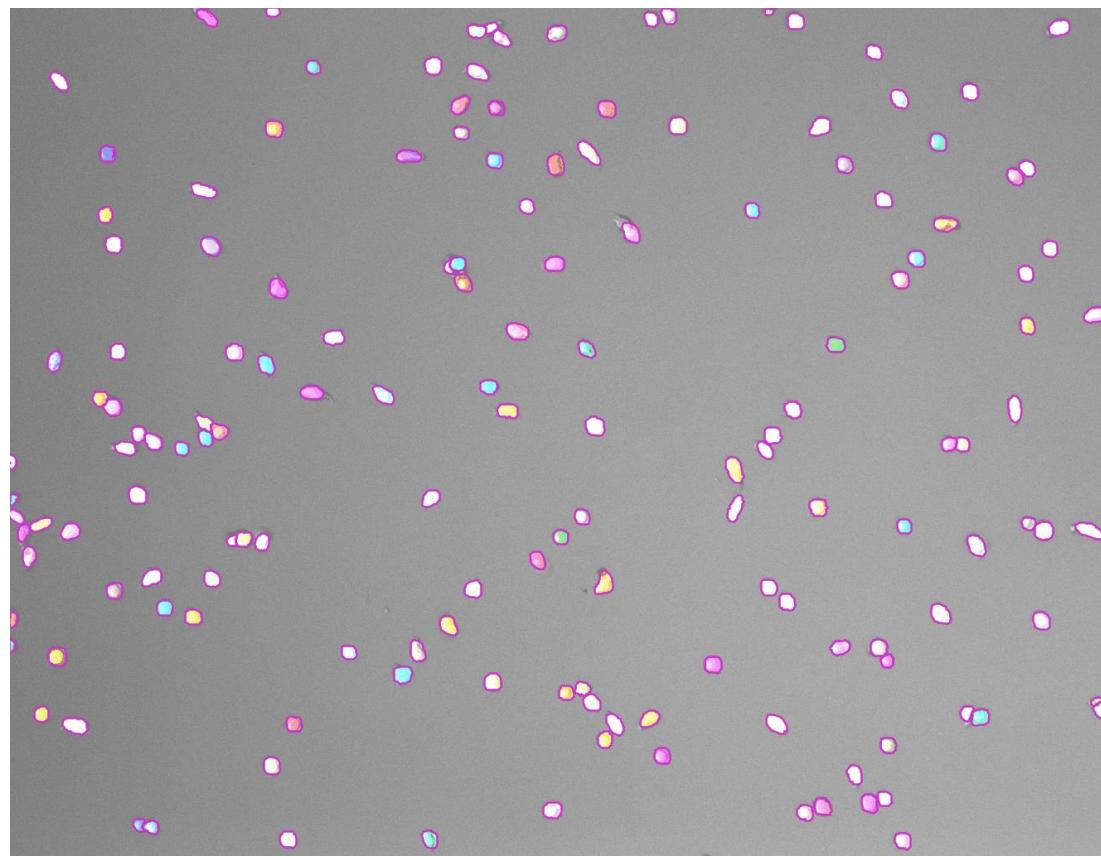
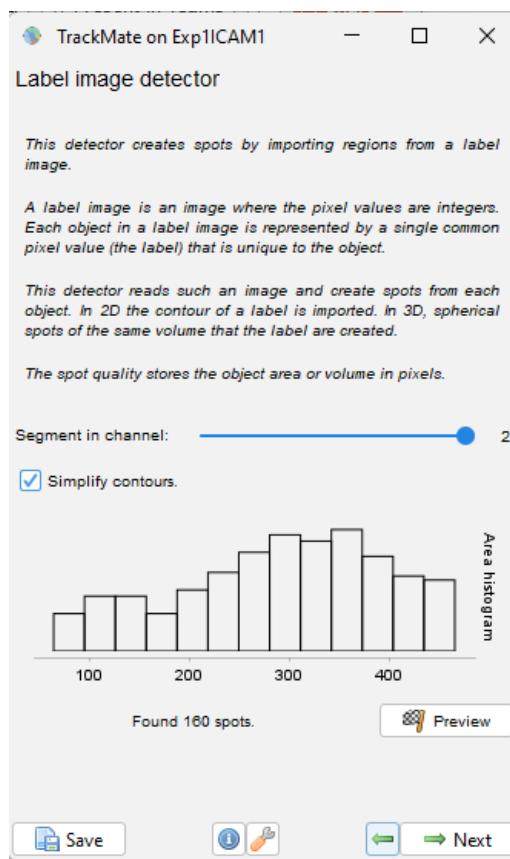
Make sure
your
calibration
is right



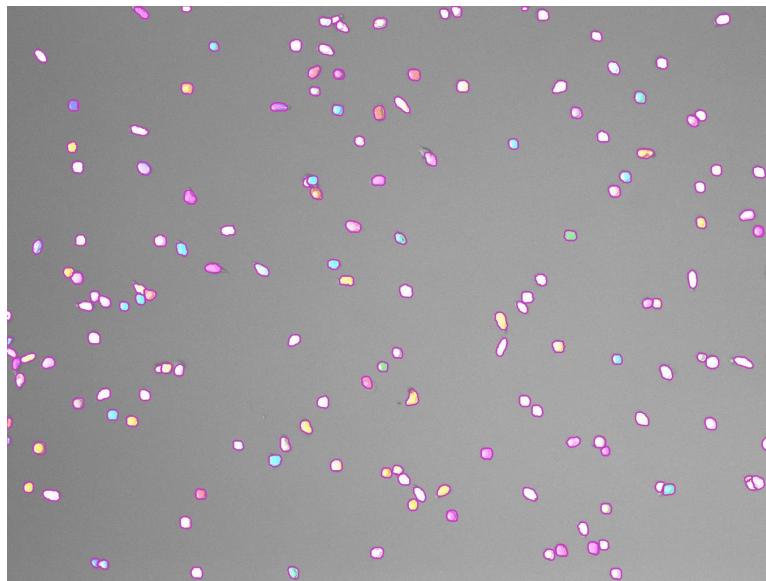
TrackMate object detection



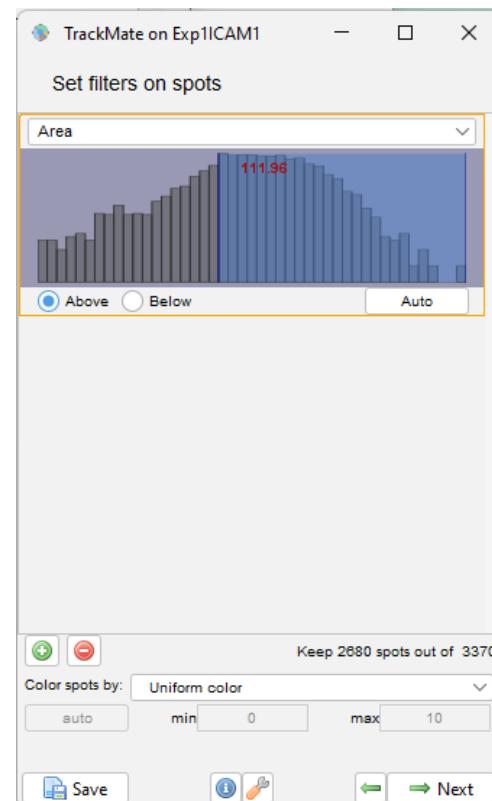
TrackMate label detector



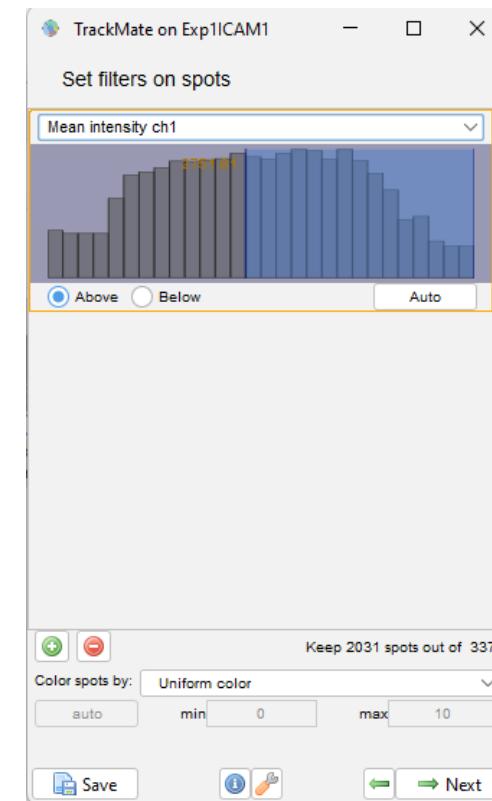
TrackMate spot filtering



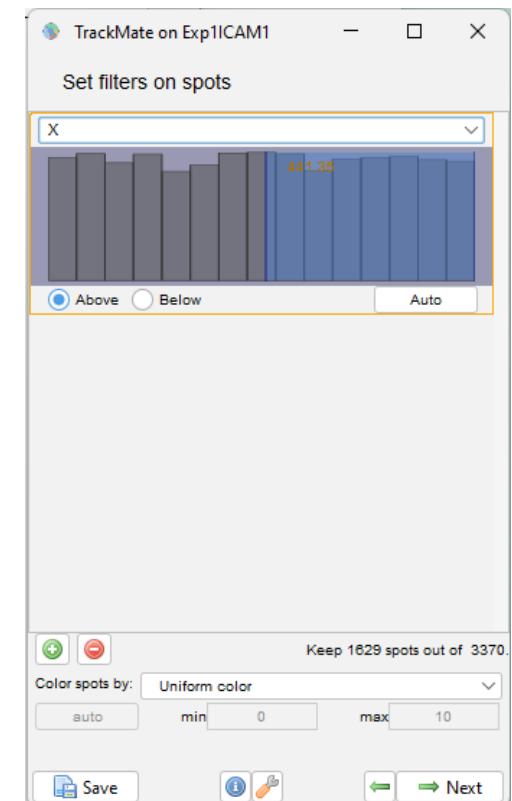
Shape /area



Intensity

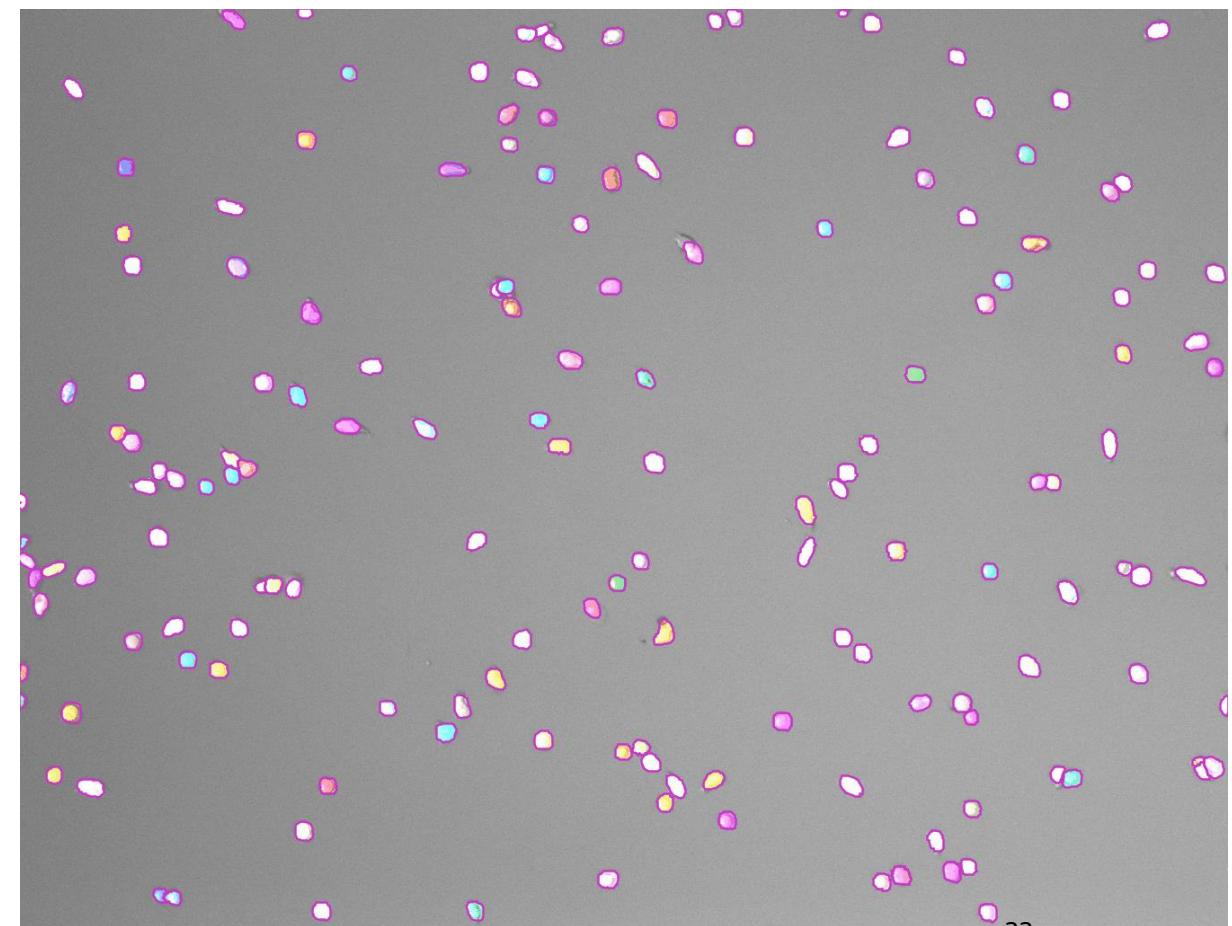
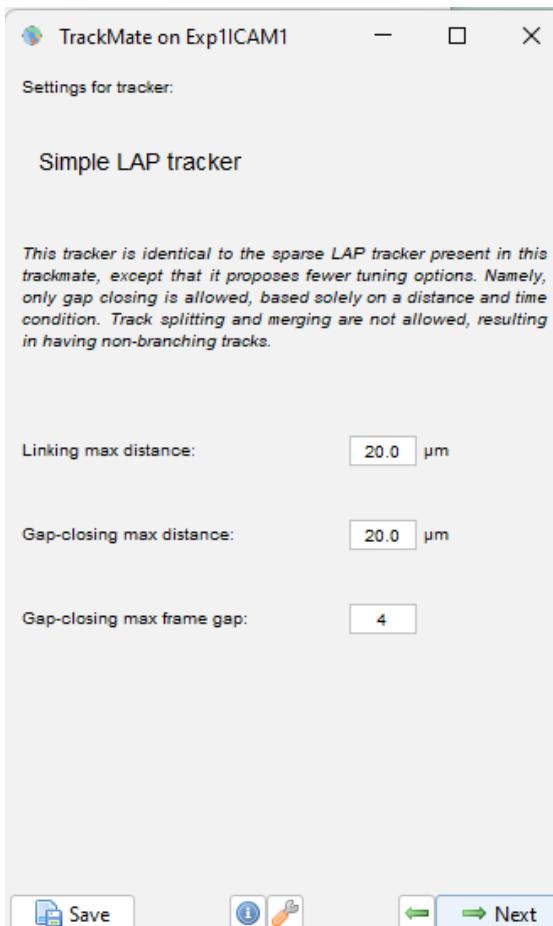
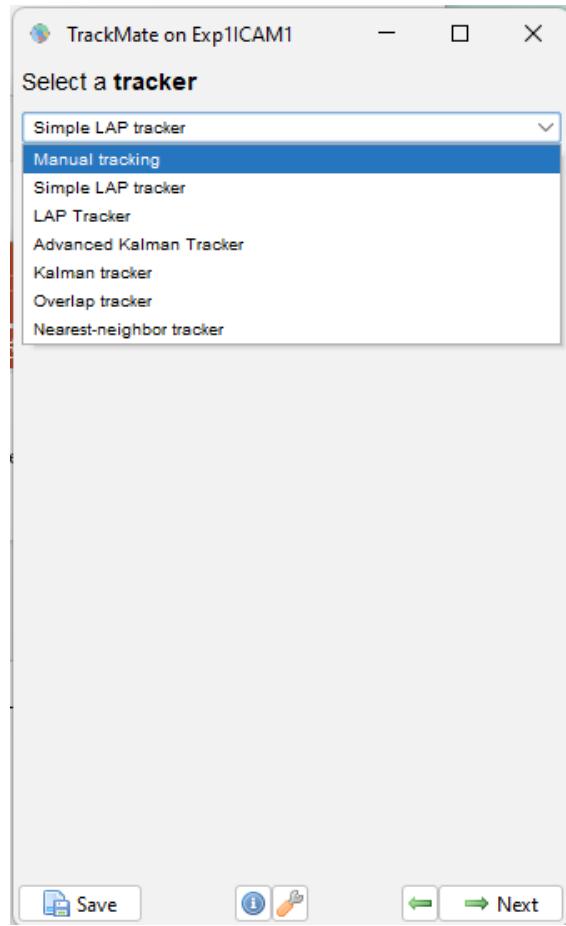


Location

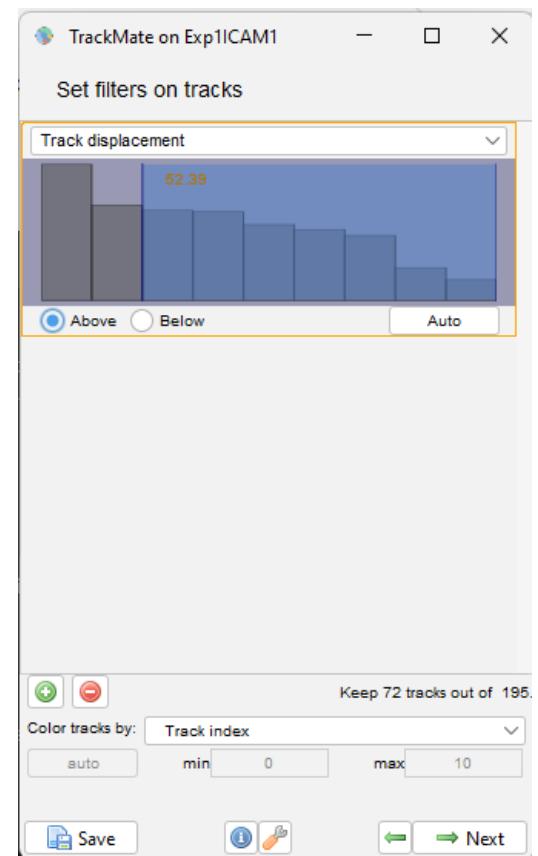
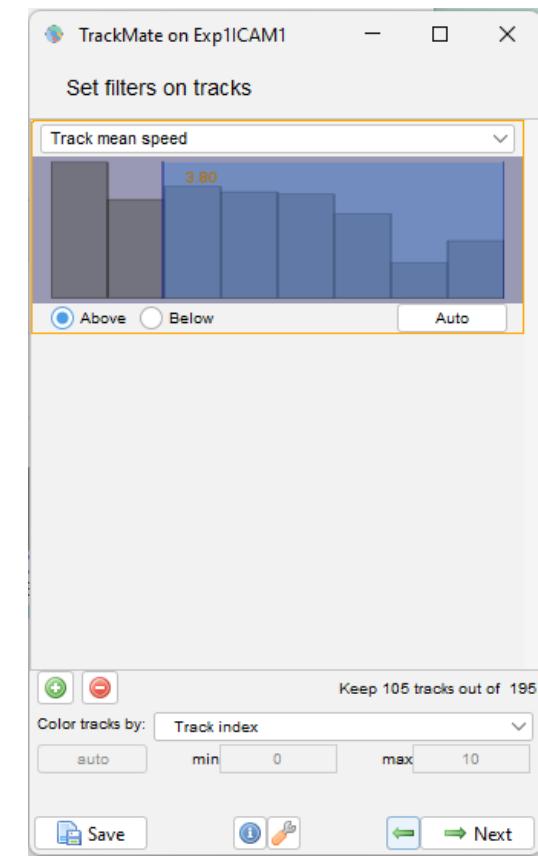
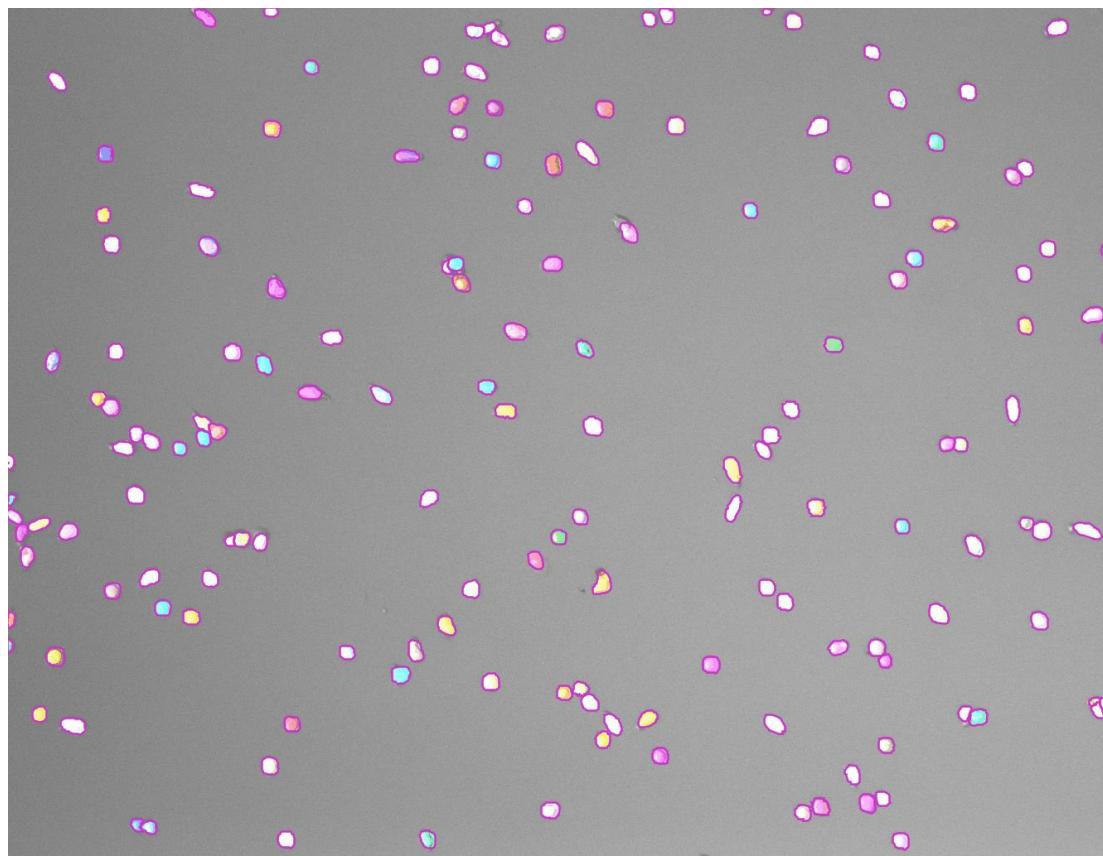


TrackMate Tracking

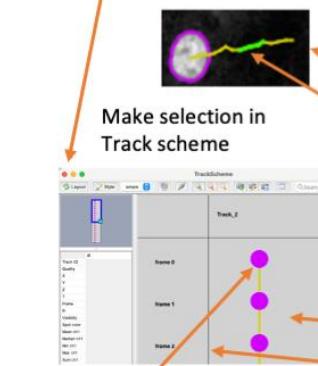
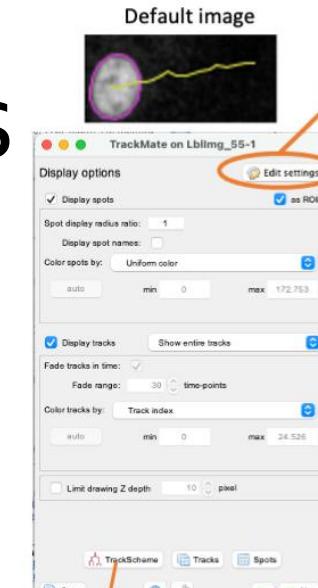
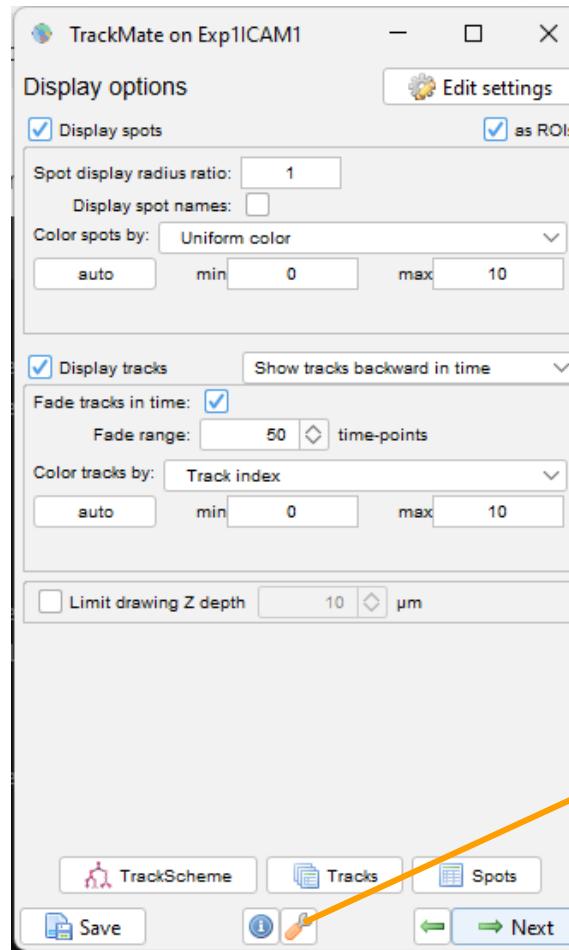
Prior knowledge of your dataset is essential



TrackMate Track Filtering



Visualization of Tracks

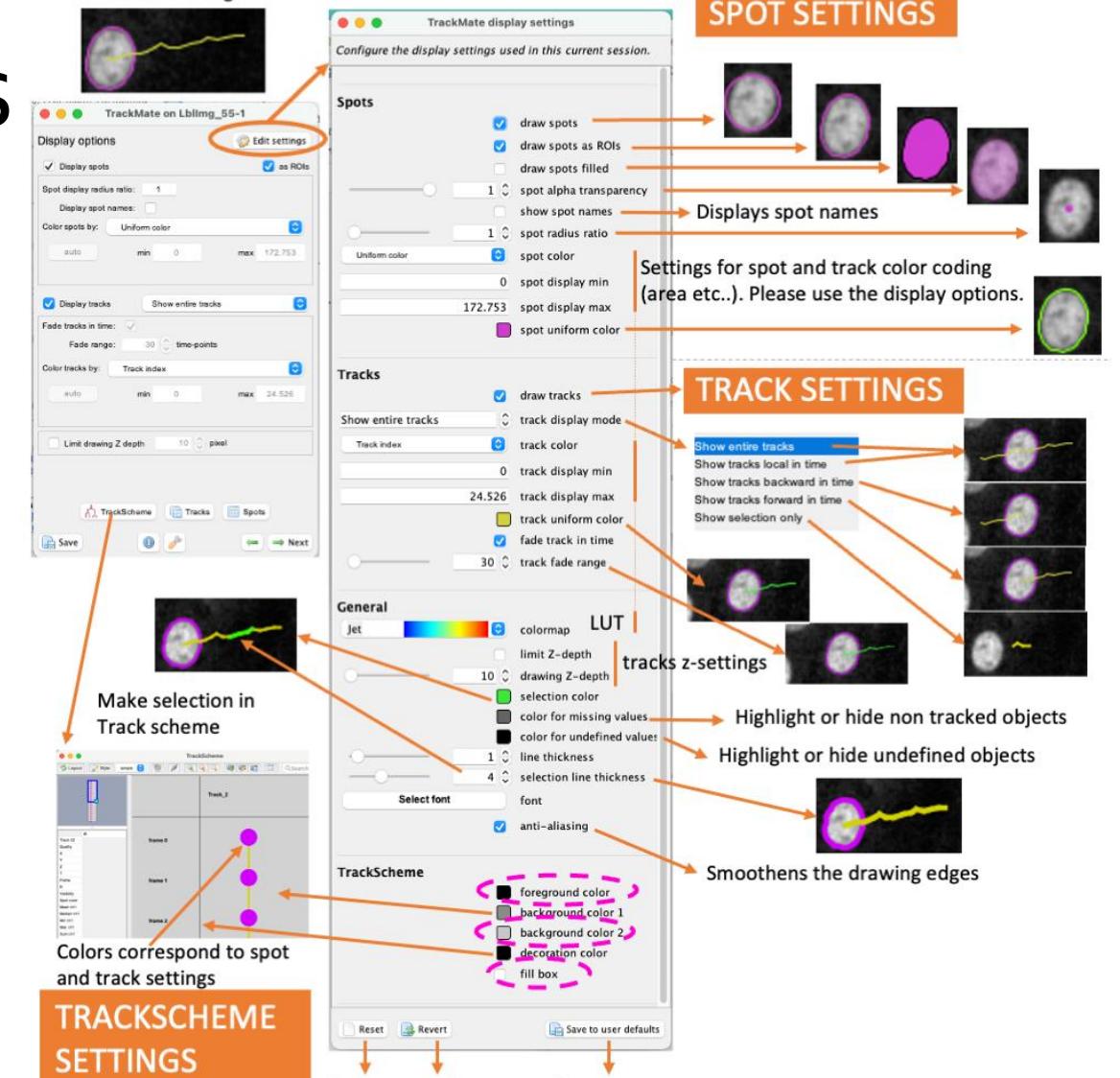


TRACKSCHEME SETTINGS

Colors correspond to spot and track settings

Reset to default undo

Save as your favorite settings

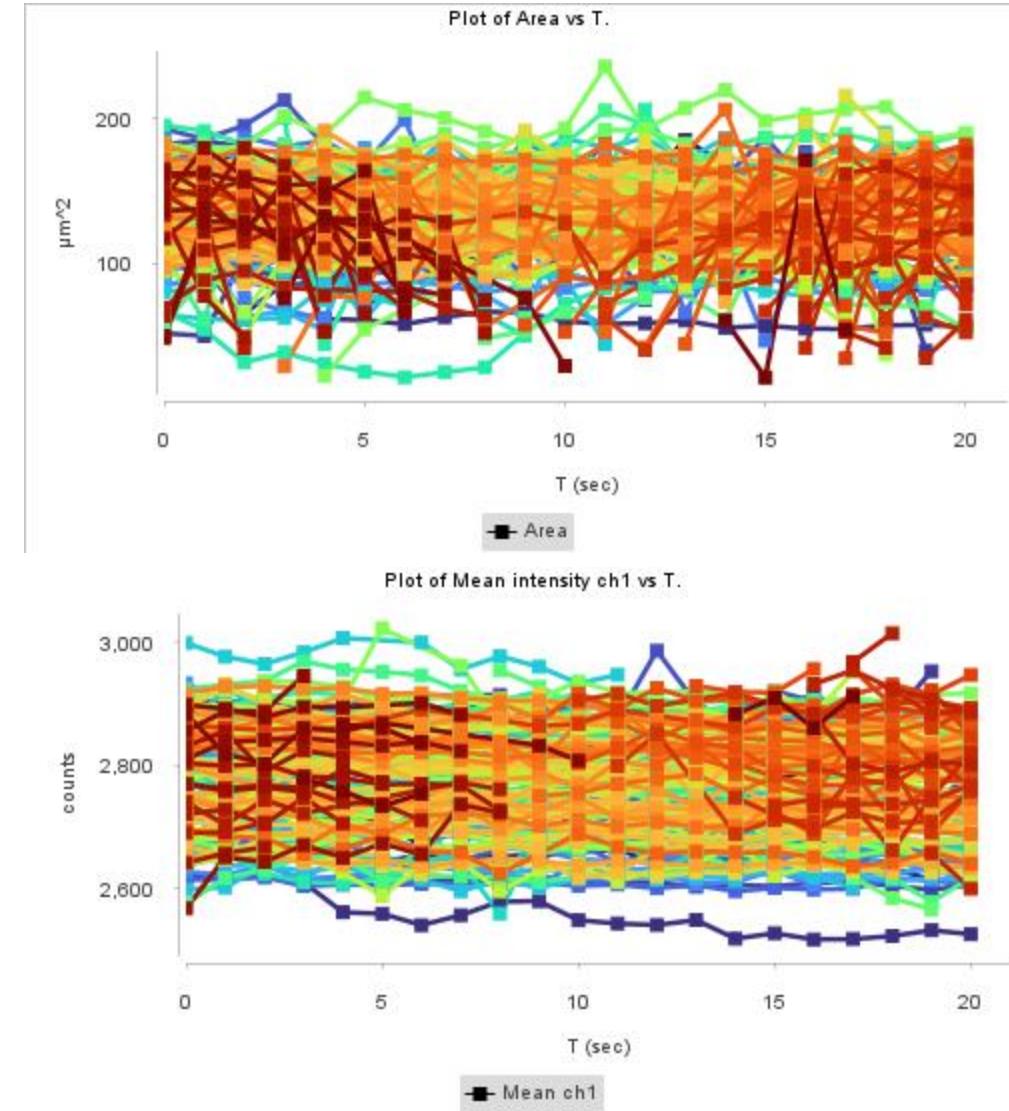


Plotting in Trackmate

The image shows two side-by-side screenshots of the Trackmate software interface, both titled "TrackMate on test_image_Exp...".

Left Screenshot: The "Plot features" dialog is open. The X-axis feature is set to "T" and the Y-axis feature is set to "Mean intensity ch1". A dropdown menu for "Radius" is visible, listing various track features. At the bottom, there are buttons for "Save" and "Next".

Right Screenshot: The "Plot features" dialog is also open. The X-axis feature is set to "T" and the Y-axis feature is set to "Mean intensity ch1". The "Radius" dropdown menu is open, showing options like "Area", "Circularity", etc. At the bottom, there are buttons for "Save" and "Next".



Exporting data in TrackMate

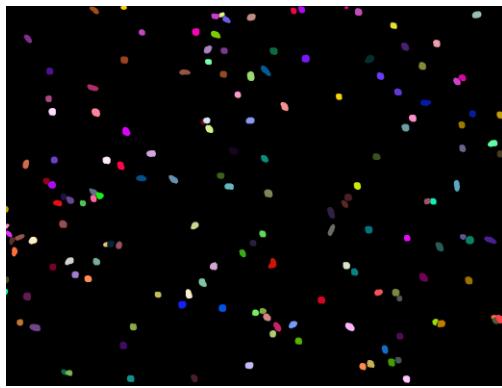
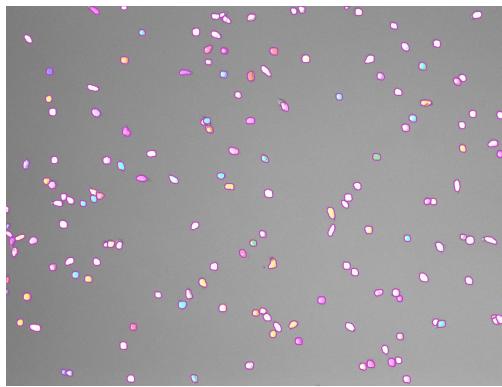
The image shows the TrackMate software interface. On the left is the 'Display options' panel, which includes settings for displaying spots (radio button selected) and tracks. It has sections for 'Spot display radius ratio' (set to 1), 'Display spot names' (unchecked), 'Color spots by' (Uniform color), and 'Display tracks' (checked). Below these are 'Fade tracks in time' (checked), 'Fade range' (set to 50), 'Color tracks by' (Track index), and 'Limit drawing Z depth' (set to 10 µm). At the bottom of the panel are tabs for 'TrackScheme', 'Tracks', and 'Spots', with 'Save' highlighted by a yellow circle. On the right is a 'Track tables' window titled 'Export to CSV'. It contains three tabs: 'Spots', 'Edges', and 'Tracks'. The 'Tracks' tab displays a table of tracking data with columns for ID, Label, Spot ID, Track ID, Quality, X (µm), Y (µm), Z (µm), T (sec), Frame, R (µm), and Vt. A yellow arrow points from the 'Save' button in the Trackmate window to the 't-cell-dataset_tracking_settings' file shown below. Another yellow arrow points from the 'Edit settings' button in the top right of the main window to the 't-cell-dataset_tracking_settings' file.

Spots	Label	Spot ID	Track ID	Quality (quality)	X (µm)	Y (µm)	Z (µm)	T (sec)	Frame	R (µm)	Vt
Edges	ID4352	4352	0	420	354.386	208.813	0	8	8	7.458	
	ID3841	3841	0	306	356.975	208.412	0	12	12	6.313	
	ID2308	2308	0	308	355.35	211.924	0	1	1	6.407	
	ID2020	2020	0	327	357.188	210.079	0	20	20	6.601	
	ID3877	3877	0	293	357.077	208.42	0	13	13	6.218	
	ID3014	3014	0	332	356.529	208.689	0	6	6	6.636	
	ID4328	4328	0	280	355.927	210.309	0	15	15	6.029	
	ID4014	4014	0	366	356.173	208.804	0	7	7	6.981	
	ID4238	4238	0	277	355.378	209.826	0	3	3	6.062	
	ID3854	3854	0	305	356.792	209.861	0	18	18	6.334	
	ID4752	4752	0	347	352.238	213.473	0	0	0	6.818	
	ID4753	4753	0	294	357.294	208.308	0	11	11	6.224	
	ID2449	2449	0	292	356.258	210.029	0	4	4	6.192	
	ID4435	4435	0	281	356.187	209.267	0	17	17	6.095	
	ID2099	2099	0	304	357.087	210.905	0	19	19	6.355	

TrackMate settings file
t-cell-dataset_tracking_settings 24/09/2024 16.01 XML File 7 730 KB

26

Exporting in TrackMate



TrackMate on test_image_Exp...

Select an action

- Capture overlay
- Export to CTC format
- Capture overlay**
- Compute distance to ROIs
- Export tracks to XML file
- Extract track stack
- Export spots to IJ ROIs
- Export to ISBI challenge file format
- Export tracks to Icy
- Export label image
- Merge a TrackMate file
- Export to MotilityLab spreadsheet
- Plot N spots vs time
- Recompute all features
- Branch hierarchy analysis
- Trim non-visible data

Execute

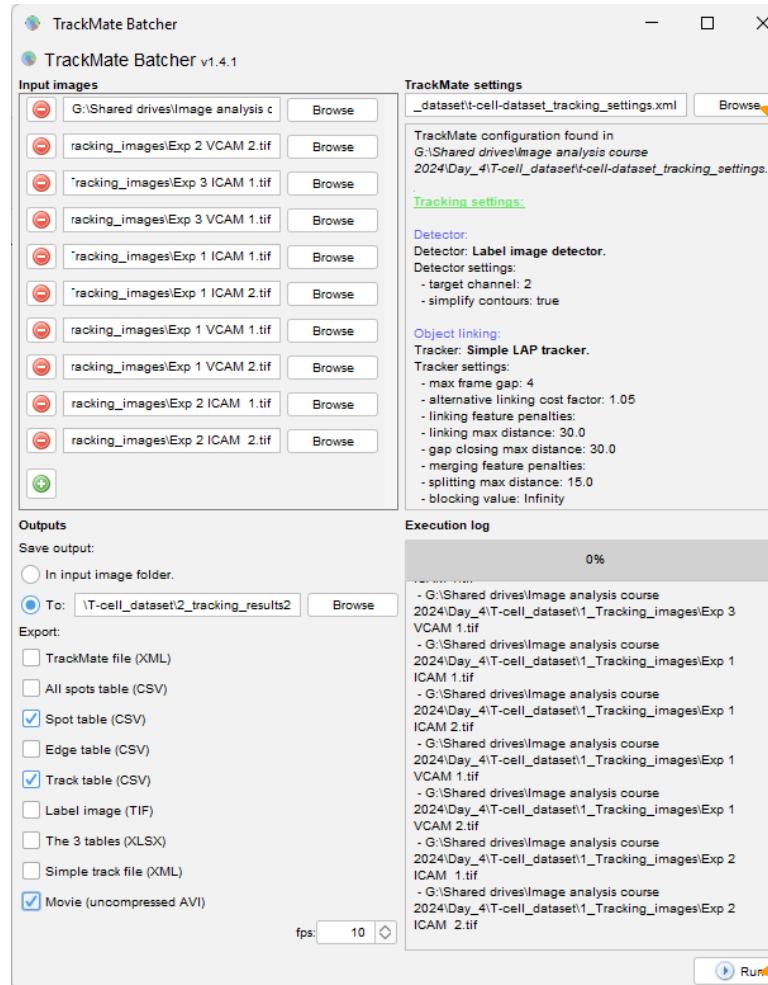
Save

Next

A screenshot of the TrackMate software interface. The window title is "TrackMate on test_image_Exp...". The main area is titled "Select an action" and lists various export and processing options. The option "Capture overlay" is highlighted with a blue selection bar. At the bottom right, there is a button labeled "Execute" which is also circled in orange. Below the list of actions are standard file and navigation buttons: "Save", "Info", "Edit", and "Next".

What if you have multiple movies to track?

Drag and drop
the files to track



Export settings

Path to TrackMate
settings file

`t-cell-dataset_tracking_settings`

Log window

Start!

Output:

Name	Date modified	Type	Size
Exp 1 ICAM 1-movie	25/09/2024 15.06	AVI File	84 677 KB
Exp 1 ICAM 1-spots	25/09/2024 15.06	Microsoft Excel C...	1 653 KB
Exp 1 ICAM 1-tracks	25/09/2024 15.06	Microsoft Excel C...	66 KB
Exp 1 ICAM 2-movie	25/09/2024 15.06	AVI File	84 677 KB
Exp 1 ICAM 2-spots	25/09/2024 15.06	Microsoft Excel C...	1 900 KB
Exp 1 ICAM 2-tracks	25/09/2024 15.06	Microsoft Excel C...	74 KB
Exp 1 VCAM 1-movie	25/09/2024 15.06	AVI File	84 677 KB
Exp 1 VCAM 1-spots	25/09/2024 15.06	Microsoft Excel C...	2 560 KB
Exp 1 VCAM 1-tracks	25/09/2024 15.06	Microsoft Excel C...	94 KB
Exp 1 VCAM 2-movie	25/09/2024 15.06	AVI File	84 677 KB
Exp 1 VCAM 2-spots	25/09/2024 15.06	Microsoft Excel C...	2 189 KB
Exp 1 VCAM 2-tracks	25/09/2024 15.06	Microsoft Excel C...	81 KB
Exp 2 ICAM 1-movie	25/09/2024 15.06	AVI File	84 677 KB
Exp 2 ICAM 1-spots	25/09/2024 15.06	Microsoft Excel C...	1 303 KB
Exp 2 ICAM 1-tracks	25/09/2024 15.06	Microsoft Excel C...	49 KB
Exp 2 ICAM 2-movie	25/09/2024 15.07	AVI File	84 677 KB
Exp 2 ICAM 2-spots	25/09/2024 15.07	Microsoft Excel C...	1 350 KB
Exp 2 ICAM 2-tracks	25/09/2024 15.07	Microsoft Excel C...	50 KB
Exp 2 VCAM 1-movie	25/09/2024 15.07	AVI File	84 677 KB
Exp 2 VCAM 1-spots	25/09/2024 15.07	Microsoft Excel C...	2 058 KB
Exp 2 VCAM 1-tracks	25/09/2024 15.07	Microsoft Excel C...	75 KB
Exp 2 VCAM 2-movie	25/09/2024 15.07	AVI File	84 677 KB

Trackmate Batcher!!!!

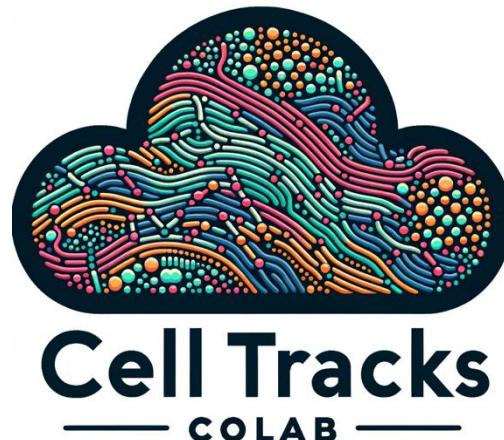
Track Analysis



- Multiple conditions
 - Multiple repeats
 - Multiple FOVs
- Large number of objects per FOV
 - Many parameters
 - Multiple csv files



HOW DO I ANALYZE ALL THIS DATA?



Cloud-based tool for
track analysis

PLOS BIOLOGY

BROWSE PUBLISH ABOUT SEARCH advanced search

OPEN ACCESS PEER-REVIEWED

METHODS AND RESOURCES

CellTracksColab is a platform that enables compilation, analysis, and exploration of cell tracking data

Estibaliz Gómez-de-Mariscal, Hanna Grobe, Joanna W. Pylyvánaien, Laura Xénard, Ricardo Henriques, Jean-Yves Tinevez, Guillaume Jacquemet

Version 2 Published: August 8, 2024 • <https://doi.org/10.1371/journal.pbio.3002740>

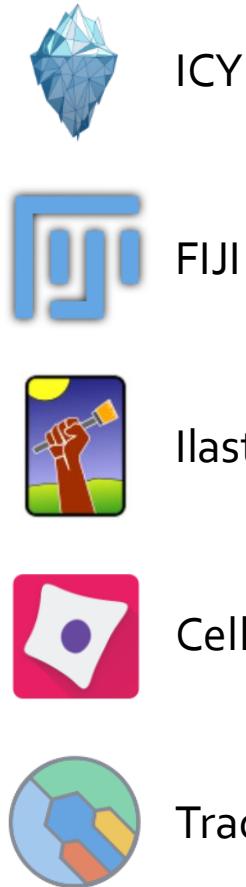
1 Save	0 Citation
1,419 View	29 Share

Download PDF Print Share Check for updates Subject Areas

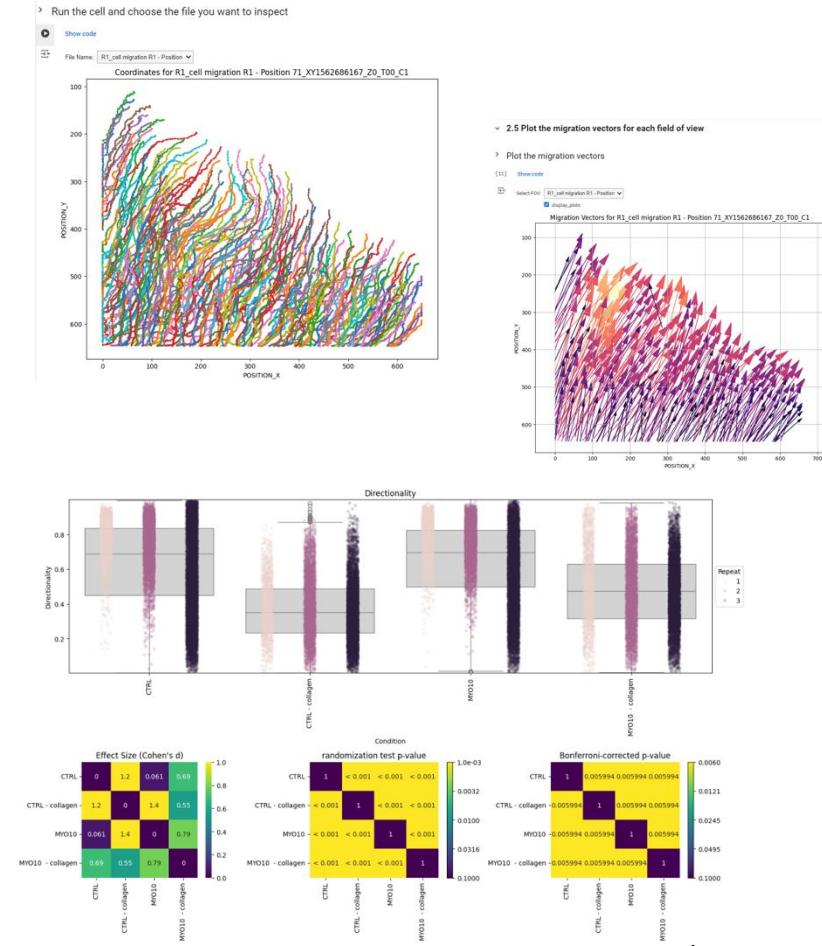
Abstract

In life sciences, tracking objects from movies enables researchers to quantify the behavior of single particles, organelles, bacteria, cells, and even whole animals. While numerous tools now allow automated tracking from video, a significant challenge persists in compiling, analyzing, and exploring the large datasets generated by these approaches. Here, we introduce

What is CellTracksColab?



Track ID	X	Y	Z	t
0	6.8	15	1.0	1
1	7.9	0.0	2.6	2
2	9.1	4.7	5.9	3
3	5.9	8.5	3.7	4
4	25	3.0	6.5	5
5	67	2.4	5.1	6
6	91	5.3	1.3	7
...



When it runs?



Google Colab



Locally using Google Colab



Locally using Jupyter

<https://github.com/CellMigrationLab/CellTracksColab>

CellTracksColab Workflow

Prepare Data

Load csv files generated from multiple tracking tools

Compiling data

CellTracksColab format

Viewing tracks

Viewing, filtering and smoothing of tracks

Track Analysis

Plotting of conditions, extracting sub-populations

Requirements for CellTracksColab

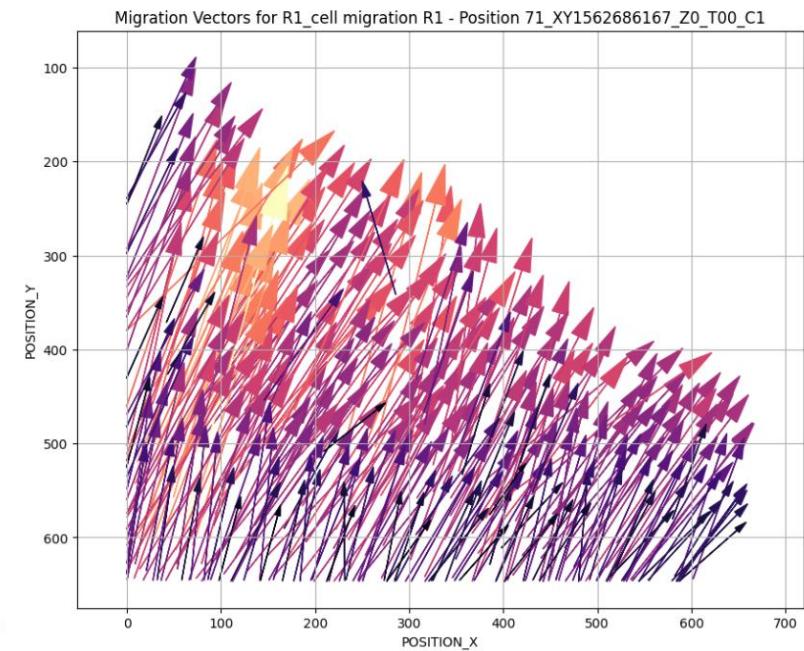
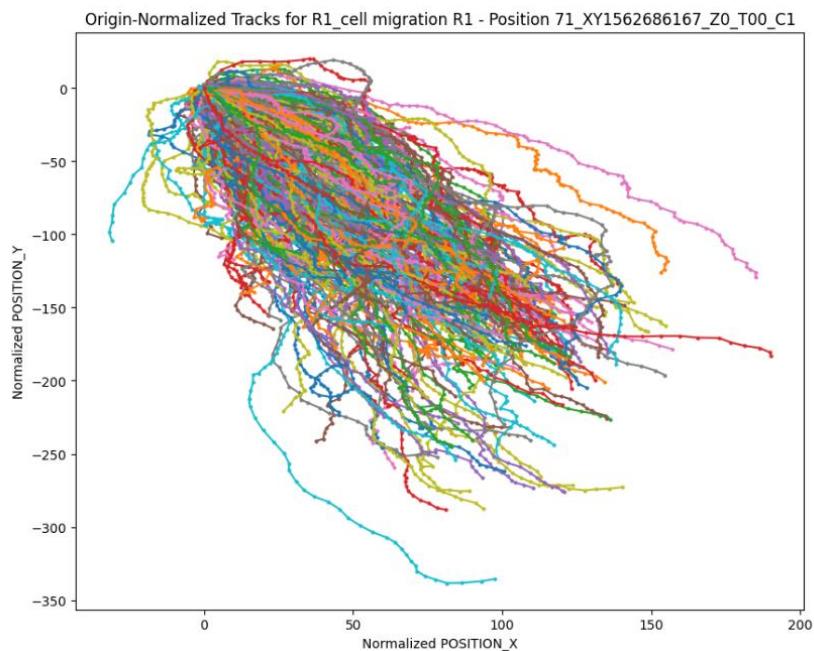
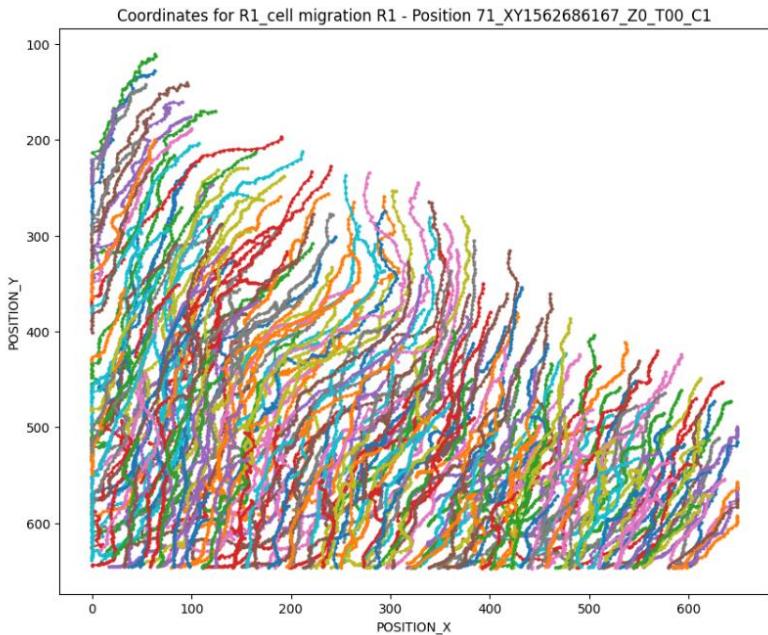
- Experiments [Folder_path]
 - Condition_1 ['condition' is derived from this folder name]
 - R1 ['repeat' is derived from this folder name]
 - FOV1.csv
 - FOV2.csv
 - R2
 - FOV1.csv
 - FOV2.csv
 - Condition_2
 - R1
 - R2

Minimum requirements for data

Track ID	X Coordinate	Y Coordinate	Z Coordinate	Time Point
0	687.991	150.047	0.0	0.0
1	1255.994	467.008	0.0	0.0
2	171.994	853.024	0.0	0.0
... (and so on)				

- represents the main folder or directory
- represents the condition folders.
- represents the repeat folders.
- represents the individual CSV files.

Track Visualization



- By repeat
- By condition

Track filtering

Smoothing Neighbors: 3

Duration: 168.75 – 940.00

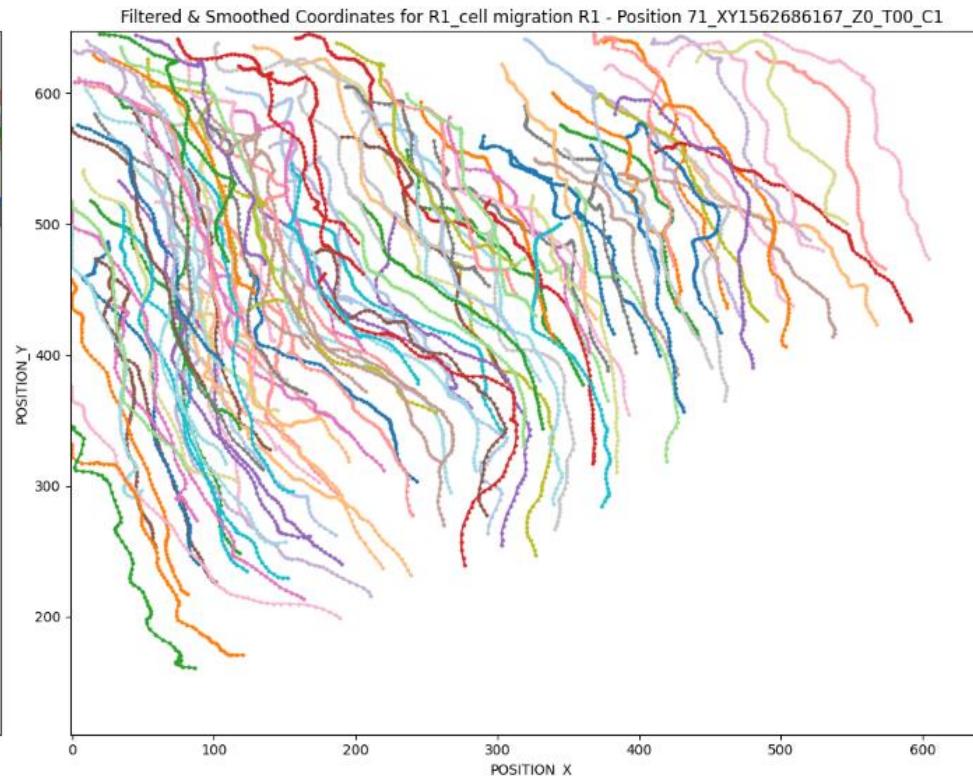
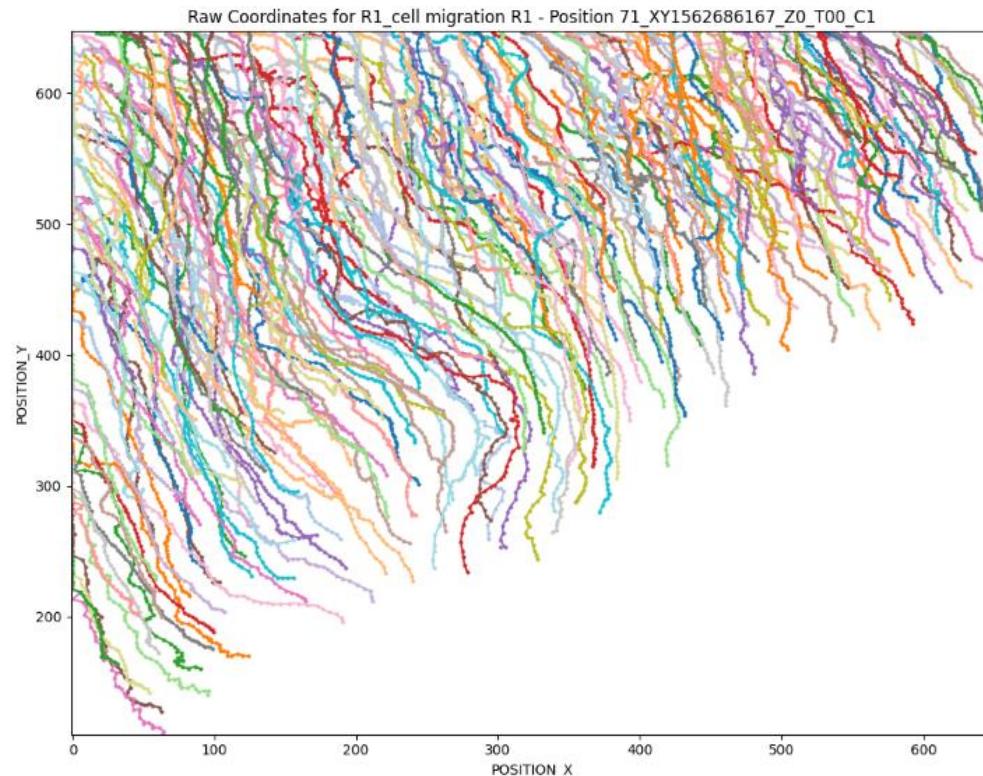
Mean Speed: 0.10 – 2.85

Max Speed: 0.20 – 4.56

Min Speed: 0.00 – 1.49

Total Distance: 226.61 – 934.07

Apply Filters



Track features

These metrics can be computed in the TrackMate, Custom, or Viewer notebooks:

- **Track Duration:** The total duration of the track.
- **Mean Speed:** The average speed of the track.
- **Max Speed:** The maximum speed recorded for the track.
- **Min Speed:** The minimum speed recorded for the track.
- **Speed Standard Deviation:** The standard deviation of the speeds recorded for the track.
- **Total Distance Traveled:** The cumulative distance traveled by the track.
- **Directionality:** Provides a measure of the overall direction of a track, indicating the straightness of the path taken.
- **Tortuosity:** Measures how convoluted or twisted a path is, with a value of 1 indicating a straight path and higher values indicating more twists and turns.
- **Total Turning Angle:** Indicates the cumulative amount of turning along the path, with higher values indicating more turning.
- **Spatial Coverage:** Represents the area (2D) or volume (3D) covered by the object's movement.

Spot features

These metrics are derived from the information provided by [TrackMate](#) in the spots table:

- **Intensity Metrics:** Mean, median, min, max, total, and standard deviation of intensities in different channels.
 - Examples: MEAN_INTENSITY_CH1, MEDIAN_INTENSITY_CH1, MIN_INTENSITY_CH1, MAX_INTENSITY_CH1, TOTAL_INTENSITY_CH1, STD_INTENSITY_CH1
- **Shape Metrics:** Ellipse parameters, area, perimeter, circularity, solidity, and shape index.
 - Examples: ELLIPSE_X0, ELLIPSE_Y0, ELLIPSE_MAJOR, ELLIPSE_MINOR, ELLIPSE_THETA, ELLIPSE_ASPECTRATIO, AREA, PERIMETER, CIRCULARITY, SOLIDITY, SHAPE_INDEX

The following is a comprehensive list of potential metrics that can be computed:

- MEAN_INTENSITY_CH1, MEDIAN_INTENSITY_CH1, MIN_INTENSITY_CH1, MAX_INTENSITY_CH1, TOTAL_INTENSITY_CH1, STD_INTENSITY_CH1, CONTRAST_CH1, SNR_CH1
- ELLIPSE_X0, ELLIPSE_Y0, ELLIPSE_MAJOR, ELLIPSE_MINOR, ELLIPSE_THETA, ELLIPSE_ASPECTRATIO, AREA, PERIMETER, CIRCULARITY, SOLIDITY, SHAPE_INDEX
- MEAN_INTENSITY_CH2, MEDIAN_INTENSITY_CH2, MIN_INTENSITY_CH2, MAX_INTENSITY_CH2, TOTAL_INTENSITY_CH2, STD_INTENSITY_CH2, CONTRAST_CH2, SNR_CH2
- MEAN_INTENSITY_CH3, MEDIAN_INTENSITY_CH3, MIN_INTENSITY_CH3, MAX_INTENSITY_CH3, TOTAL_INTENSITY_CH3, STD_INTENSITY_CH3, CONTRAST_CH3, SNR_CH3
- MEAN_INTENSITY_CH4, MEDIAN_INTENSITY_CH4, MIN_INTENSITY_CH4, MAX_INTENSITY_CH4, TOTAL_INTENSITY_CH4, STD_INTENSITY_CH4, CONTRAST_CH4, SNR_CH4

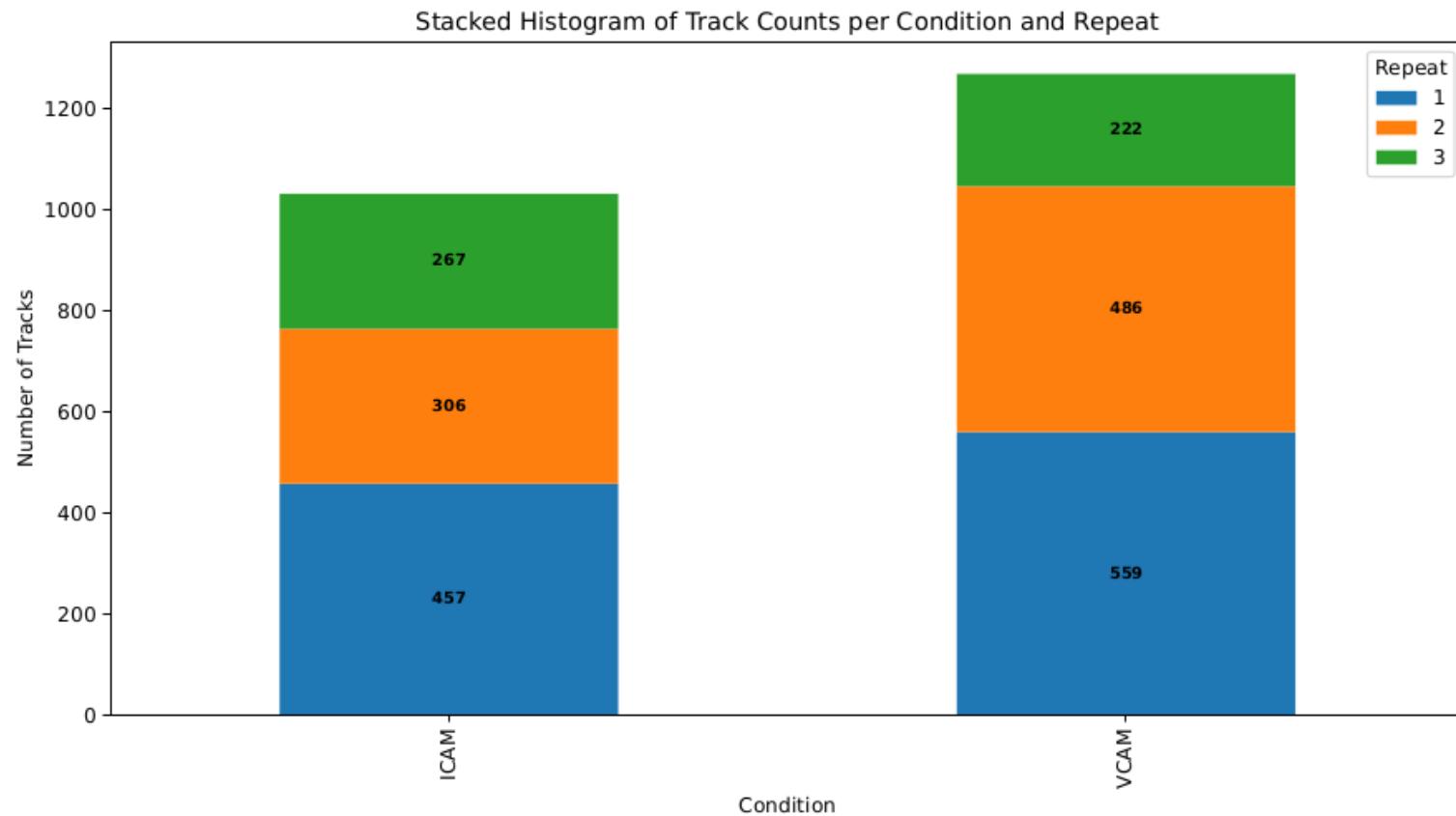
Quality Control

Purpose

- Ensuring a balanced dataset is crucial in cell tracking and similar biological analyses.
- This means that each biological repeat should carry equal weight in the analysis.

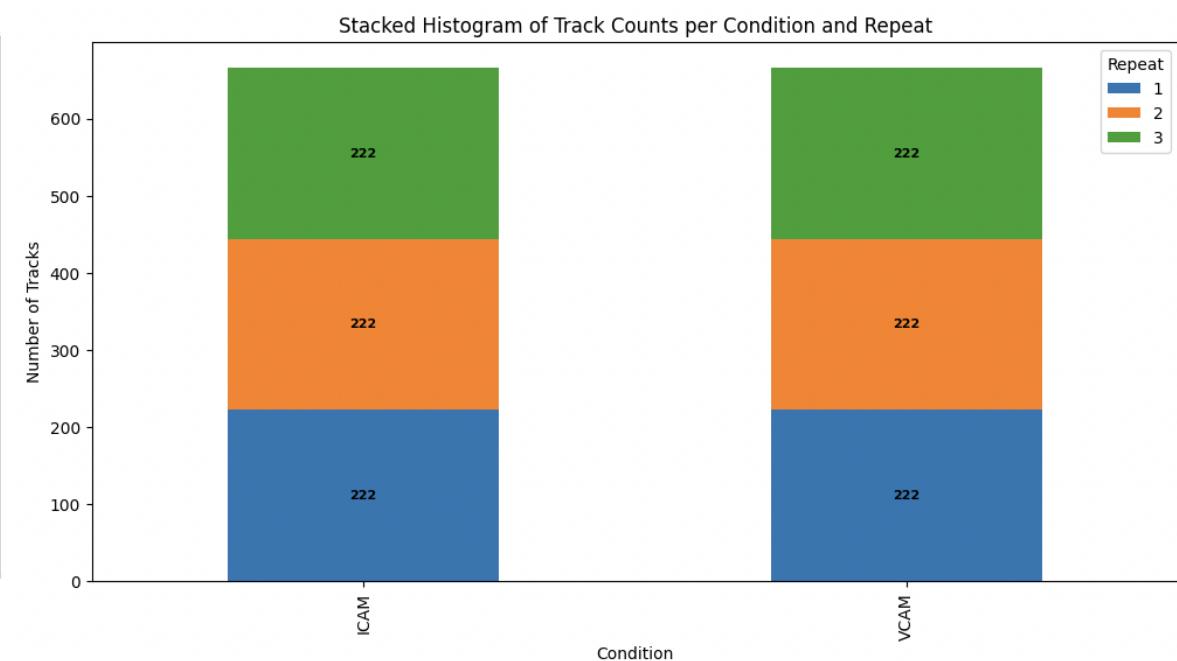
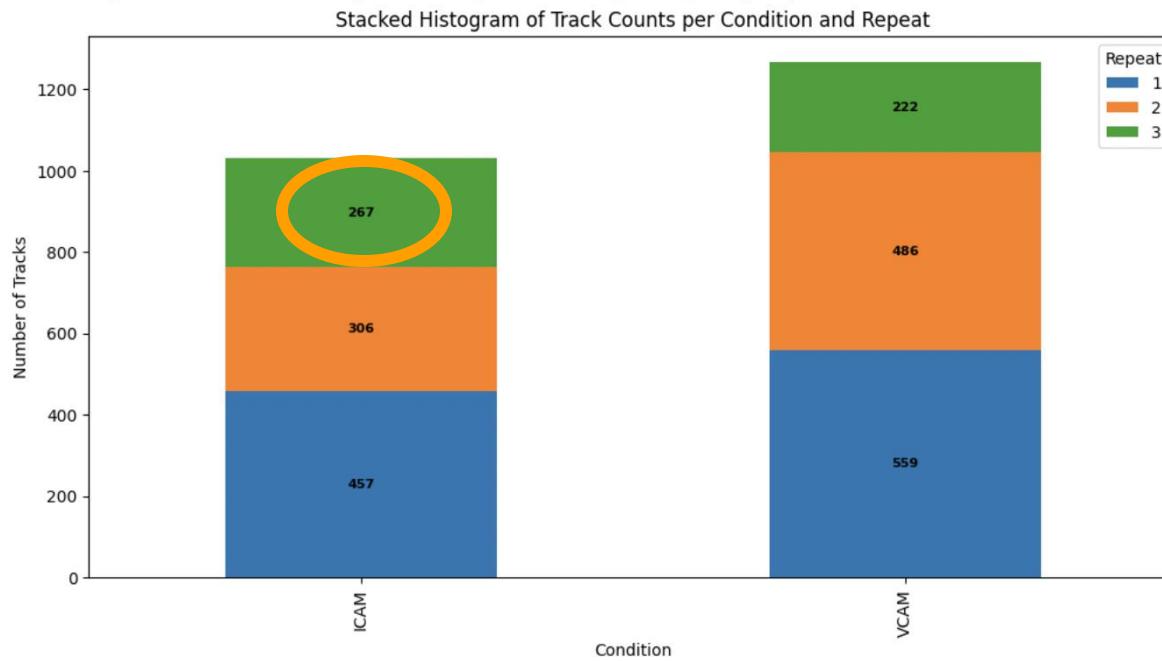
A balanced dataset is essential for:

- Capturing True Biological Variation: Equal weighting ensures accurate representation.
- Reducing Sampling Bias: Balancing the dataset helps avoid overemphasizing characteristics from any single repeat, which might not represent the broader biological context.



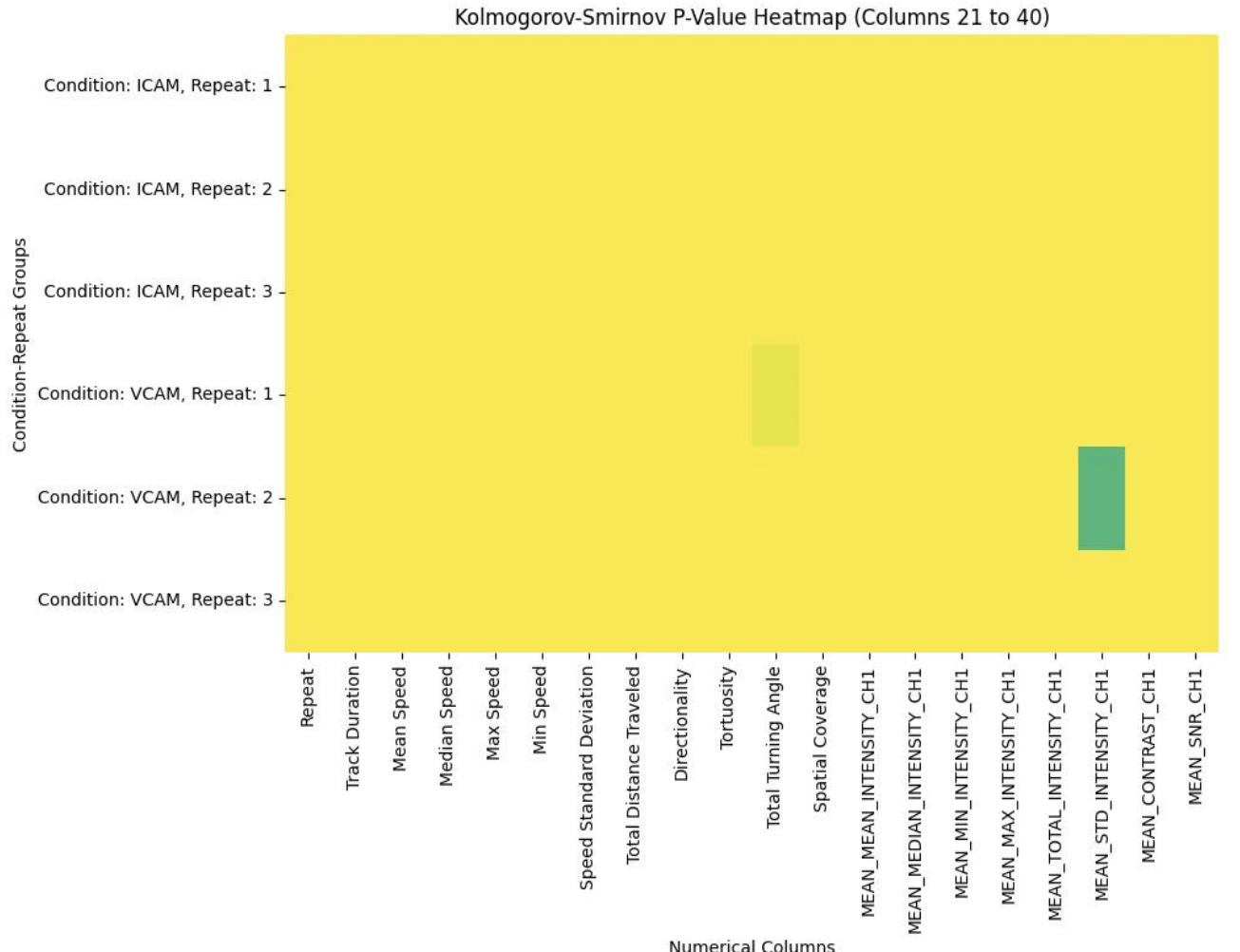
Note: If your data is imbalanced, consider balancing it to prevent skewing your results.

Data balancing



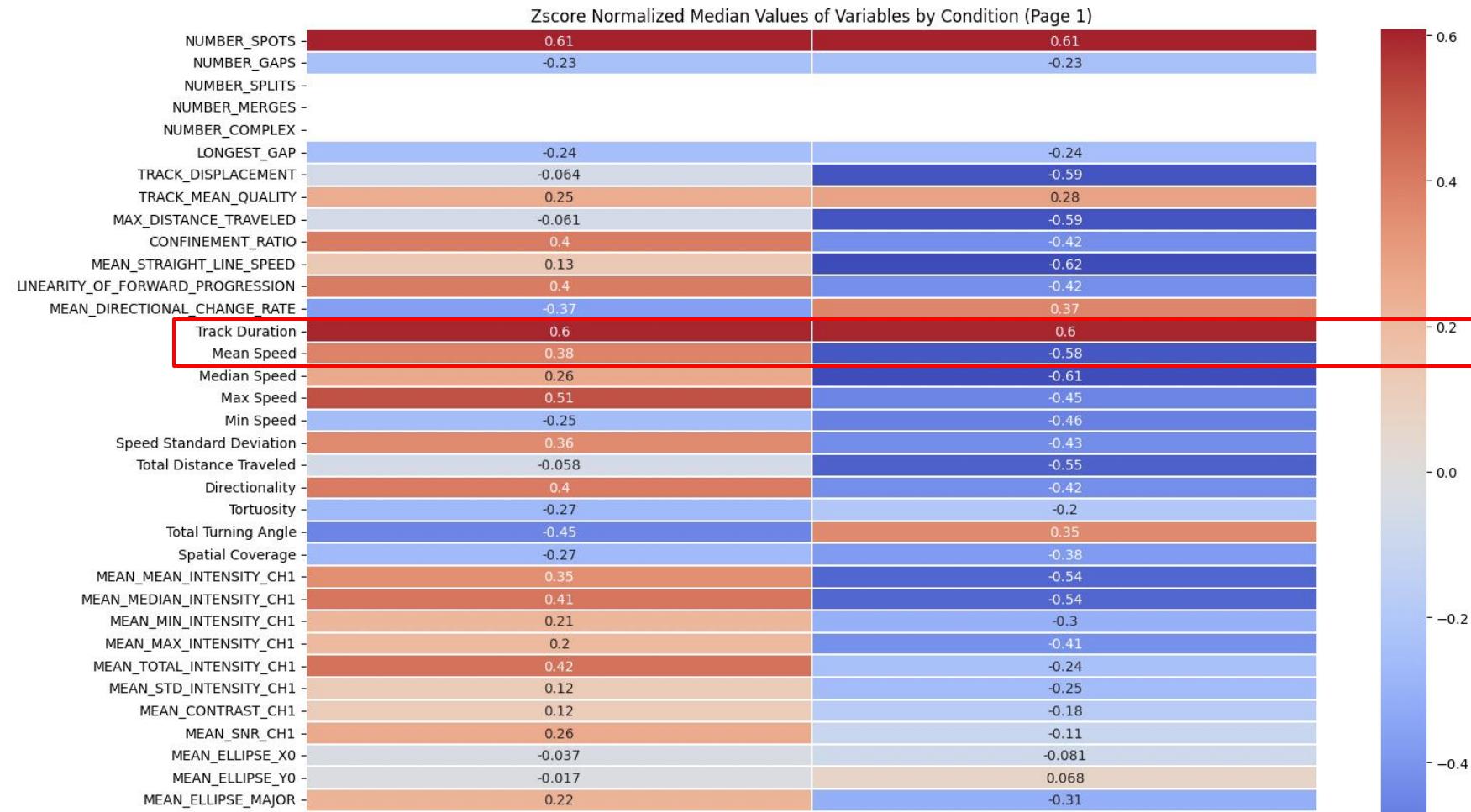
- randomly selecting tracks from these larger groups

Effect of data balancing

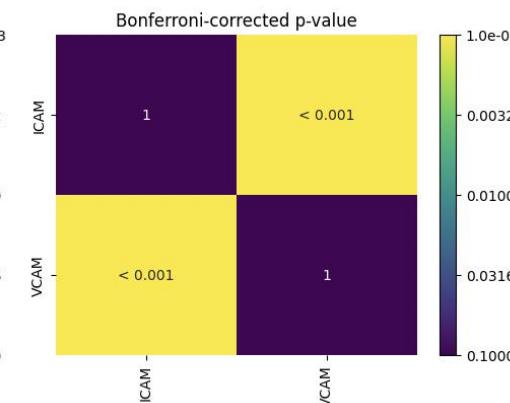
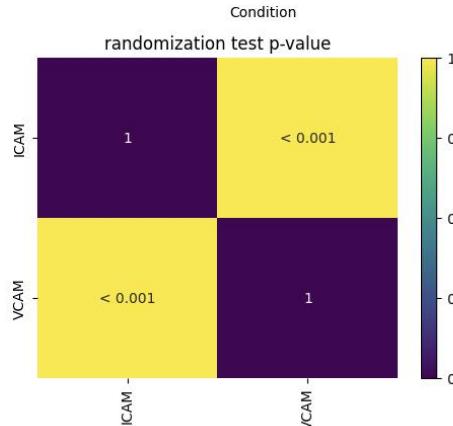
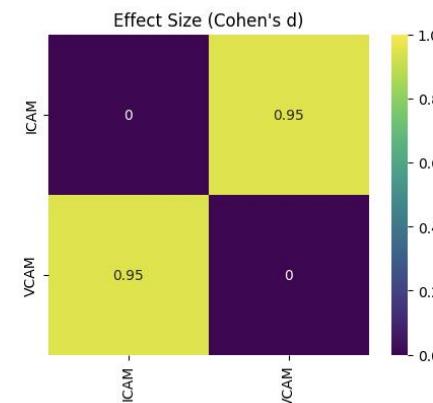
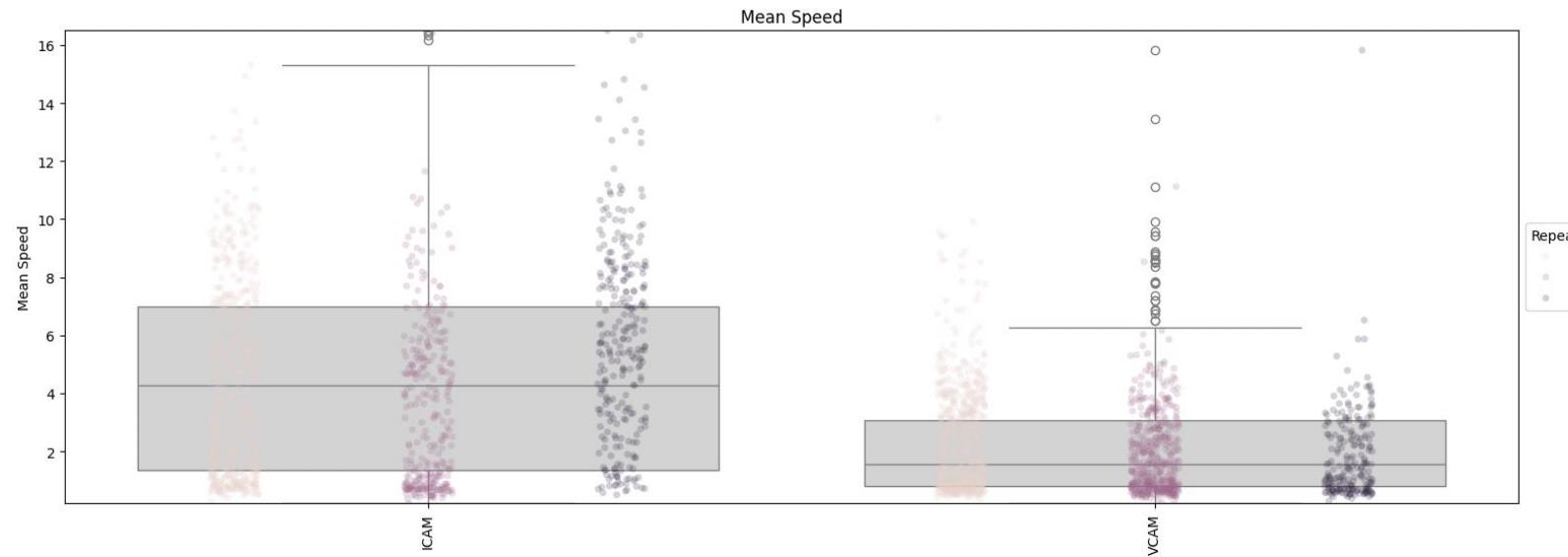


- Compares the distribution of each numerical column in the original and resampled datasets to assess if there is a significant change
- Presents p-values visually
 - providing an easy way to identify significant differences in distributions
- **High P-Values (Yellow):**
 - Indicate that the downsampling process likely did not significantly alter the distribution of that numerical column for the specific condition-repeat group.
- **Low P-Values (Green/blue):**
 - Suggest that the downsampling process may have affected the distribution significantly.

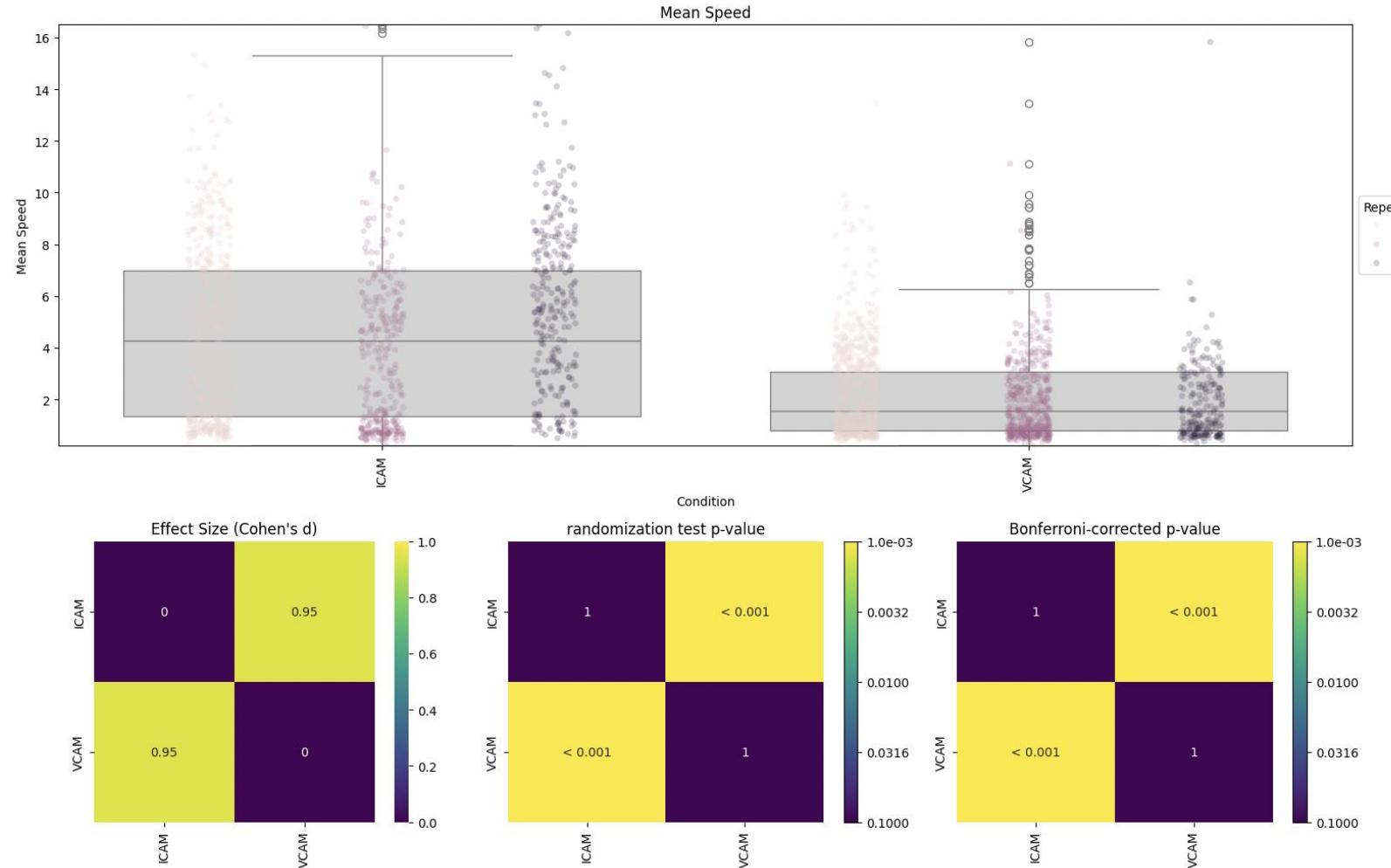
Plot entire dataset at heatmap



Plotting of metrics (unbalanced)



Plotting of metrics (balanced)



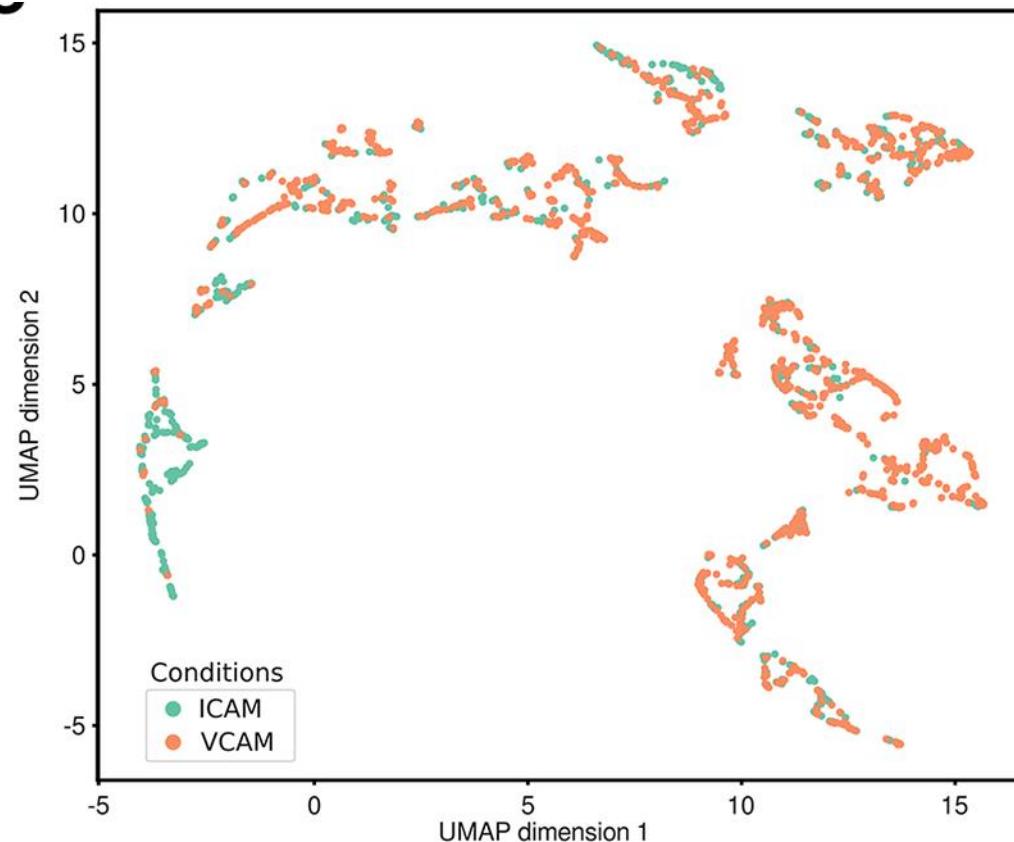
WANT TO COMPARE MANY FEATURES AND FIND SUB- POPULATIONS?

Time for dimension reduction techniques!

Exploring high-dimensional data

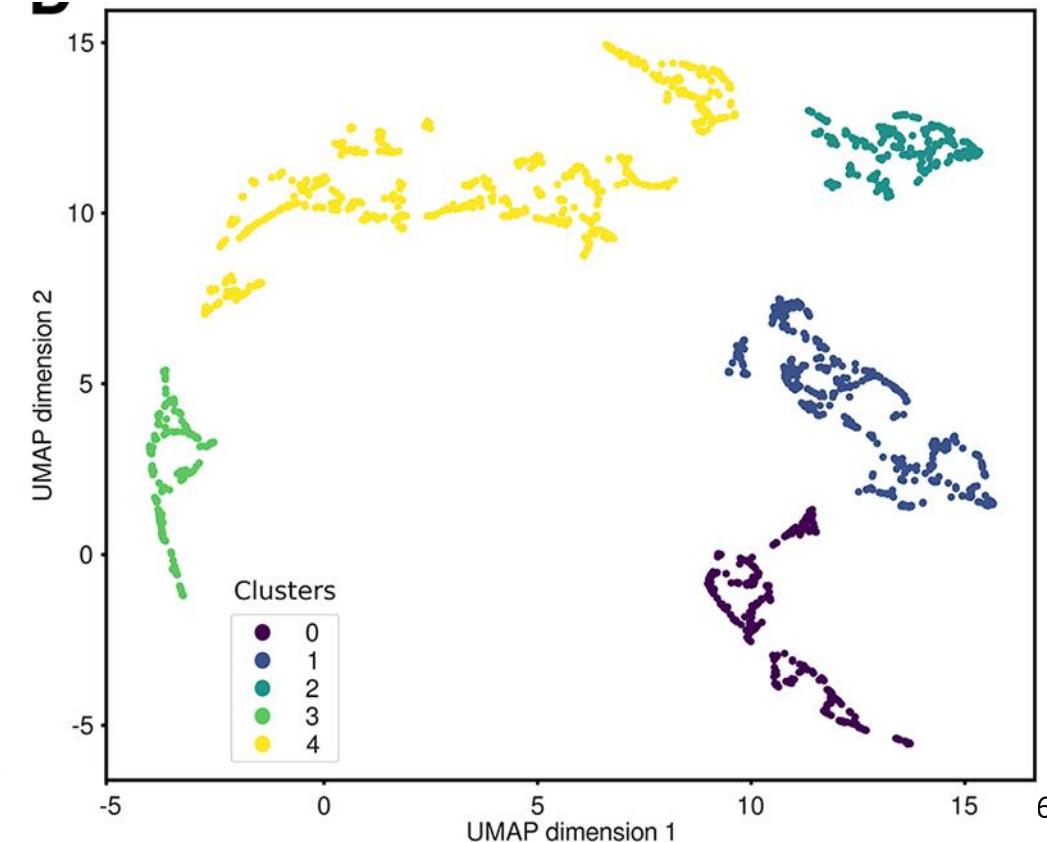
UMAP and t-SNE:

reducing the number of dimensions of complex data by taking data from its original high-dimensional space and shrink it down to 2D



HDBSCAN clustering data from UMAP/t-SNE output:

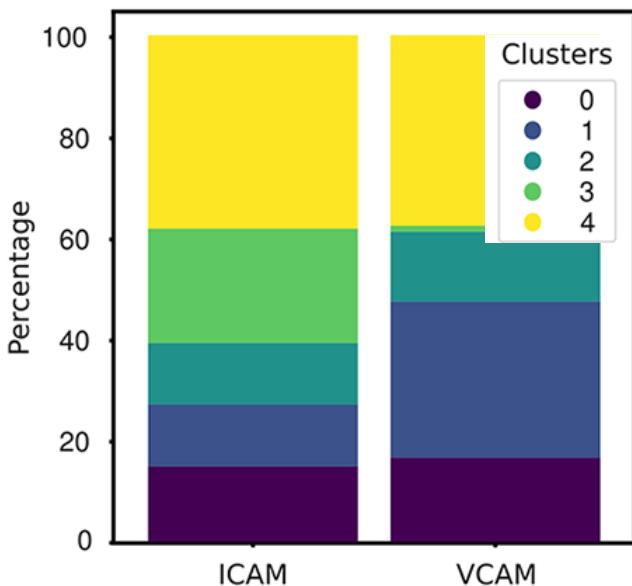
determines clusters based on density and similarity, identifying groups of similar cell tracks.



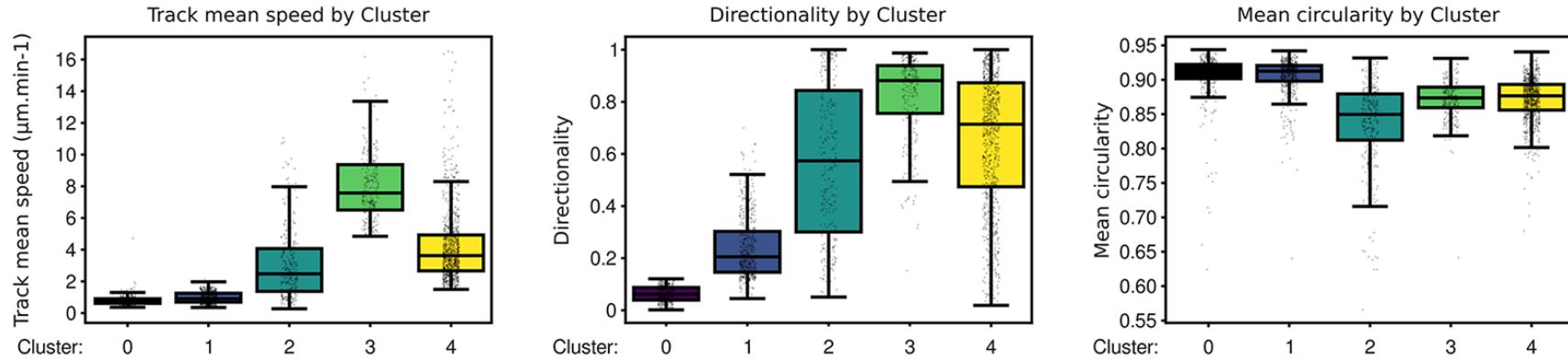
Data fingerprinting and sub-populations

Fingerprinting:

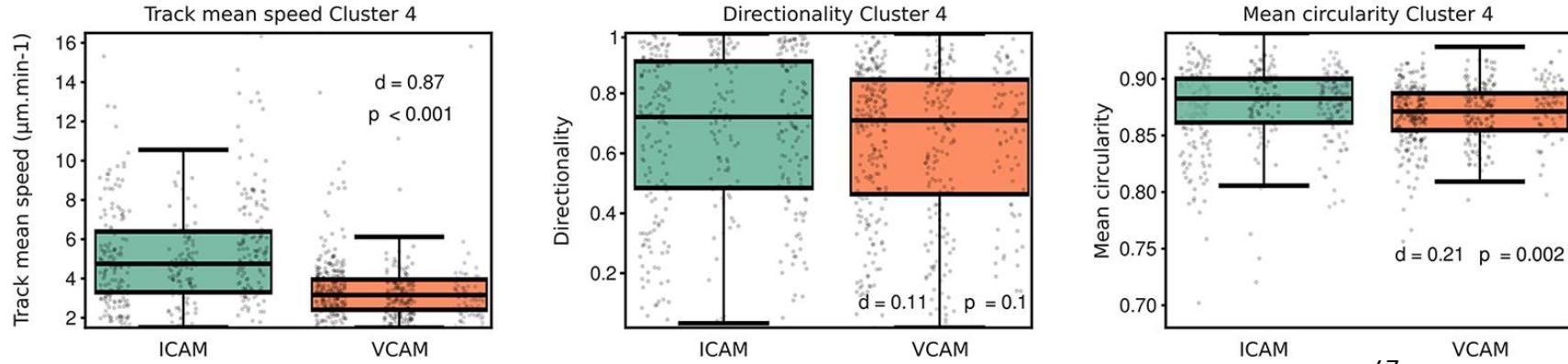
Each cluster represents a group of similar cells or behaviours. Fingerprinting helps create a unique profile for each group.



By cluster



By condition



Conclusions

- TrackMate and CellTracksColab provide powerful tools for analyzing cell behavior and tracking particles in from large amount of biological data
- Allow identification of sub-populations in data and measure their features to better understand the data.

