

Workshop prep

(materials: https://github.com/CellMigrationLab/I2K_2024)

1. Open Fiji and activate the TrackMate Helper update site. Restart Fiji.



2. Download
 - Image for tracking settings
 - T-cell dataset (TrackMate batcher input)
 - Tracking results (CellTracksColab input)

<https://zenodo.org/records/13969009>

0_Tracking_settings.zip md5:262dc0c4f74f86d2c6fd5ff4154fc4e3	38.8 MB	Preview	Download
1_TrackMate_batcher_input.zip md5:471ac8870694db6f9a28ebcaefbd57cb	366.7 MB	Preview	Download
2_CellTracksColab_input.zip md5:62989f9c9cfb95575f42850b44a678b	44.4 MB	Preview	Download
3_CellTracksColab_results.zip md5:cfae4f3b7051b7e9bdc79102e43655c	16.3 MB	Preview	Download

3. Unzip the *2_CellTracksColab_input.zip* and upload to your google drive and create a new folder for results.

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, Hanna Grobe and Robert Haase

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.

Ershov, D., Phan, M. S., Pylvänäinen, J. W., Rigaud, S. U., Le Blanc, L., Charles-Orszag, A., Conway, J. R. W., Laine, R. F., Roy, N. H., Bonazzi, D., Duménil, G., Jacquemet, G., & Tinevez, J. Y. (2022). **TrackMate 7: integrating state-of-the-art segmentation algorithms into tracking pipelines.** *Nature methods*, 19(7), 829–832. <https://doi.org/10.1038/s41592-022-01507-1>



Cell Tracks
— COLAB —

What is CellTracksColab?

Cloud-based tool for track analysis.

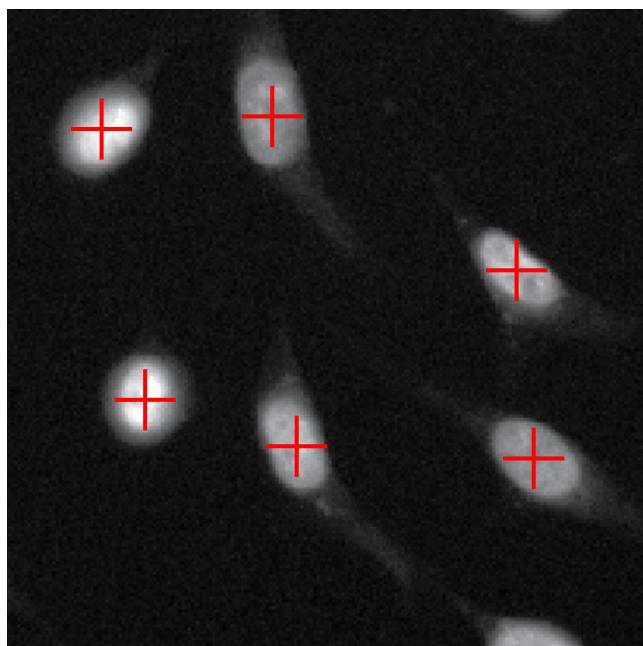
- **Key Features:**
 - **Cloud-based:** No installation required; runs on Google Colab notebooks
 - **Accessible:** Suitable for users without advanced computing infrastructure
 - **Export and Visualize:** Outputs track data, graphs, and visualizations in real-time.

Gómez-de-Mariscal, E., Grobe, H., Pylvänäinen, J. W., Xénard, L., Henriques, R., Tinevez, J. Y., & Jacquemet, G. (2024). **CellTracksColab is a platform that enables compilation, analysis, and exploration of cell tracking data.** PLoS biology, 22(8), e3002740. <https://doi.org/10.1371/journal.pbio.3002740>

What is Particle and Object Tracking?

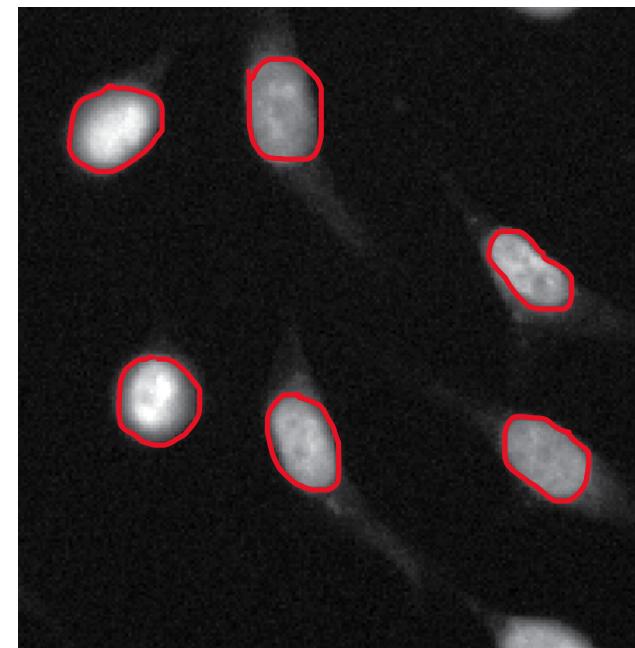
Particle tracking

Tracking the centroid of each particle's signal



Object tracking

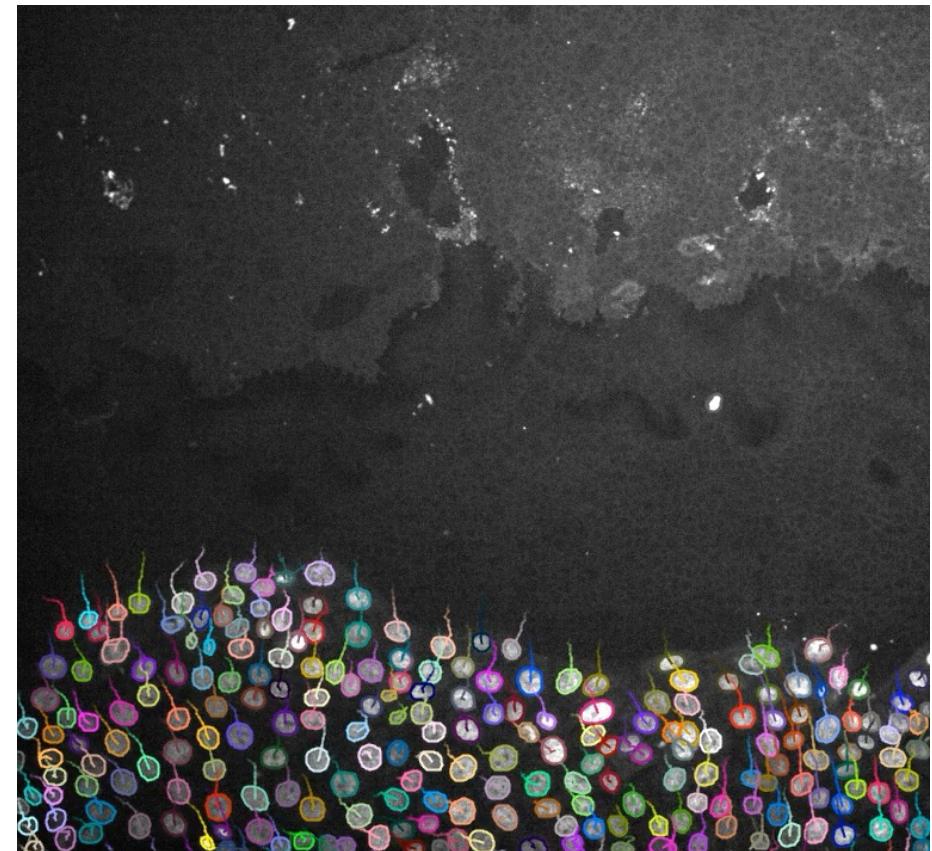
Tracking the entire shape and size of the object



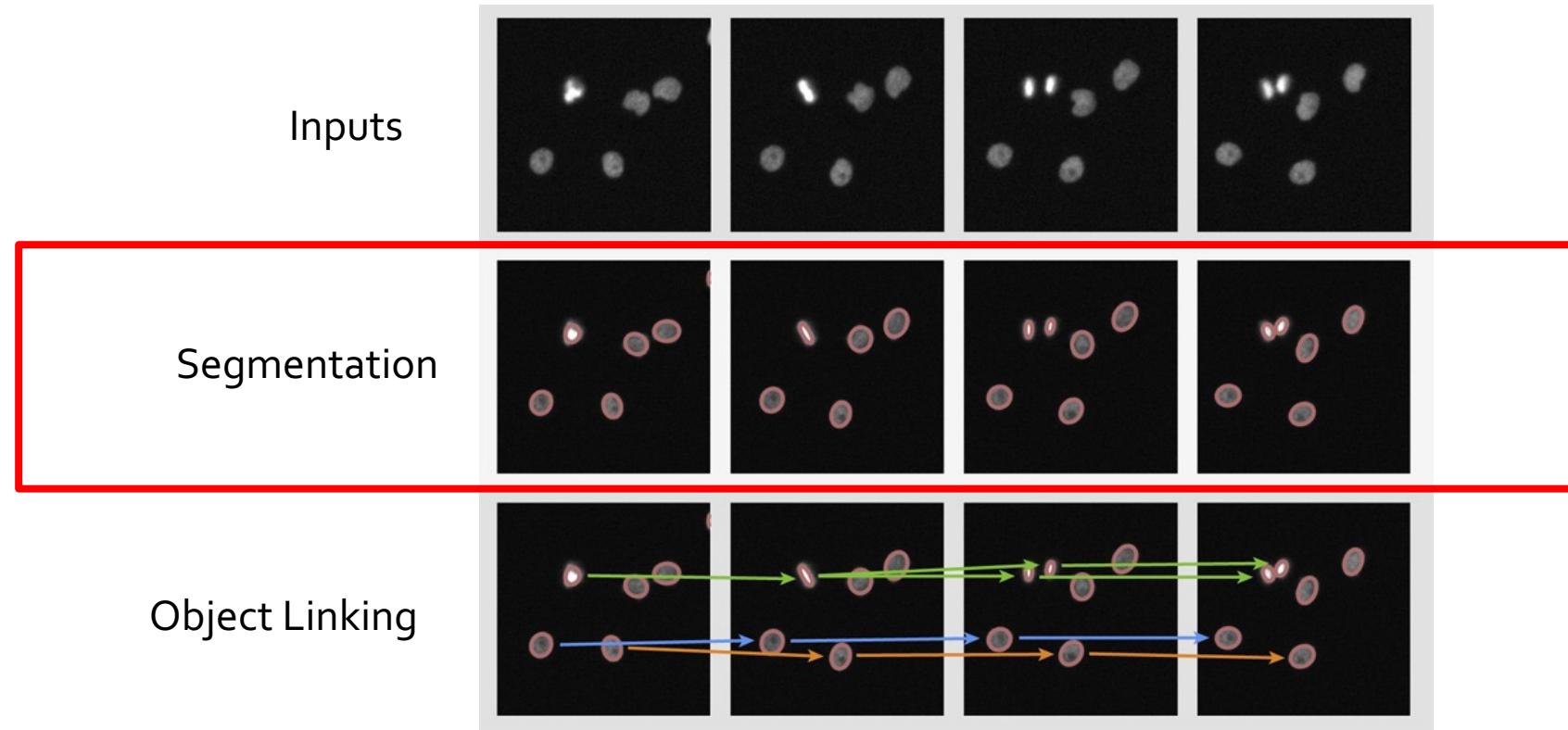
Source: Konrad Schindler, Computer Vision Lab, ETH Zuerich
<http://www.vision.ee.ethz.ch/datasets/>

Applications in Cell Biology

- **Object Tracking:**
 - Tracking individual proteins in live cells
 - Cell motility and migration studies
 - Tracking cell division over time
 - Measuring organelle movement or shape changes over time
 - Measuring signal changes over time

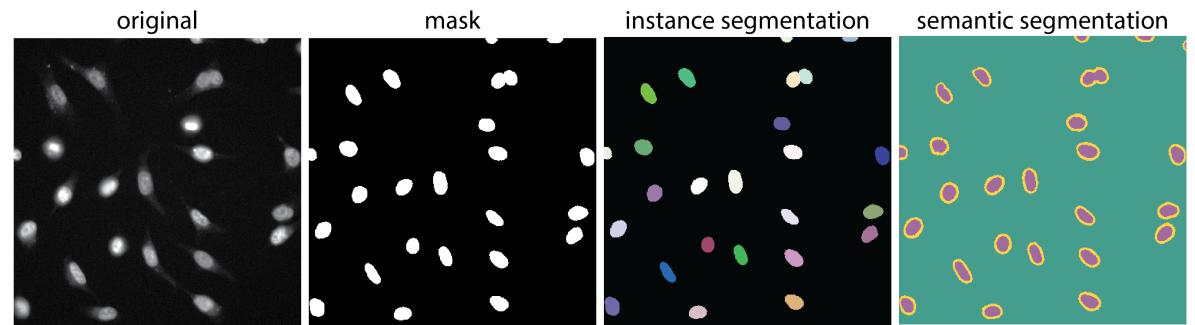
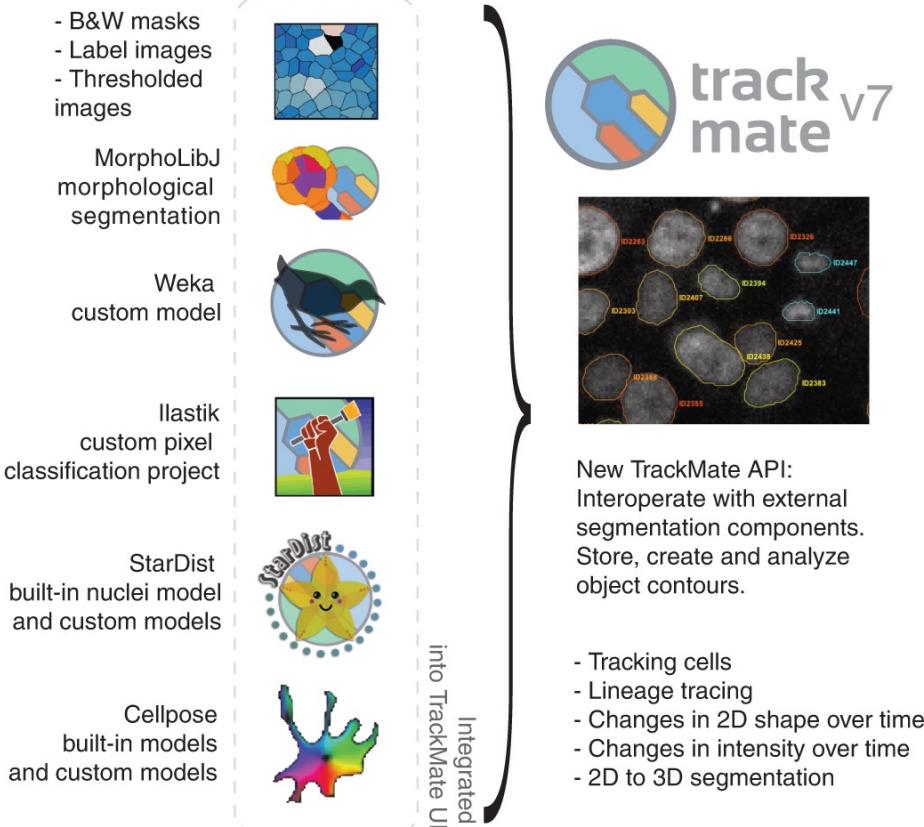


Tracking principle in TrackMate



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

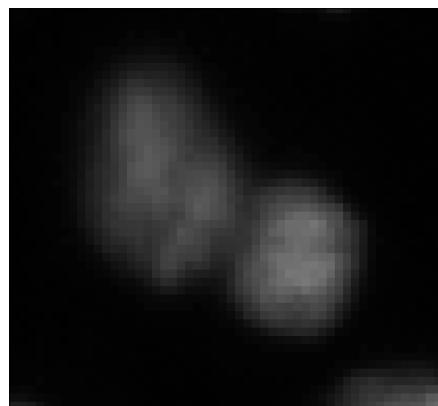
Segmentation



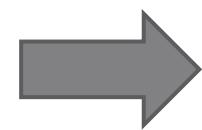
- Intensity-based segmentation
- Feature-based segmentation
- Using a pixel classifier (WEKA, ilastik, ect)
- Using Deep-Learning (e.g. Cellpose, StarDist)

Segmentation

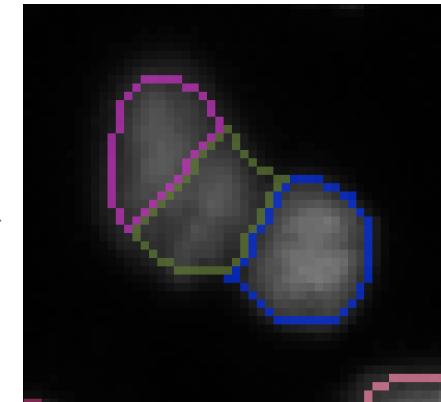
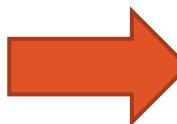
In the tracking context, object differentiation is important



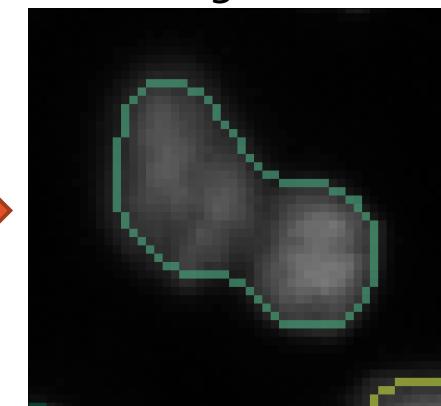
Raw image (nuclei)



Instance segmentation

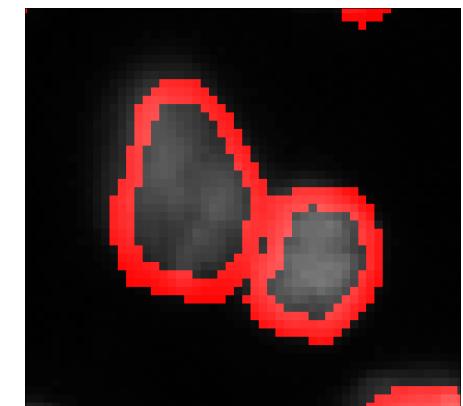


False objects
(over-segmentation)



Missed objects
(under-segmentation)

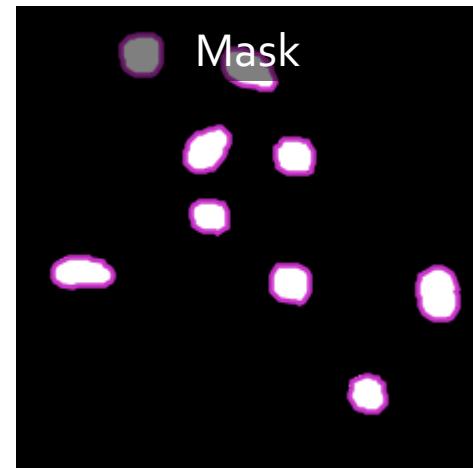
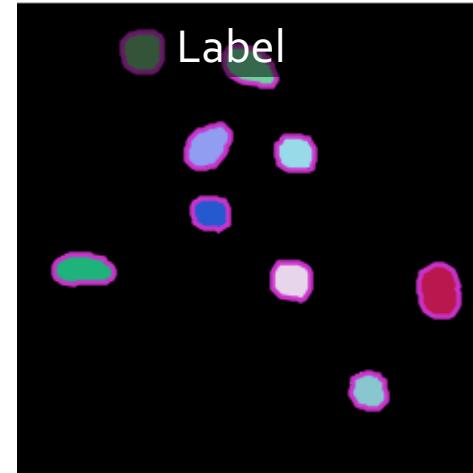
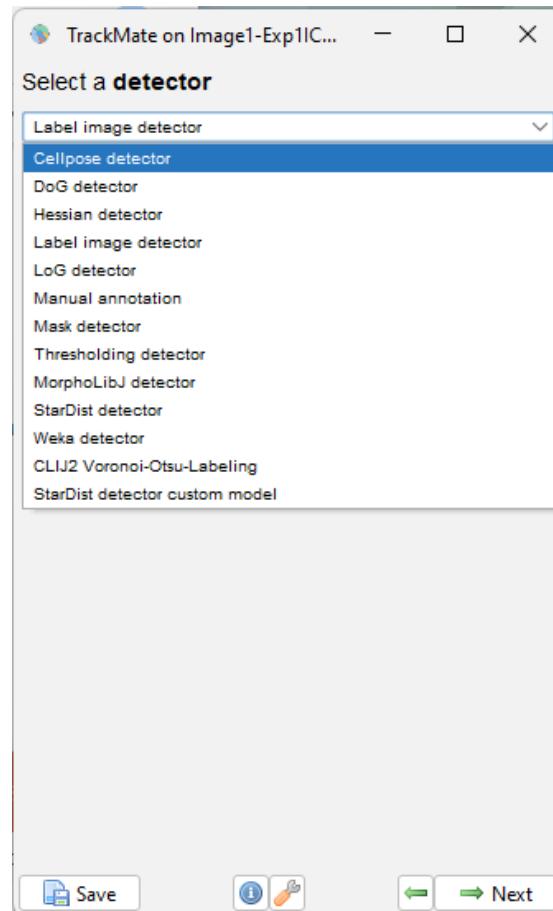
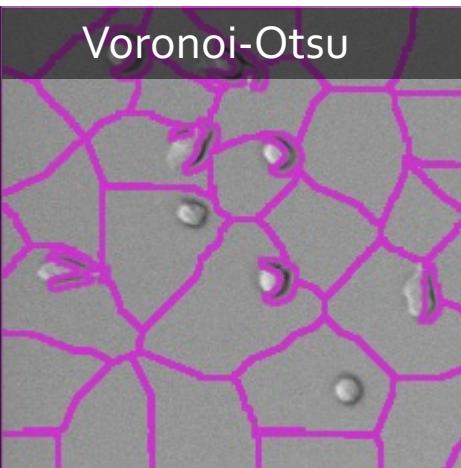
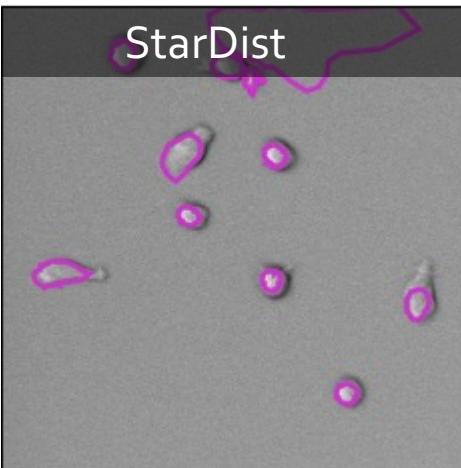
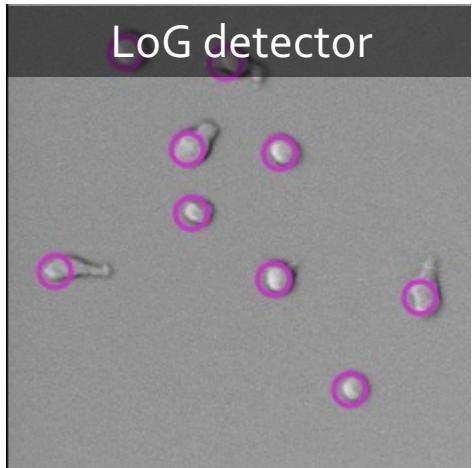
Outline precision can be of secondary interest



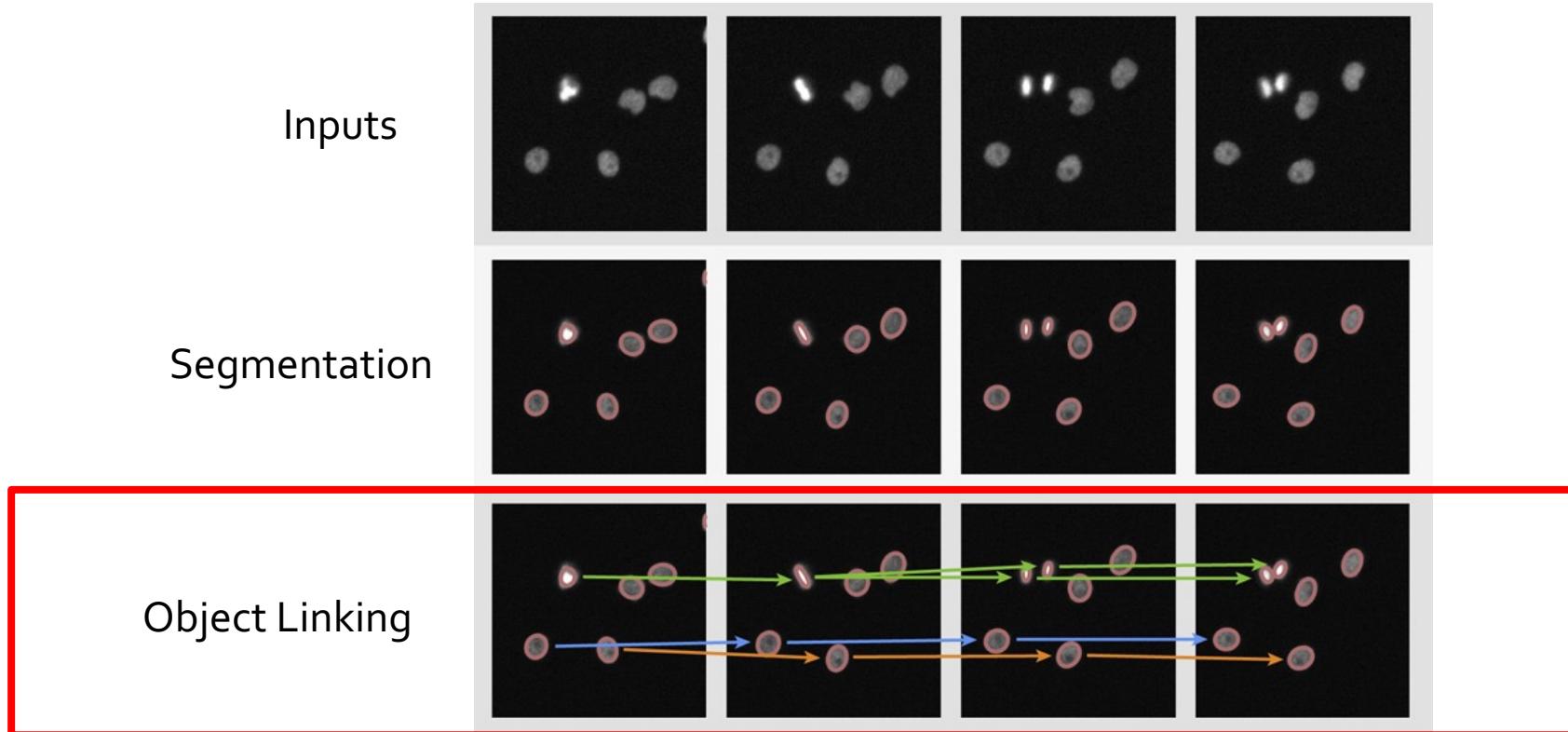
"Ground truth"
(manual annotation)

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/BBBC002>]

TrackMate object detection



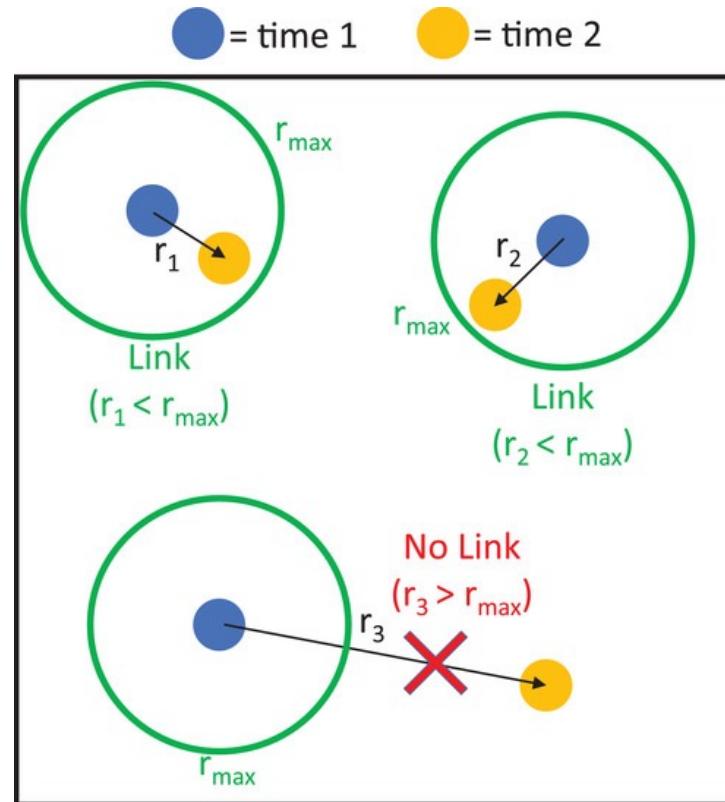
Tracking principle in TrackMate



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

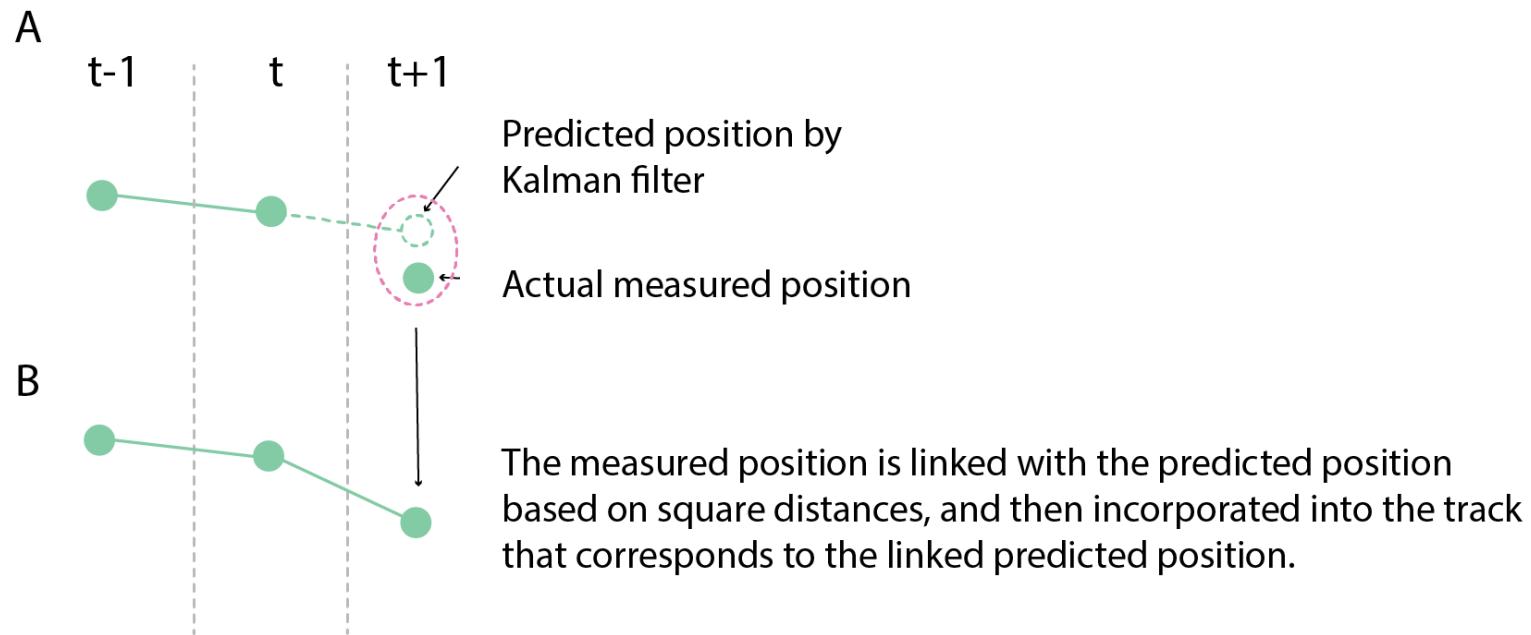
Tracking algorithm example - LAP tracker

Object linking using a search radius (LAP tracker)



Tracking algorithm example – Kalman tracker

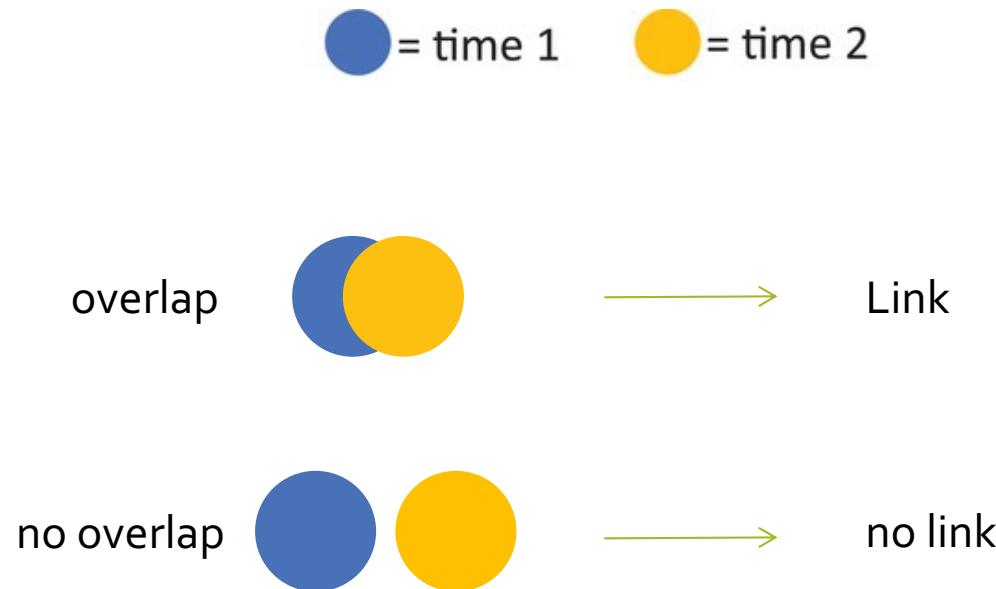
- The **Kalman tracker** is a mathematical tool used to estimate the position of moving objects over time, even when the measurements are noisy or incomplete.



Tracking algorithm example - overlap tracker

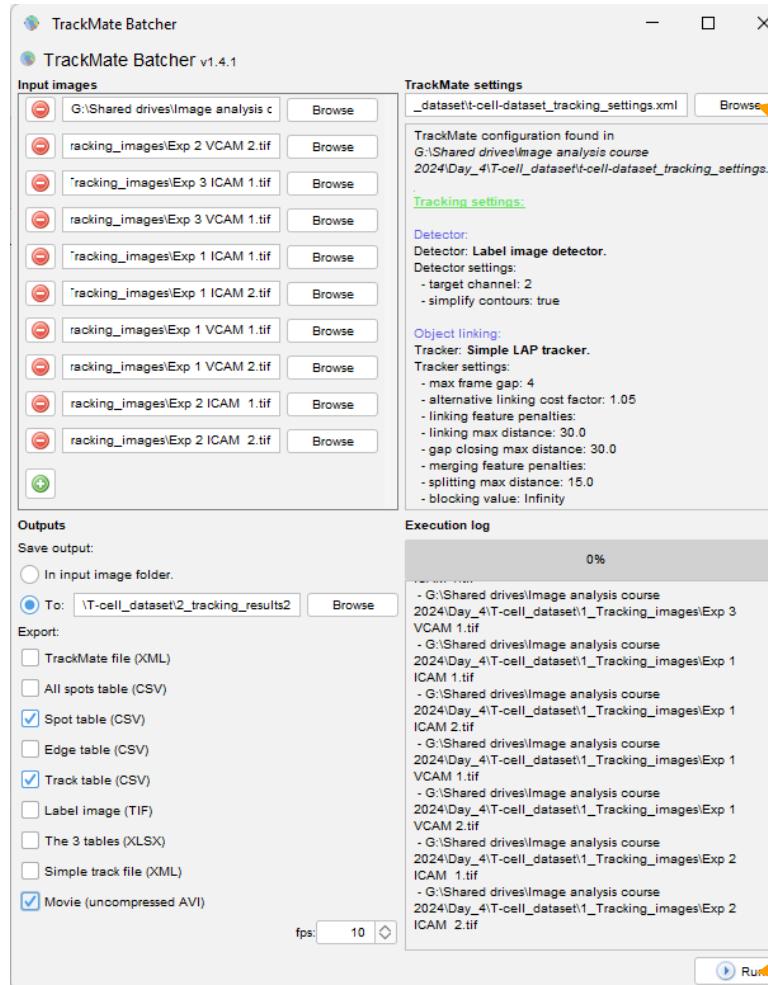
Object linking using an overlap (The overlap tracker)

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



What if you have multiple movies to track?

Drag and drop
the files to track



Path to TrackMate
settings file

t-cell-dataset_tracking_settings

Log window

Start!

Output:

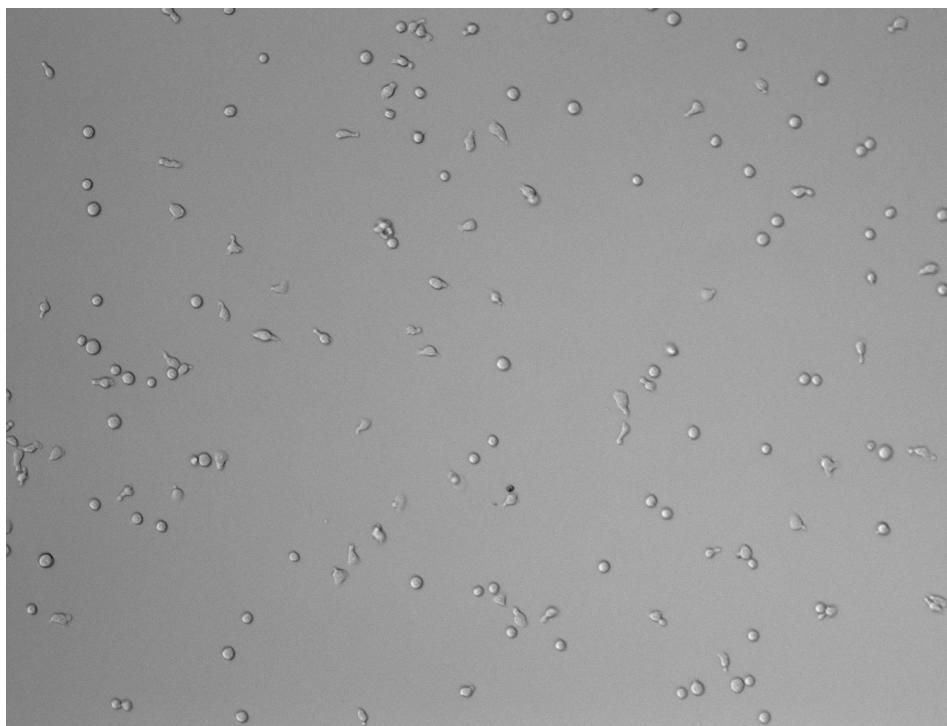
Name	Date modified	Type	Size
Exp 1 ICAM 1-movie	25/09/2024 15.06	AVI File	84 677 KB
Exp 1 ICAM 1-spots	25/09/2024 15.06	Microsoft Excel C...	1 653 KB
Exp 1 ICAM 1-tracks	25/09/2024 15.06	Microsoft Excel C...	66 KB
Exp 1 ICAM 2-movie	25/09/2024 15.06	AVI File	84 677 KB
Exp 1 ICAM 2-spots	25/09/2024 15.06	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!!!!

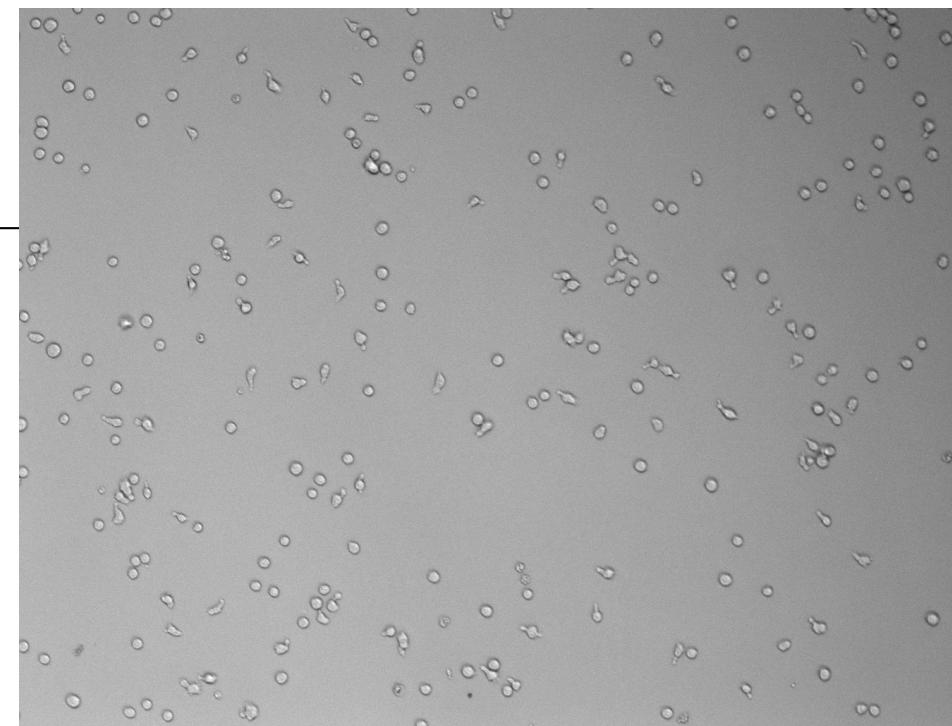
OUR TASK TODAY

How does the coating on glass bottom dishes affect the migration of T-cells?

ICAM



VCAM



Task 1 – tracking of T cell dataset

1. Use the *Image for tracking settings* to define tracking parameters using the TrackMate interface in Fiji
2. Save these settings as an xml file (TrackMate settings file)
3. Apply these settings to all data using TrackMate Batcher
 - *Plugins > Tracking > TrackMate Batcher*
 - Folder with the tracking images
 - Export:
 - 1) Spot table
 - 2) Track table
 - 3) Movie

0_Tracking_settings.zip md5:262dc0c4f74f86d2c6fd5ff4154fc4e3	38.8 MB	Preview	Download
1_TrackMate_batcher_input.zip md5:471ac8870694db69a28ebcaefdf57cb	366.7 MB	Preview	Download
2_CellTracksColab_input.zip md5:62989f9c9cfb955755f42850b44a678b	44.4 MB	Preview	Download
3_CellTracksColab_results.zip md5:cf0ae4f3b7051b7e9bdc79102e43656c	16.3 MB	Preview	Download

<https://zenodo.org/records/13969009>

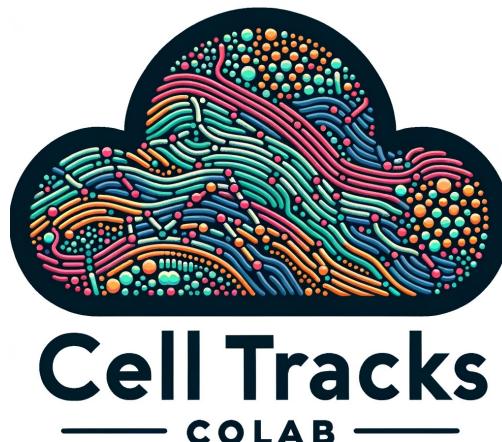
Track Analysis



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

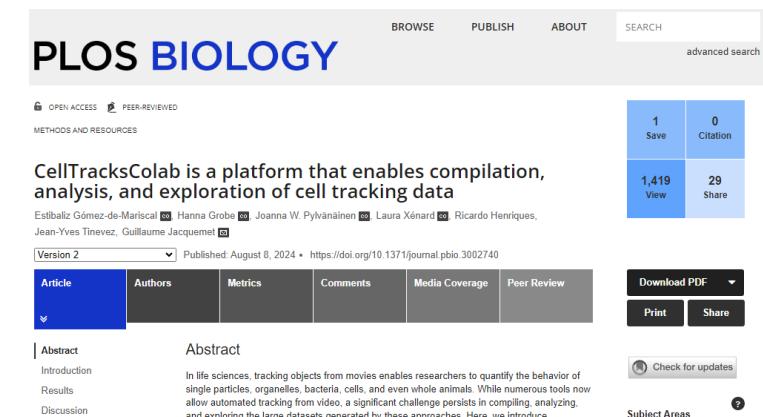


HOW DO I ANALYZE ALL THIS DATA?

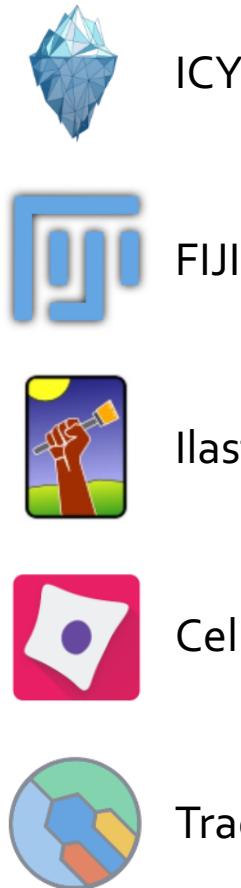


Cloud-based tool for
track analysis

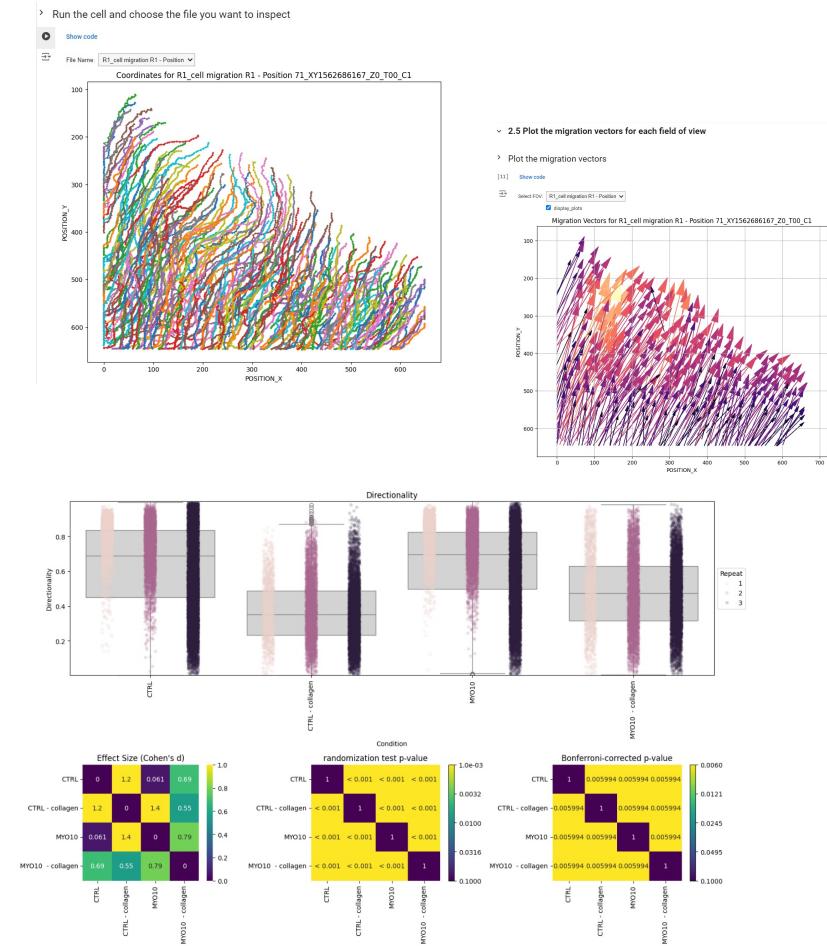
CellTracksColab Gómez-de-Mariscal, E., Grobe, H., Pylvänäinen, J. W., Xénard, L., Henriques, R., Tinevez, J. Y., & Jacquemet, G. (2024). **CellTracksColab is a platform that enables compilation, analysis, and exploration of cell tracking data.** PLoS biology, 22(8), e3002740. <https://doi.org/10.1371/journal.pbio.3002740>

A screenshot of a PLOS BIOLOGY article page. At the top, the journal name "PLOS BIOLOGY" is in blue, with "BROWSE", "PUBLISH", and "ABOUT" buttons to its right. A search bar with "SEARCH" and "advanced search" options is also at the top. Below the header, there's a summary section with "OPEN ACCESS" and "PEER-REVIEWED" status, and a "METHODS AND RESOURCES" link. The main title is "CellTracksColab is a platform that enables compilation, analysis, and exploration of cell tracking data". Below the title, author names are listed: Estibaliz Gómez-de-Mariscal, Hanna Grobe, Joanna W. Pylvänäinen, Laura Xénard, Ricardo Henriques, Jean-Yves Tinevez, Guillaume Jacquemet. The article version is "Version 2" and it was published on August 8, 2024. To the right of the article summary, there are metrics: 1 Save, 0 Citation, 1,419 View, and 29 Share. Below these metrics are buttons for "Download PDF", "Print", and "Share". There's also a "Check for updates" button and a "Subject Areas" dropdown. The main content area shows the first few sections of the article: "Abstract", "Introduction", "Results", and "Discussion".

What is CellTracksColab?

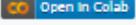


Track ID	X	Y	Z	t
0	6.8	15	1.0	1
1	7.9	0.0	2.6	2
2	9.1	4.7	5.9	3
3	5.9	8.5	3.7	4
4	25	3.0	6.5	5
5	67	2.4	5.1	6
6	91	5.3	1.3	7
...

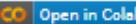
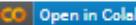


Available notebooks

Simple analysis notebooks

Notebook	Purpose	Required File Format	Link
CellTracksColab - TrackMate	Load and analyze TrackMate data. More info on how to prepare the data here .	CSV or XML files	 Open In Colab
CellTracksColab - Custom	Analyze data from CellProfiler, ICY, ilastik, or Fiji Manual Tracker. More info on how to prepare the data here .	CSV files	 Open In Colab
CellTracksColab - Viewer	Load and share data in the CellTracksColab format.	CellTracksColab format	 Open In Colab

Advanced analysis notebooks

Notebook	Purpose	Required File Format	Link
CellTracksColab - Dimensionality Reduction	Utilize advanced dimensionality reduction techniques.	CellTracksColab format	 Open in Colab
CellTracksColab - Track Spatial Clustering Analysis	Dive deeper into your dataset with track clustering analysis.	CellTracksColab format	 Open in Colab
CellTracksColab - Distance to ROI	Analyze movement tracks in relation to designated ROIs.	CellTracksColab format	 Open in Colab

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

CellTracksColab -
TrackMate

CellTracksColab -
Custom

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.

CellTracksColab -
TrackMate

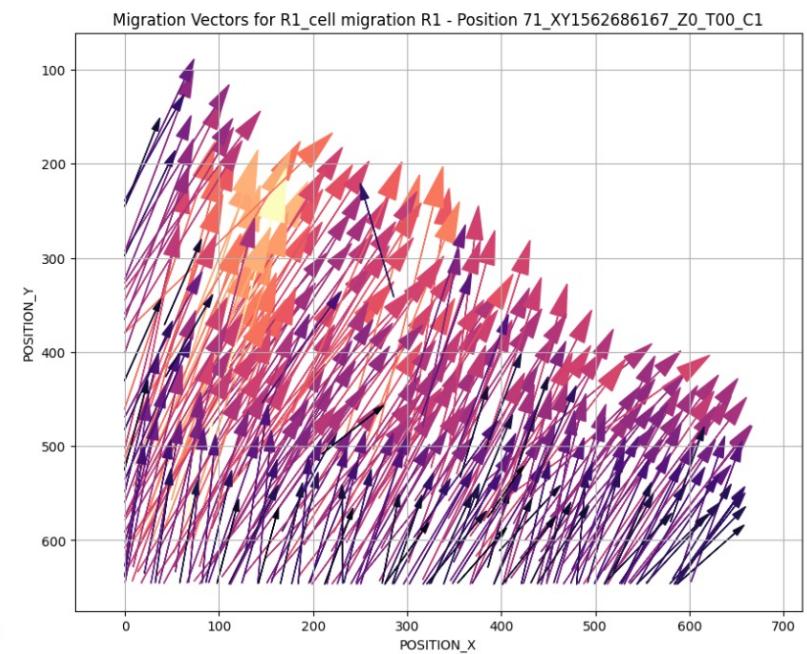
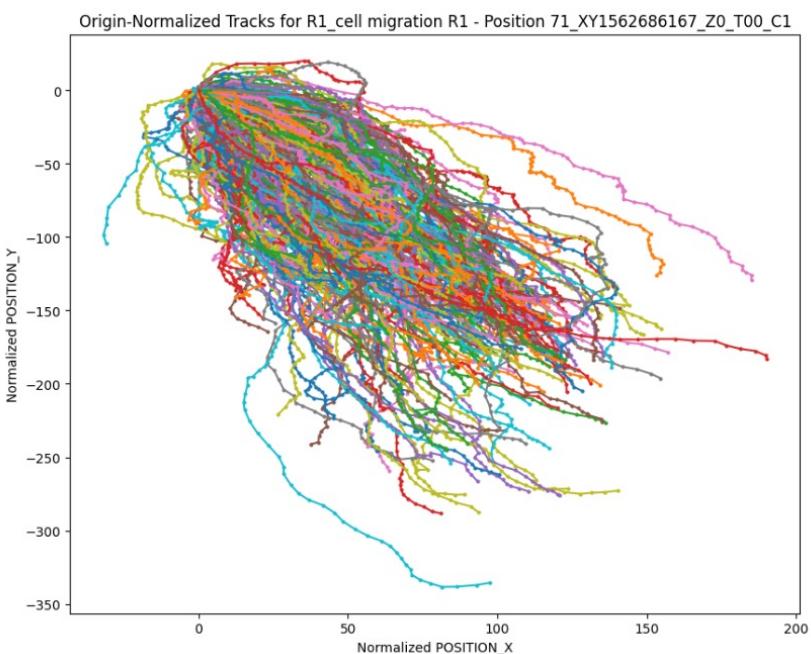
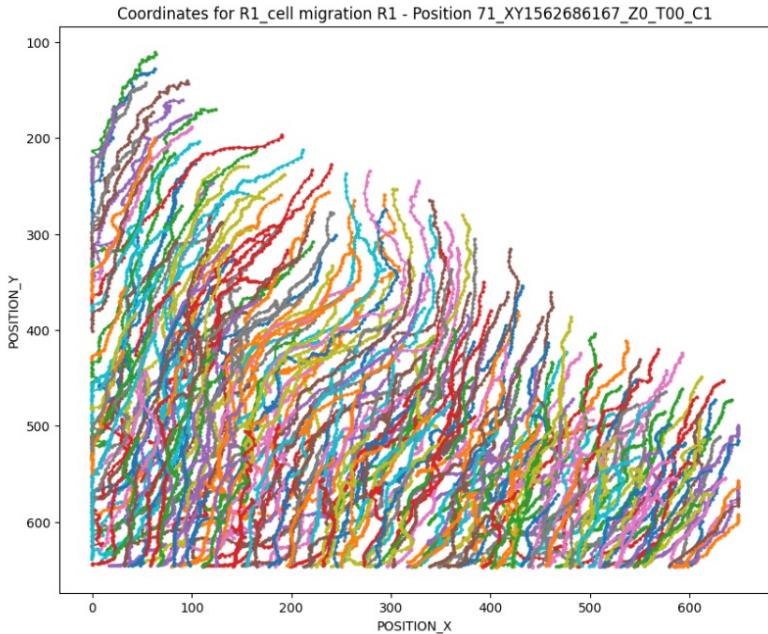
CellTracksColab -
Custom

Track Visualization

CellTracksColab -
TrackMate

CellTracksColab -
Custom

CellTracksColab -
Viewer



- By repeat
- By condition

Track filtering

Smoothing Neighbors: 3

Duration: 168.75 – 940.00

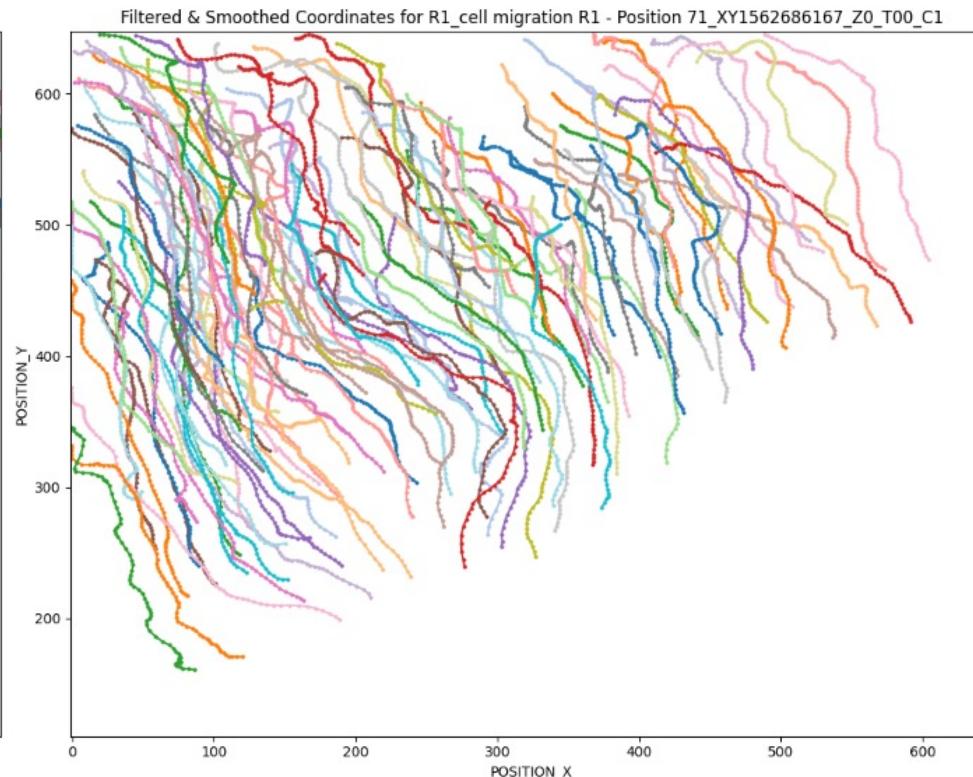
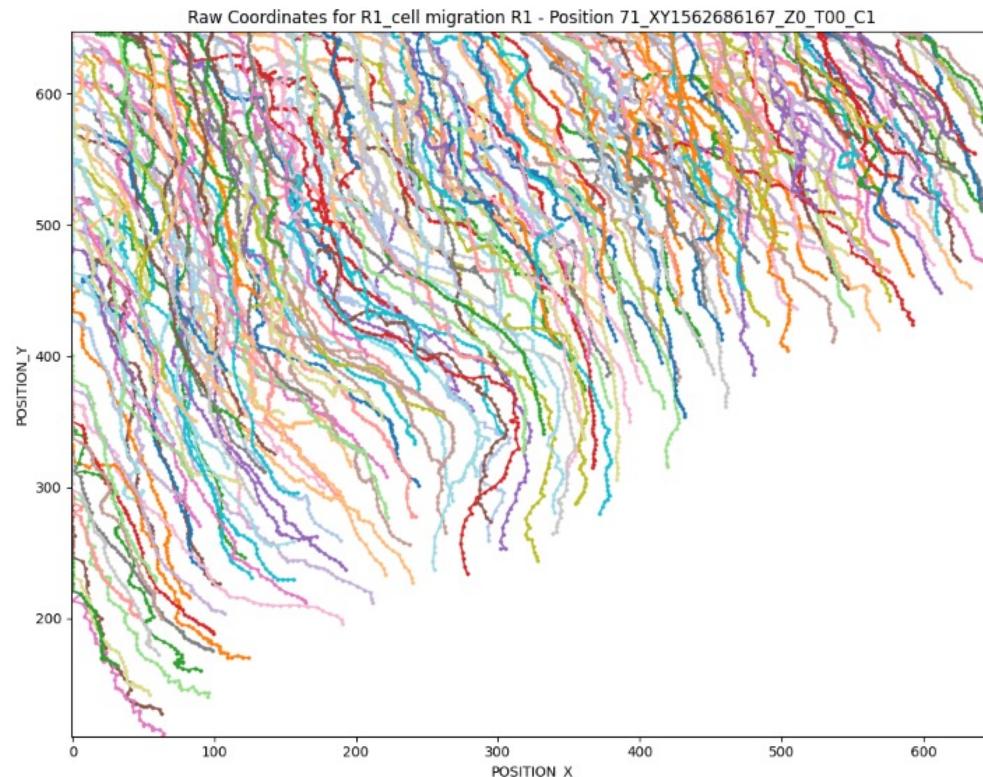
Mean Speed: 0.10 – 2.85

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.

CellTracksColab - TrackMate
CellTracksColab - Custom
CellTracksColab - Viewer

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`

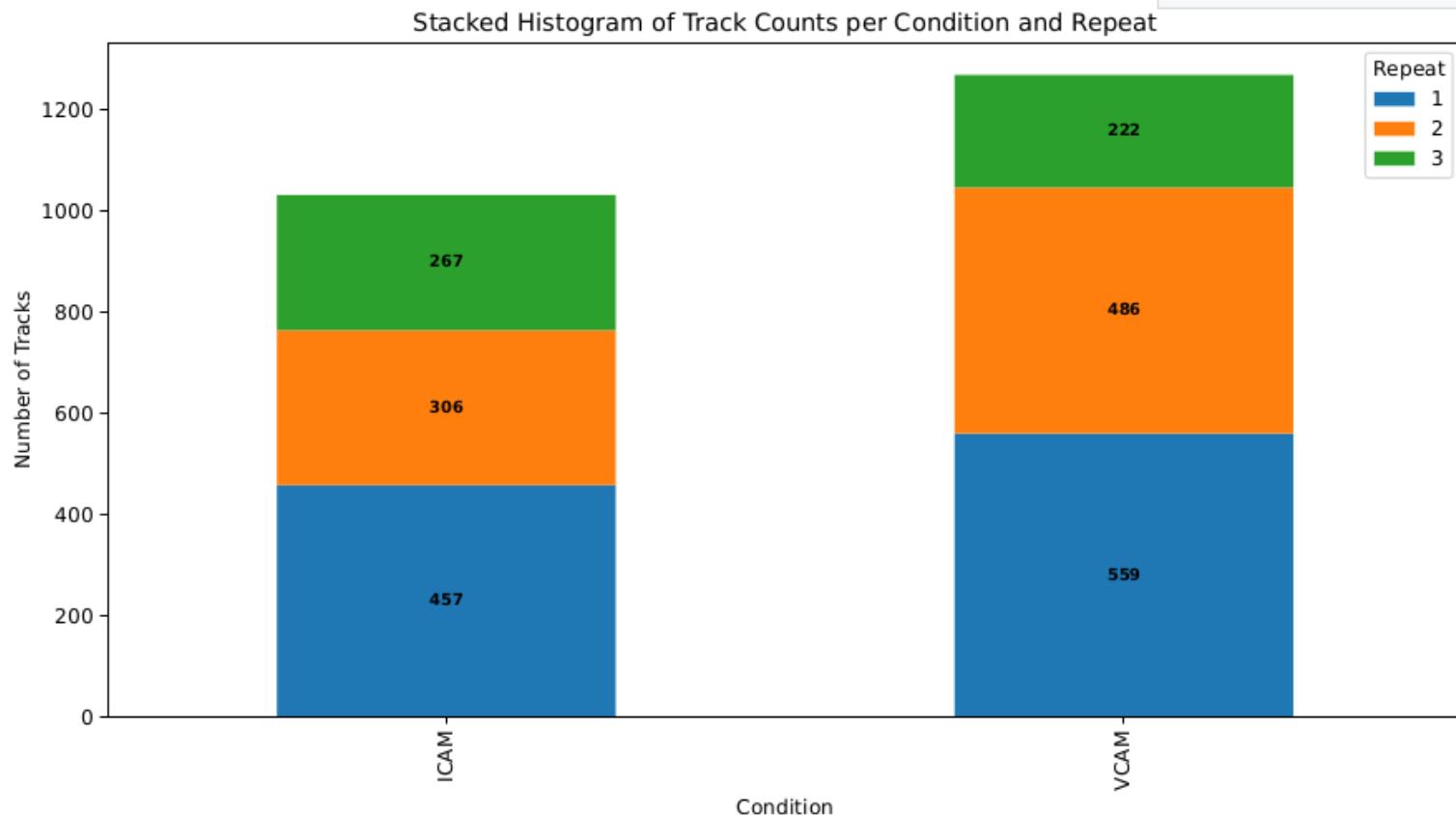
Quality Control

Purpose

- Each biological repeat should carry equal weight in the analysis

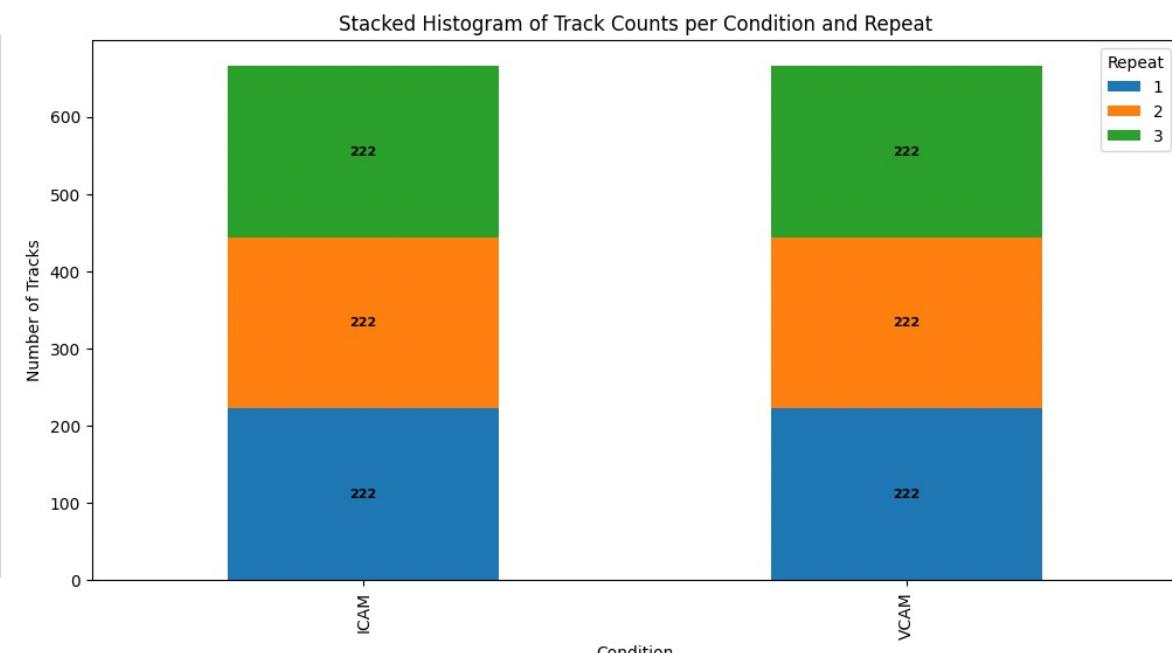
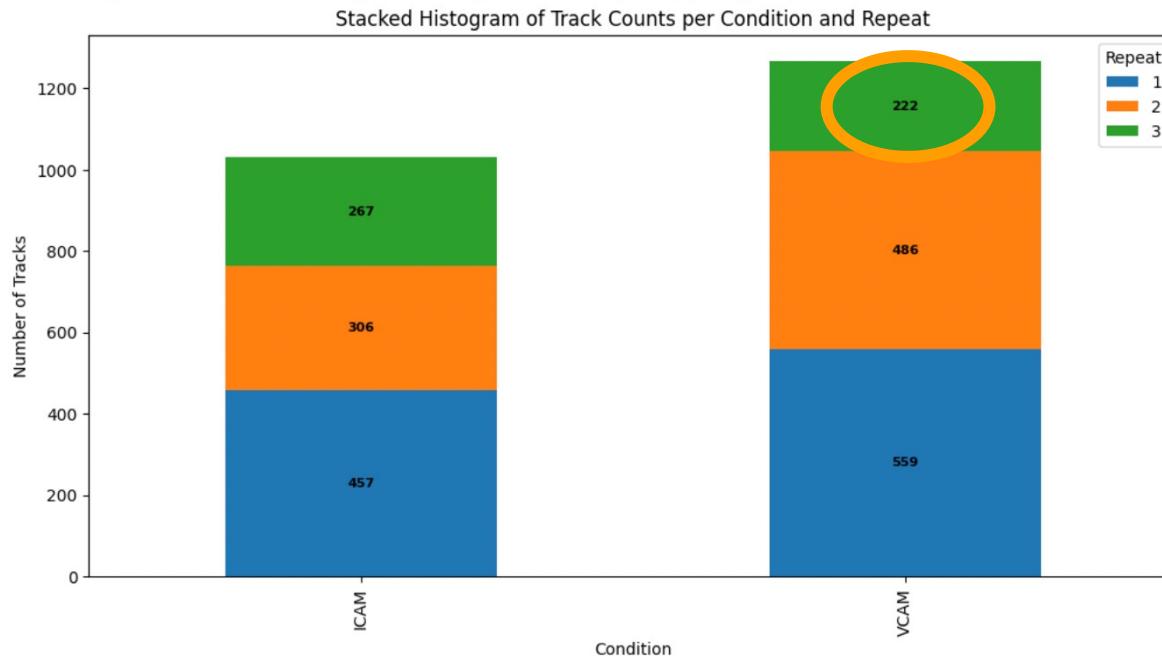
A balanced dataset is essential for:

- 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.



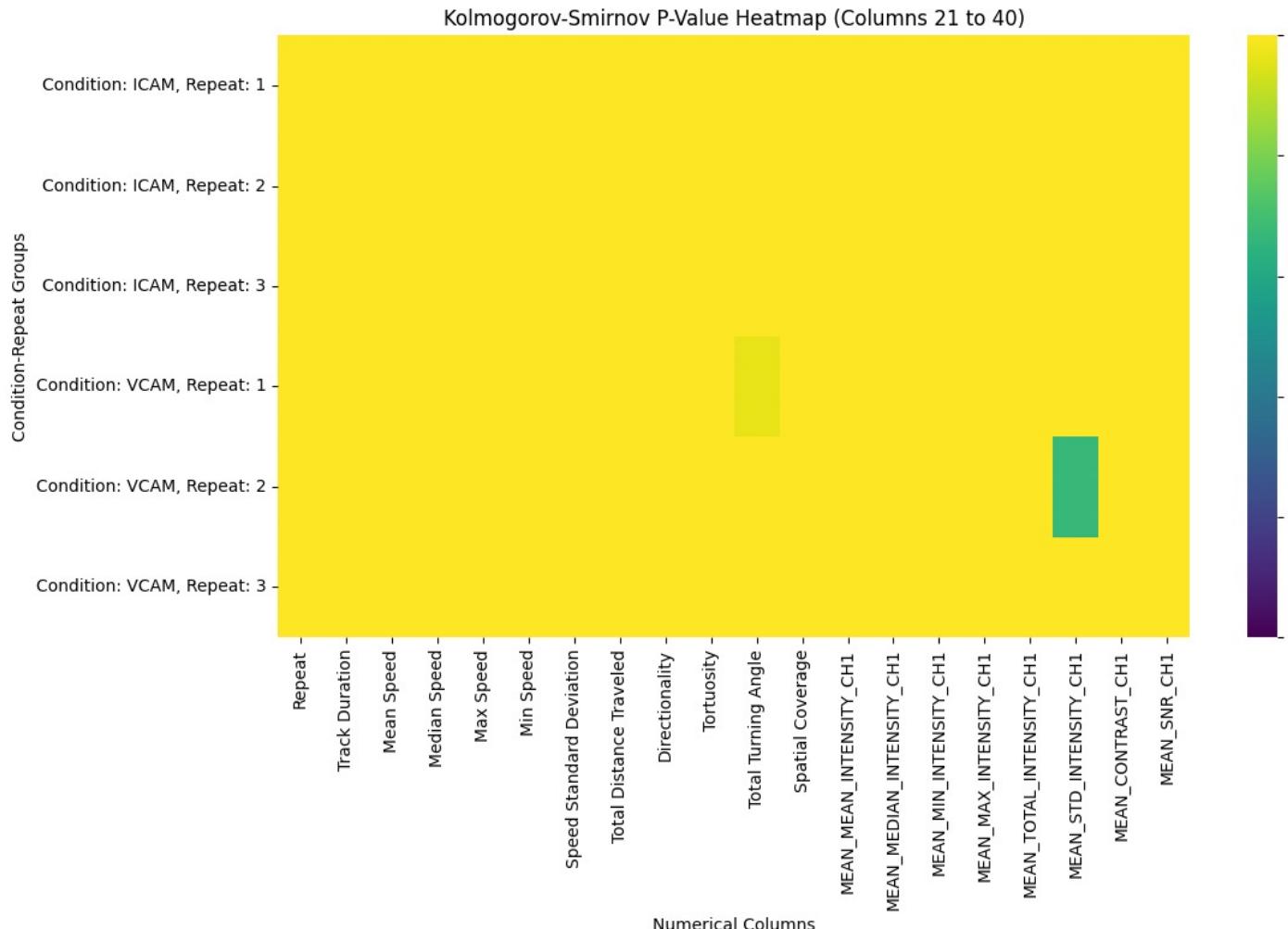
Note: If your data is imbalanced, consider balancing it to prevent skewing your results.

Data balancing



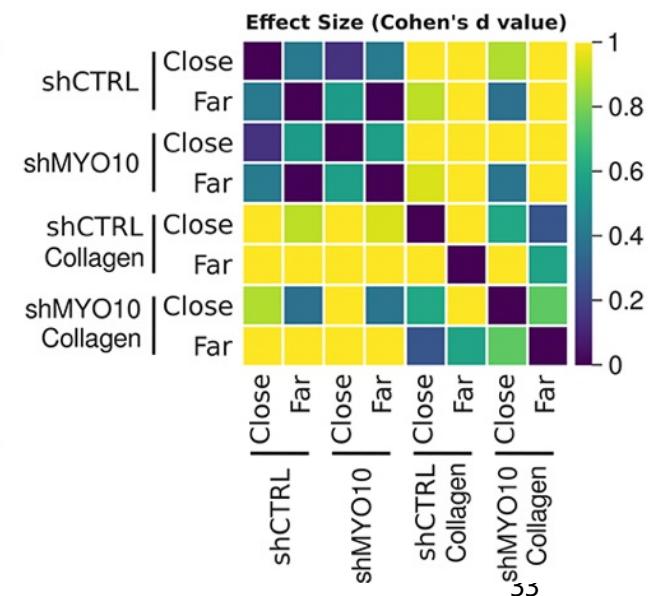
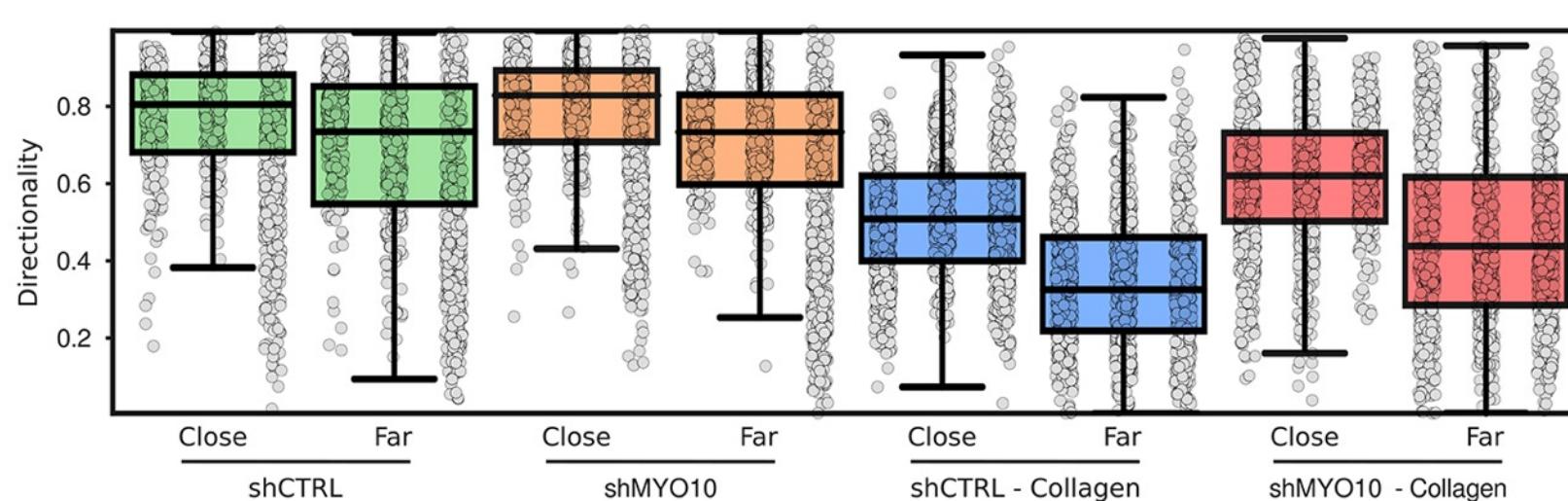
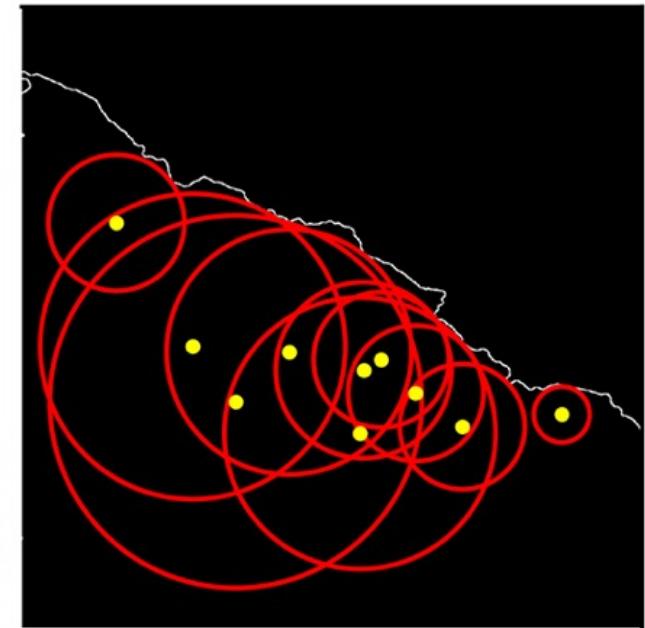
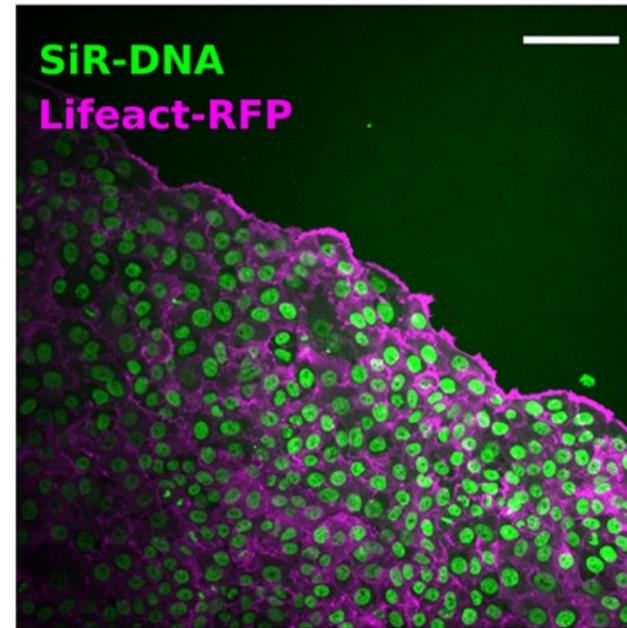
- randomly selecting tracks from these larger groups

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
- **High P-Values (Yellow):**
 - Down-sampling 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 down-sampling process may have affected the distribution significantly.

Distance to ROI



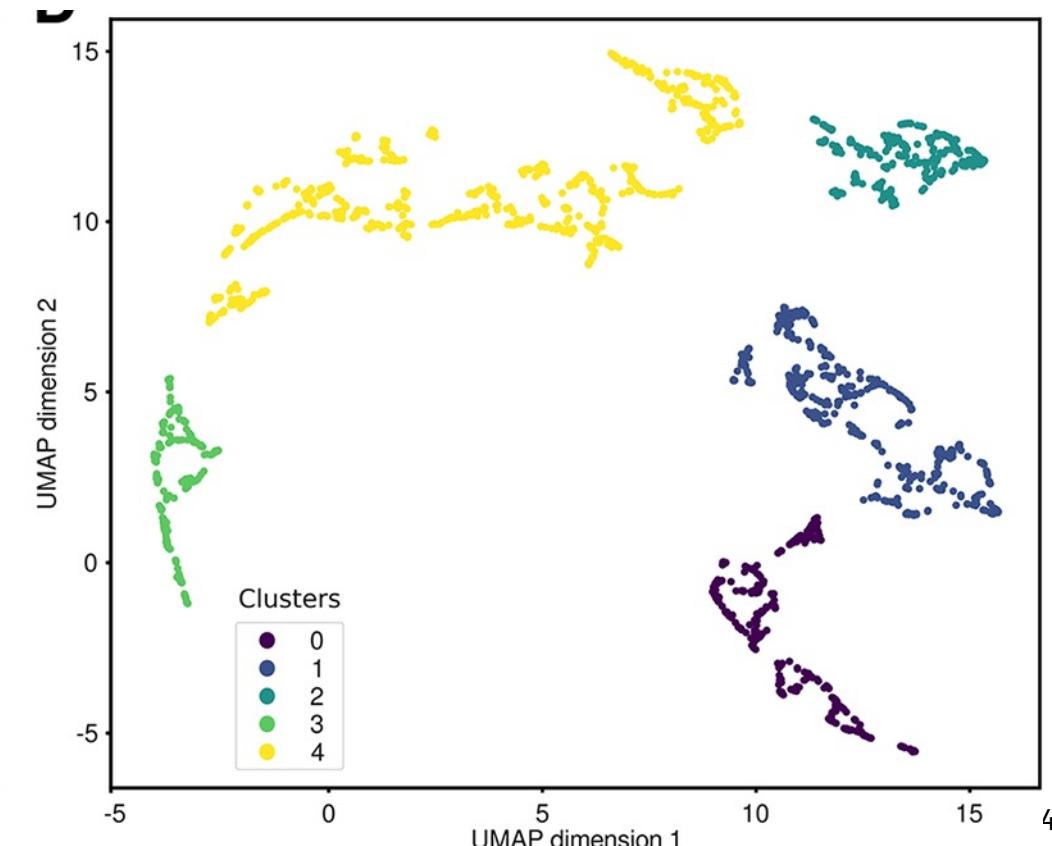
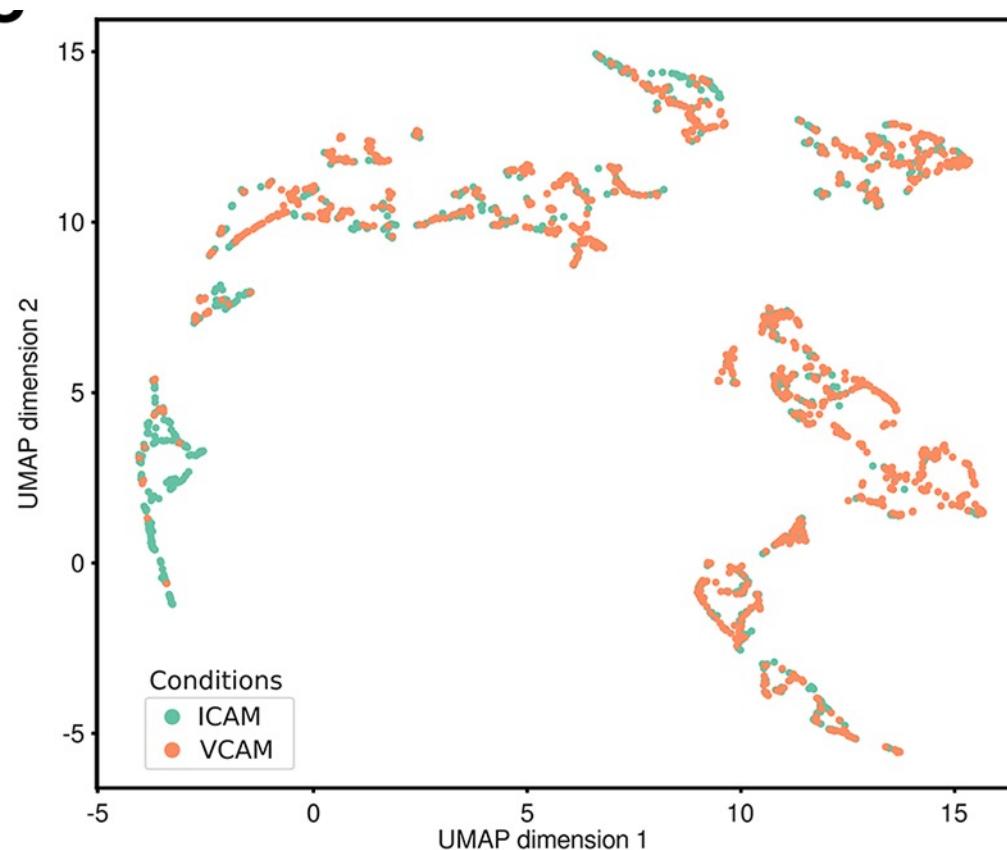
Exploring high-dimensional data

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:

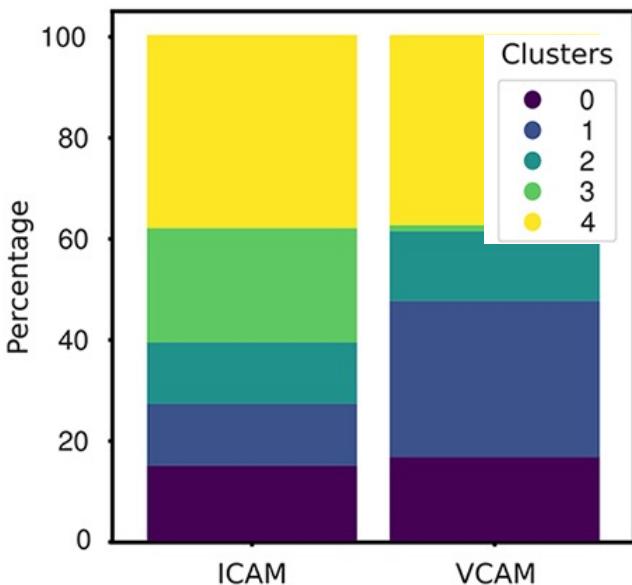
determines clusters based on density and similarity, identifying groups of similar cell tracks.



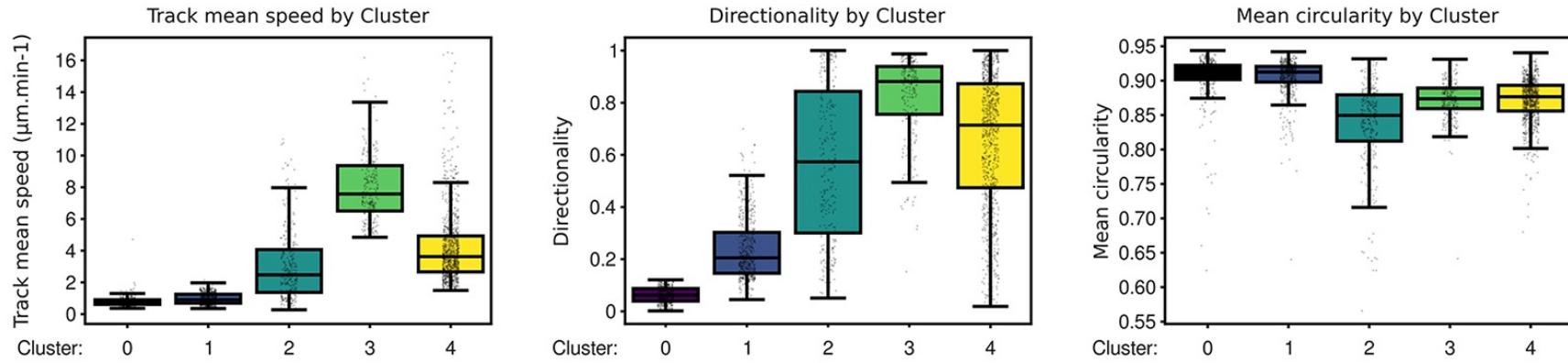
Data fingerprinting and sub-populations

Fingerprinting:

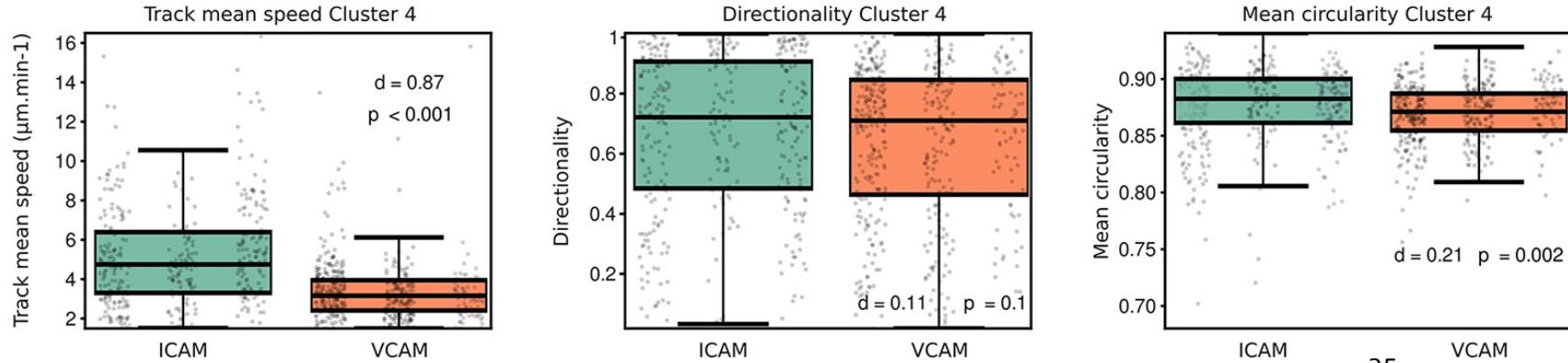
Each cluster represents a group of similar cells or behaviours. Fingerprinting helps create a unique profile for each group.



By cluster



By condition



Task 2 – Track analysis using CellTracksColab

1. Upload your tracking results to your Google Drive (or use the pre-made dataset). Make sure the folders are correctly organized.



2. Create a folder for results
3. Open The CellTracksColab TrackMate notebook
4. Make a copy of the notebook to your drive
5. Run all cells visualize and generate plots.

A screenshot of a Zenodo dataset page. It shows four files listed:

- 0_Tracking_settings.zip (38.8 MB) with 'Preview' and 'Download' buttons
- 1_TrackMate_batcher_input.zip (366.7 MB) with 'Preview' and 'Download' buttons
- 2_CellTracksColab_input.zip (44.4 MB) with 'Preview' and 'Download' buttons. This file is highlighted with a red box.
- 3_CellTracksColab_results.zip (16.3 MB) with 'Preview' and 'Download' buttons

<https://zenodo.org/records/13969009>

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.

