Workshop prep

(materials: https://github.com/CellMigrationLab/V4SDB_Winter_School_2025)

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

1. Download and unzip:
   - 0_Tracking_settings.zip
     - Place on your computer
   - 1_TrackMate_batcher_input.zip
     - Place on your computer
   - 2_CellTracksColab_input.zip
     - Upload to Google Drive

https://zenodo.org/records/14645477

2. Create 2 new folders:
   - Create a new folder on your computer for tracking results
   - Create a folder on your Google Drive for CellTracksColab results

ANALYSIS OF CELL BEHAVIOR USING TRACKMATE AND CELLTRACKSCOLAB

What, where, how?

Can I also use it?

Joanna Pylvänäinen, joanna.pylvanainen@abo.fi

V4SDB Winter School 2025

Materials modified from Guillaume Jacquemet, Hanna Grobe and Robert Haase

Contents of the workshop

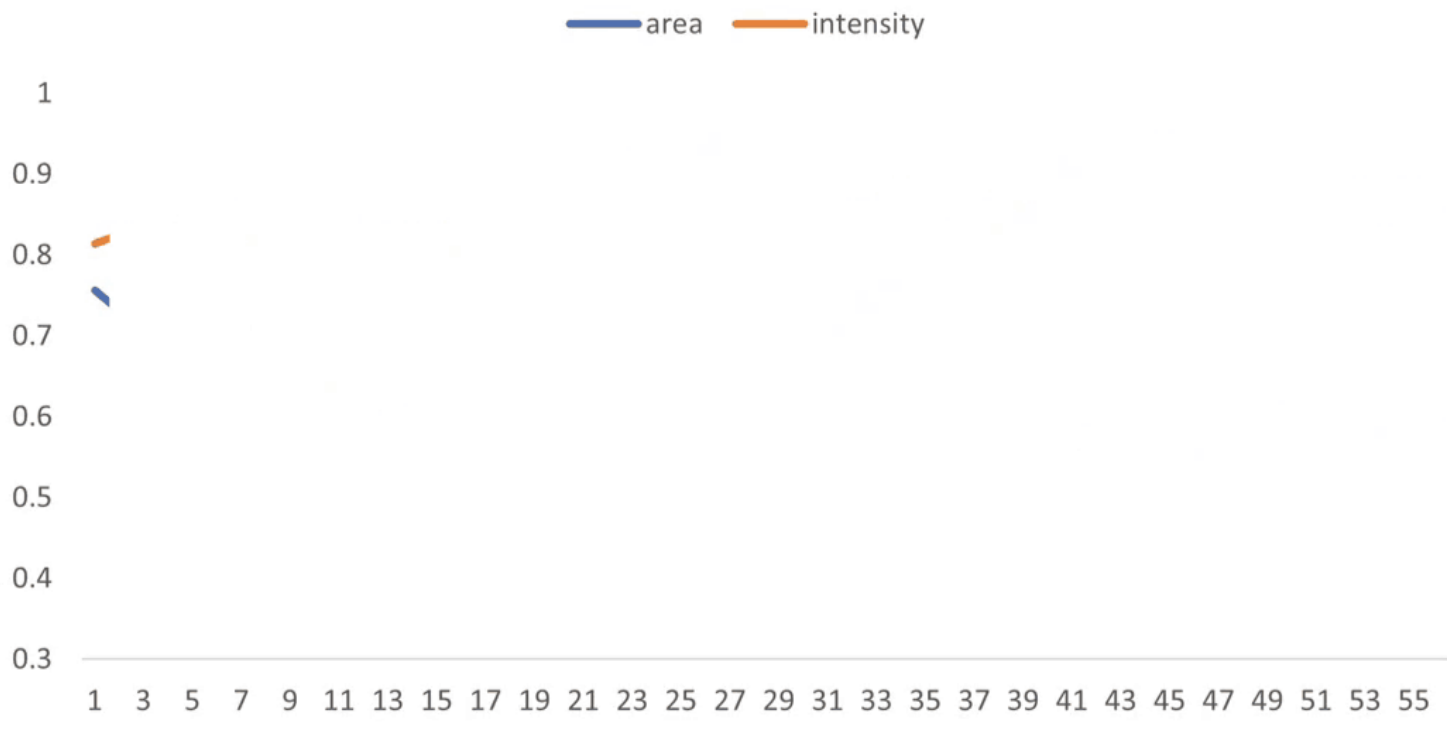
- Introduction to cell tracking in cell biology
- Introduction to TrackMate and batch processing + hands-on
- Introduction to CellTracksColab + hands-on

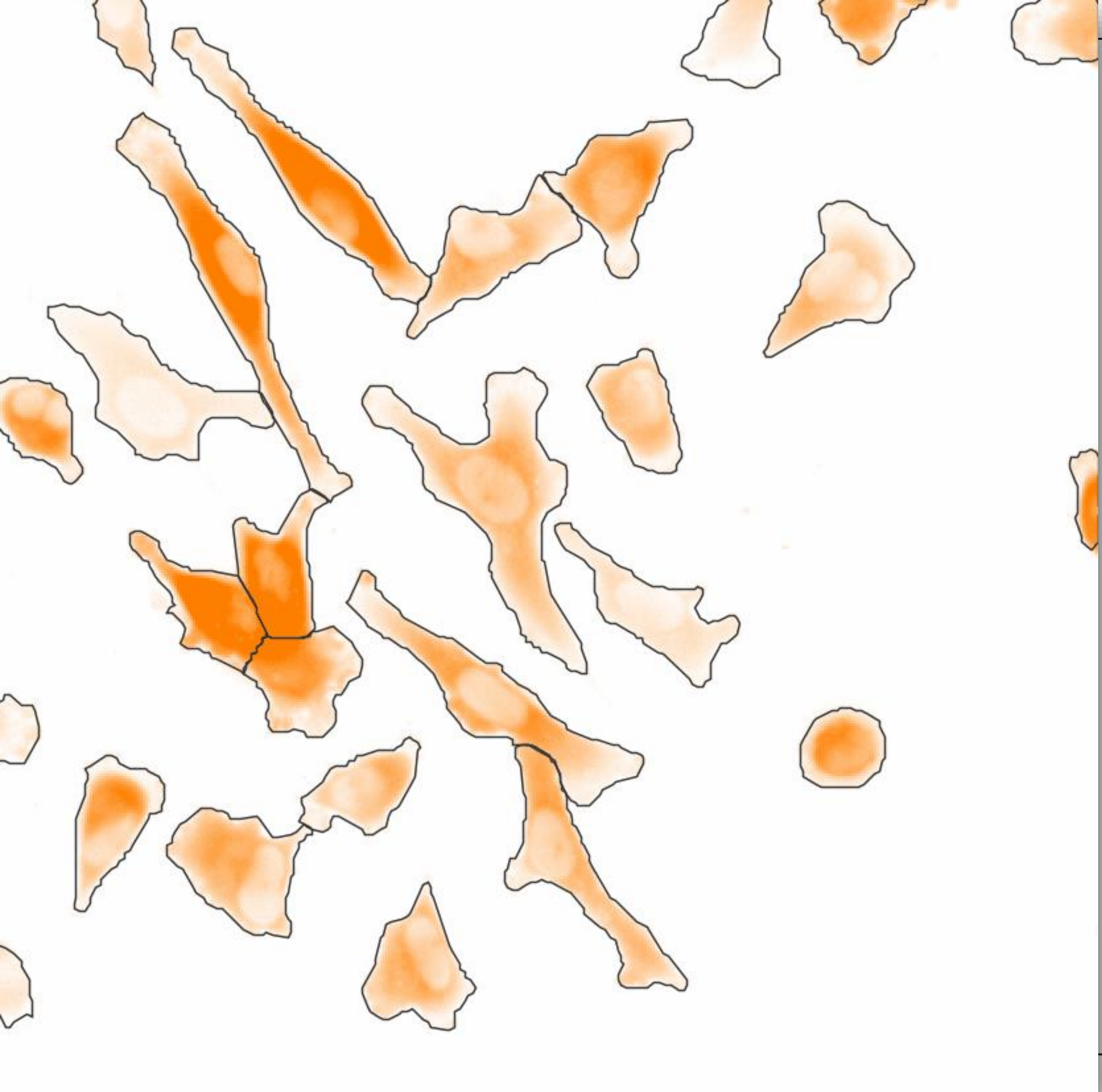
TYPICAL CELL TRACKING PROBLEM

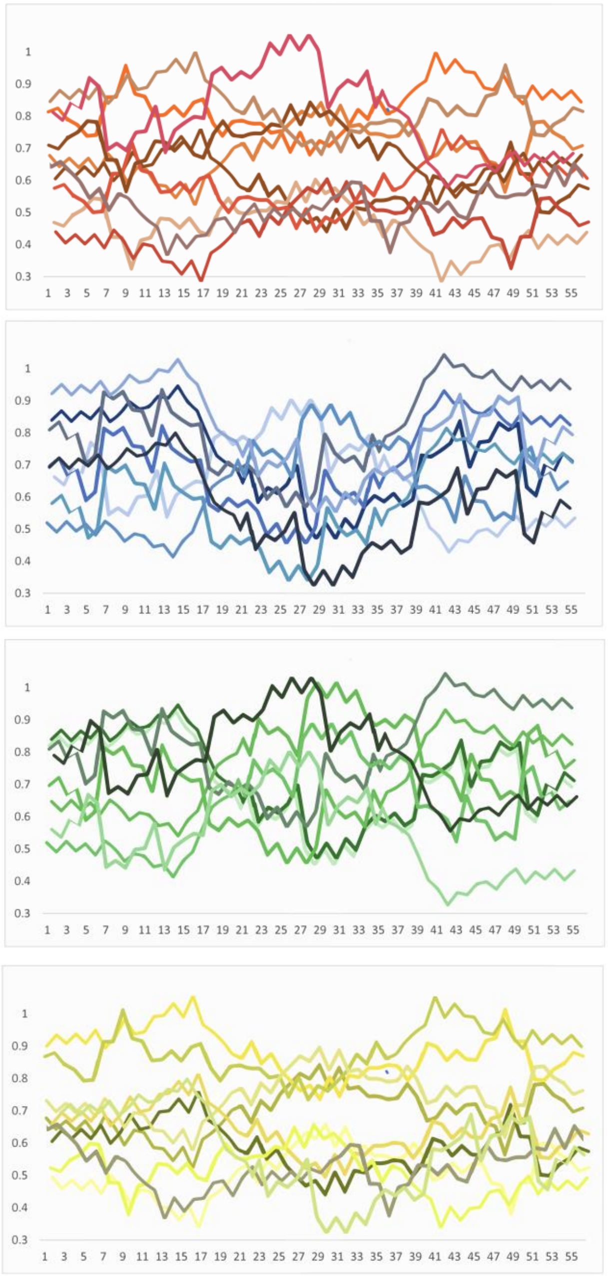
And how we approach it

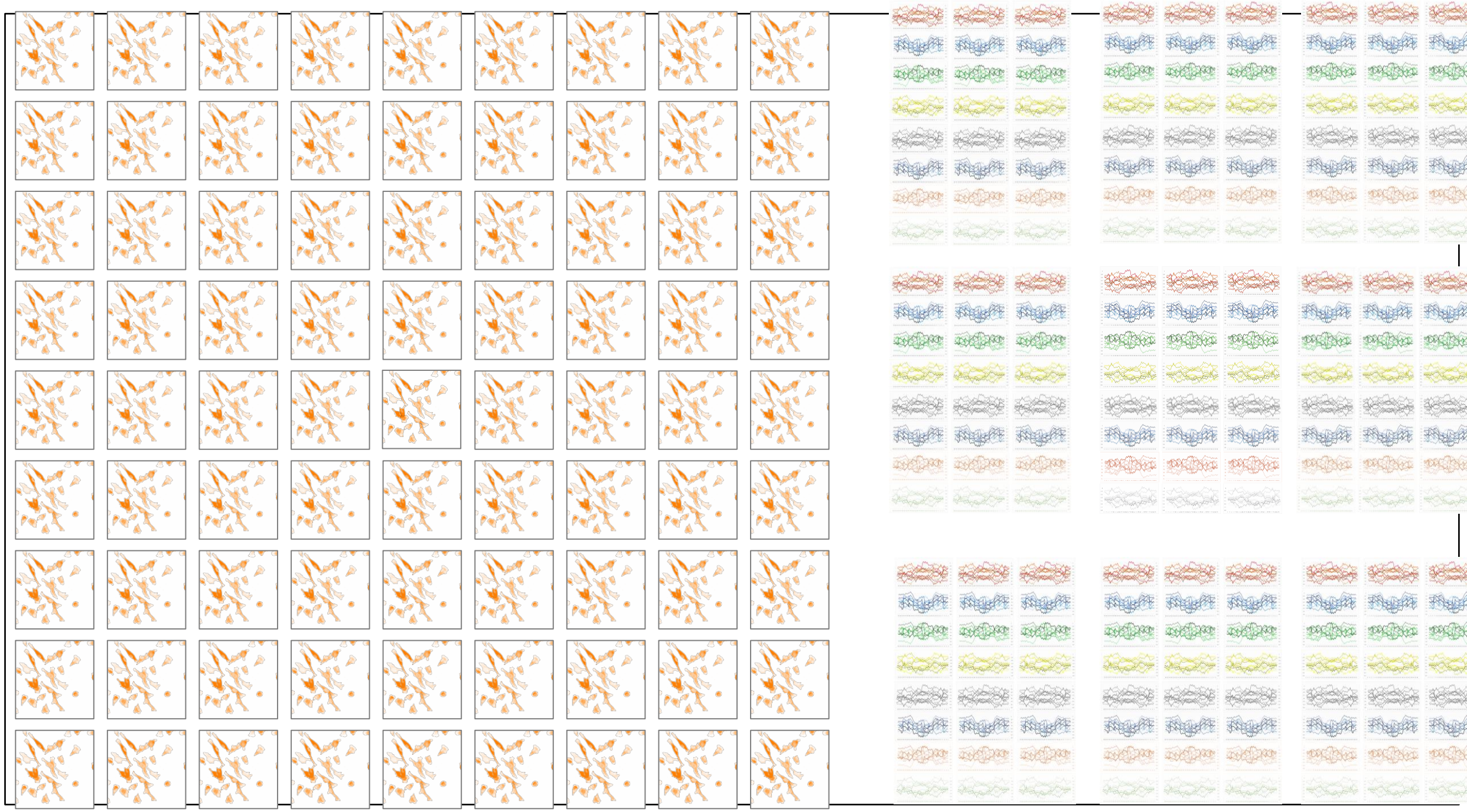
Steps of cell tracking analysis

- Segmentation
- Object linking
- Feature extraction
- Analysis





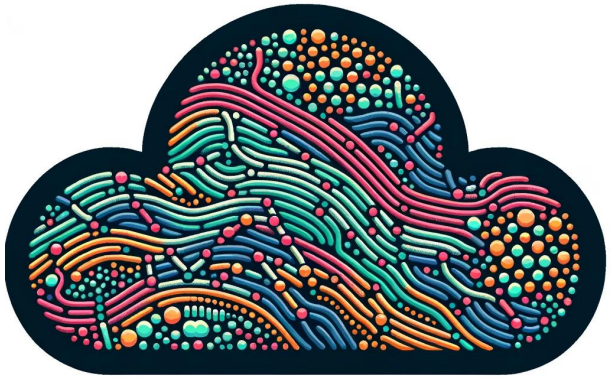

1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 43 45 47 49 51 53 55



Tools developed at Cell migration lab

trackmate

+



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

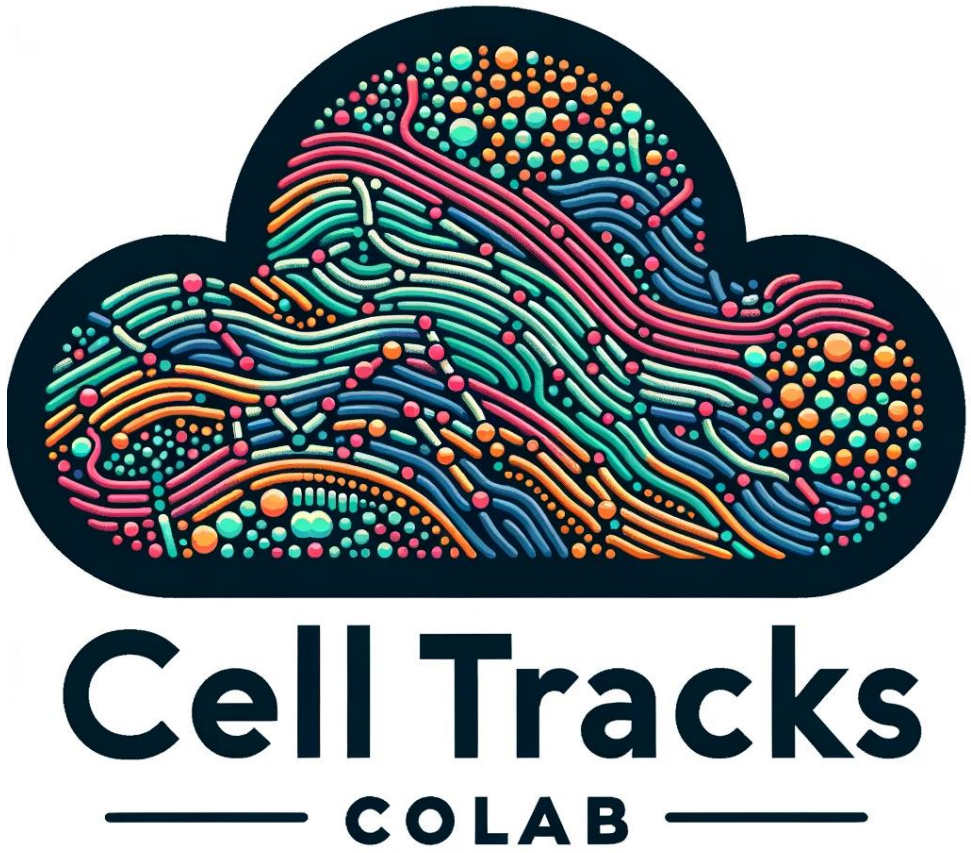
Platform tailored to simplify the exploration and analysis of cell tracking data.

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



What is CellTracksColab?

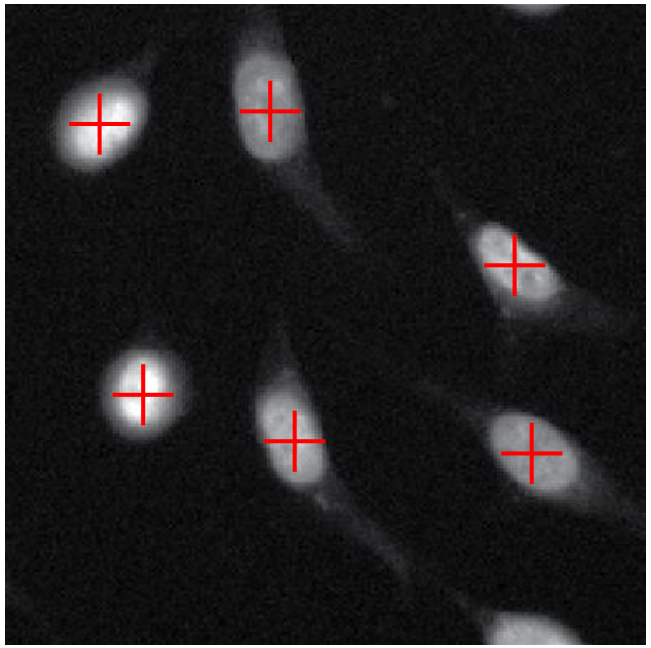
Cloud-based tool for track analysis.

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

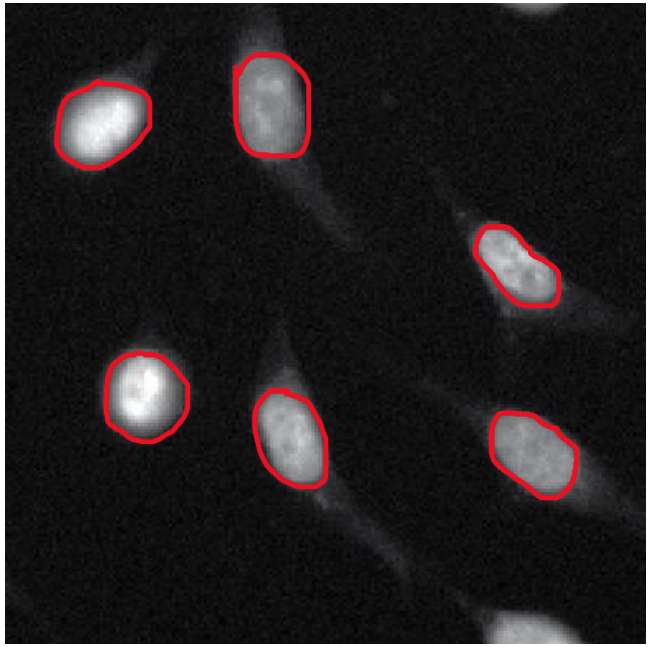
Gómez-de-Mariscal, E., Grobe, H., Pylvänäinen, J. W., Xénard, L., Henriques, R., Tinevez, J. Y., & Jacquemet, G. (2024). **CellTracksColab is a platform that enables compilation, analysis, and exploration of cell tracking data.** PLoS biology, 22(8), e3002740. https://doi.org/10.1371/journal.pbio.3002740

What is Particle and Object Tracking?

Particle tracking
Tracking the centroid of each particle's signal



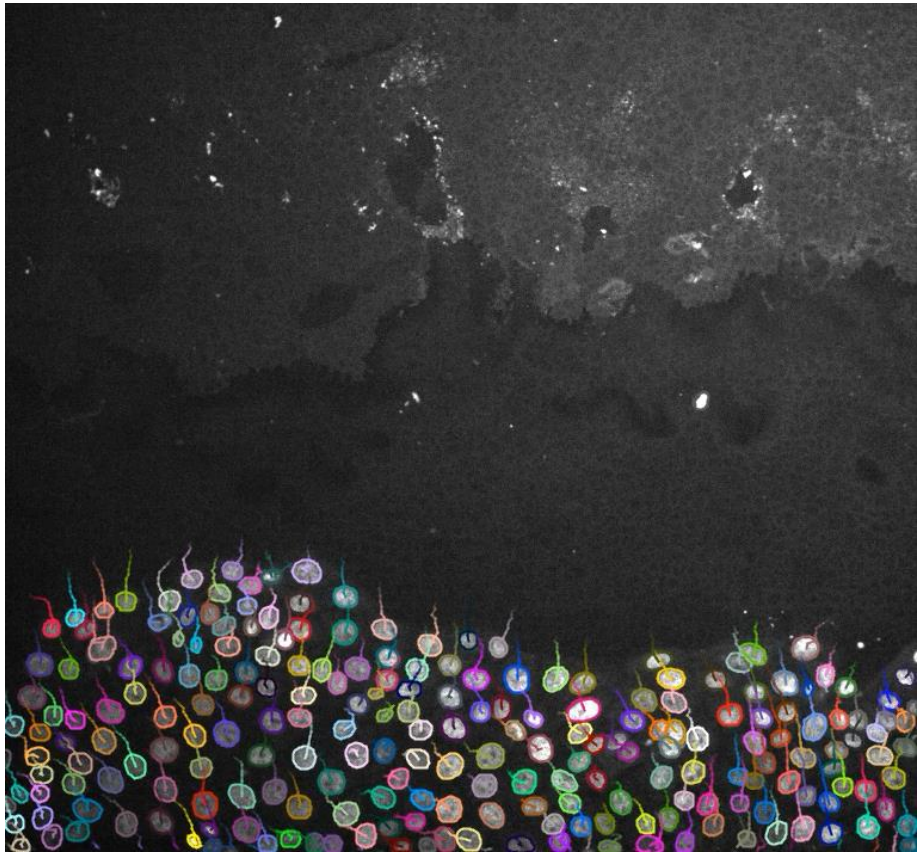
Object tracking
Tracking the entire shape and size of the object



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

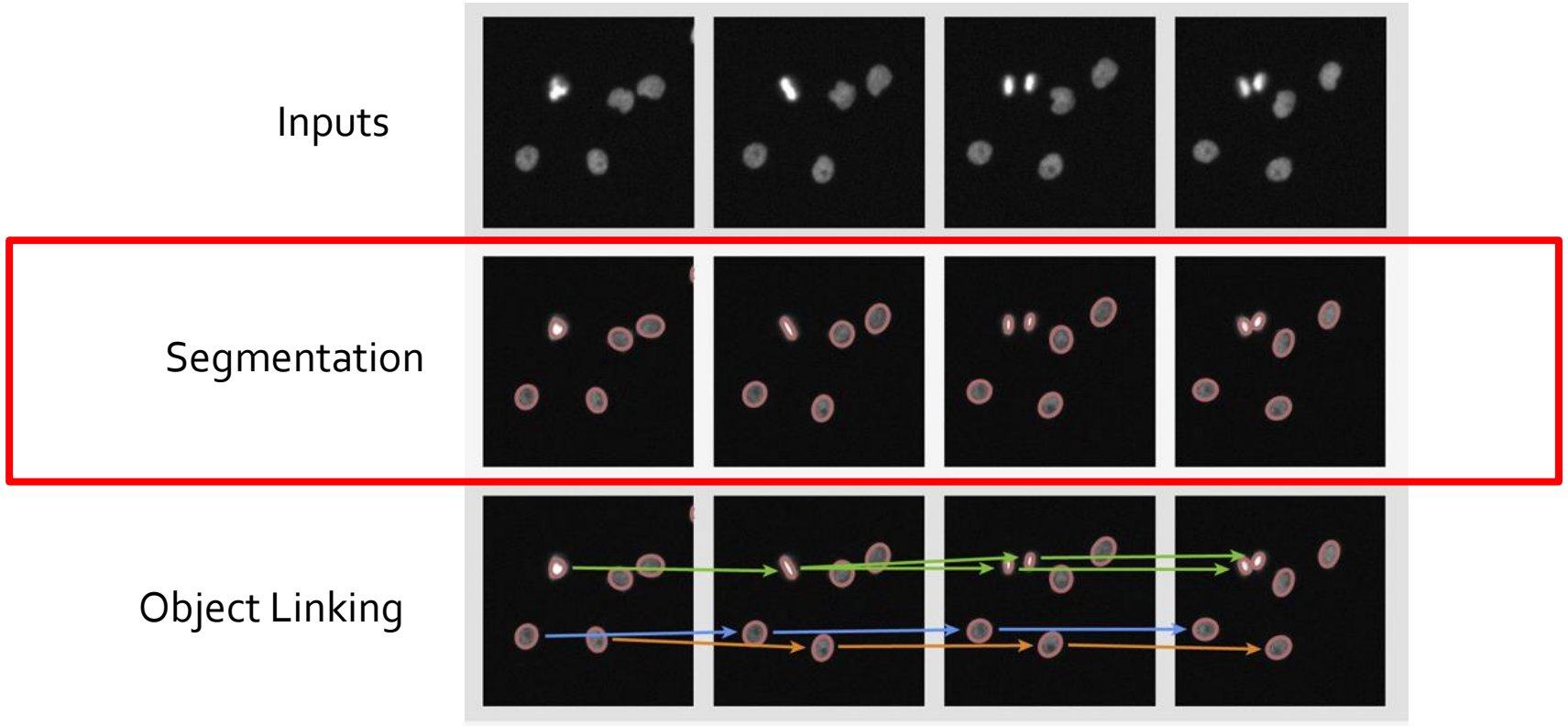
Applications in Cell Biology

- **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



Ershov et al. (Nature Methods, 2022)

Tracking principle in TrackMate



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

Segmentation

- B&W masks
- Label images
- Thresholded images

MorphoLibJ morphological segmentation

Weka custom model

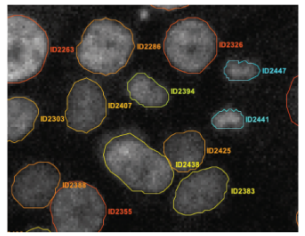
Ilastik custom pixel classification project

StarDist built-in nuclei model and custom models

Cellpose built-in models and custom models

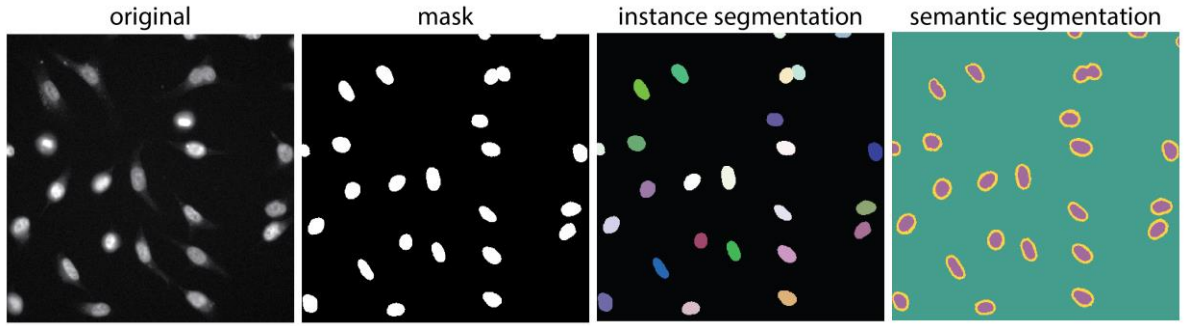
Integrated into TrackMate UI

trackmate v7



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

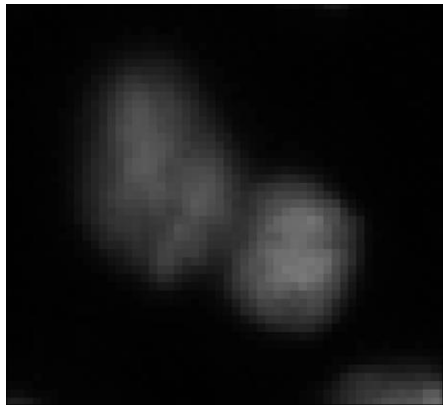
- Tracking cells
- Lineage tracing
- Changes in 2D shape over time
- Changes in intensity over time
- 2D to 3D segmentation



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

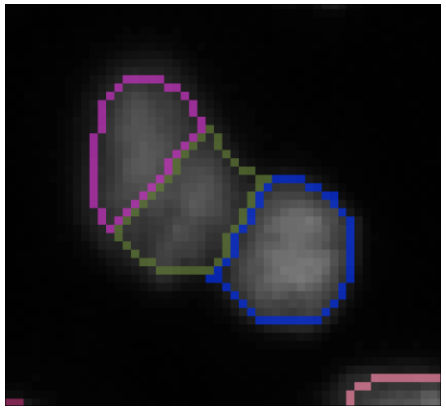
Segmentation

In the tracking context, object differentiation is important

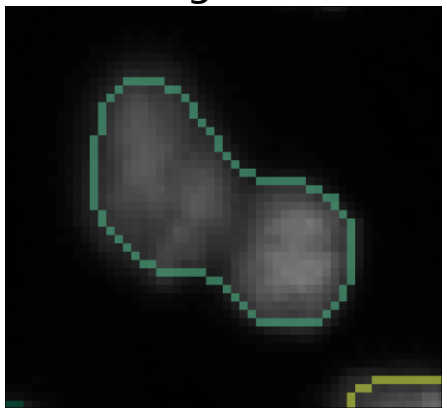


Raw image (nuclei)

Instance segmentation

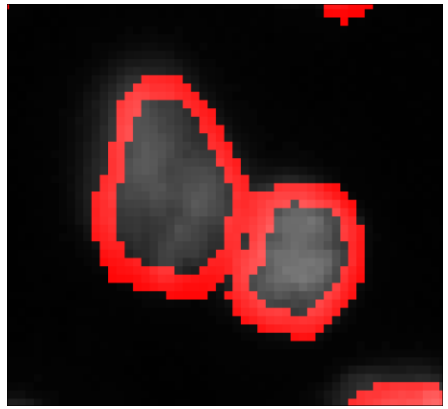


False objects
(over-segmentation)



Missed objects
(under-segmentation)

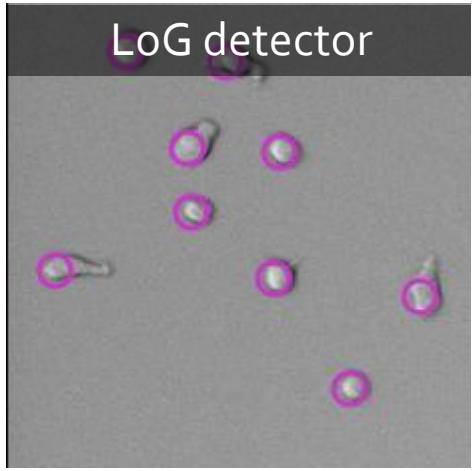
Outline precision can be of secondary interest

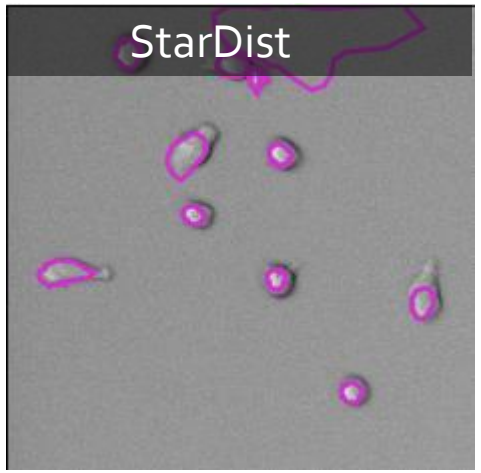


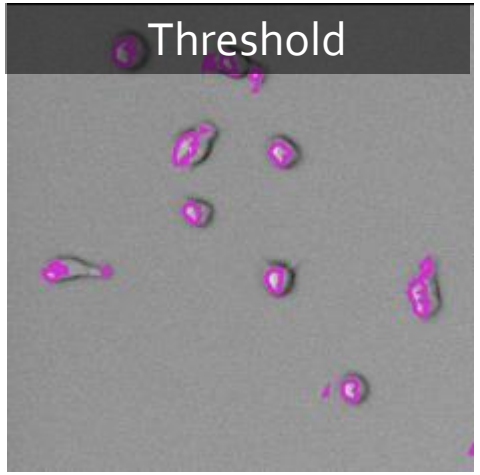
"Ground truth"
(manual annotation)

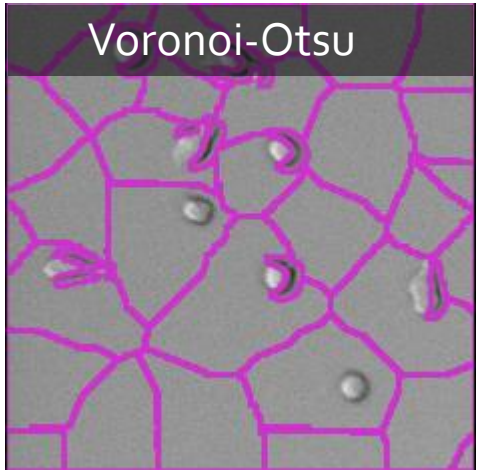
Image data source: Jones et al., *Proc. ICCV Workshop on Computer Vision for Biomedical Image Applications*, 2005 / Broad Bioimage Benchmark Collection [Ljosa et al., *Nature Methods*, 2012, https://bbbc.broadinstitute.org/BBBC007

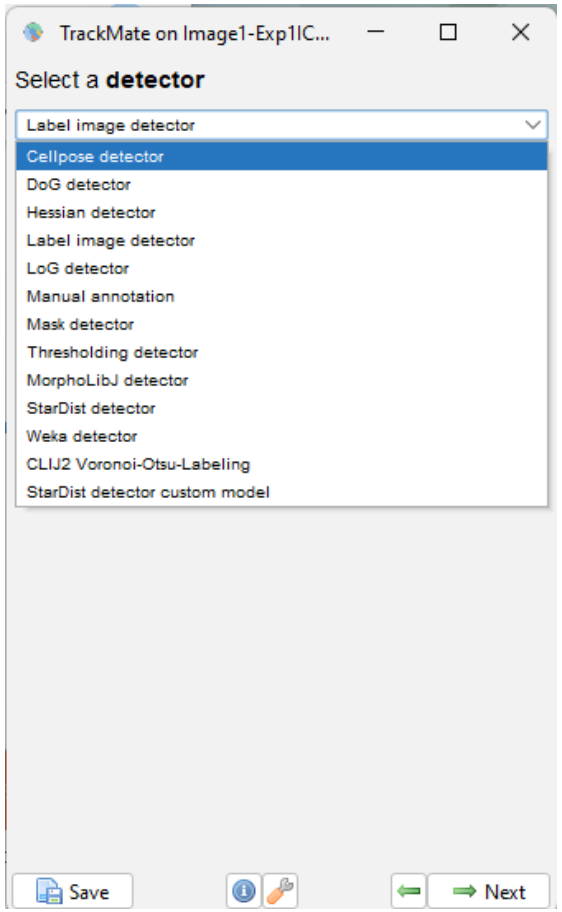
TrackMate object detection

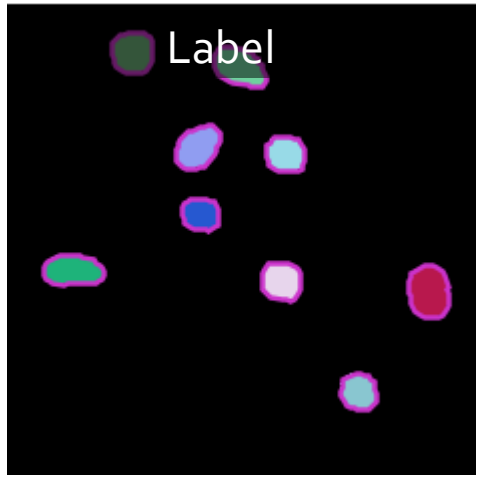


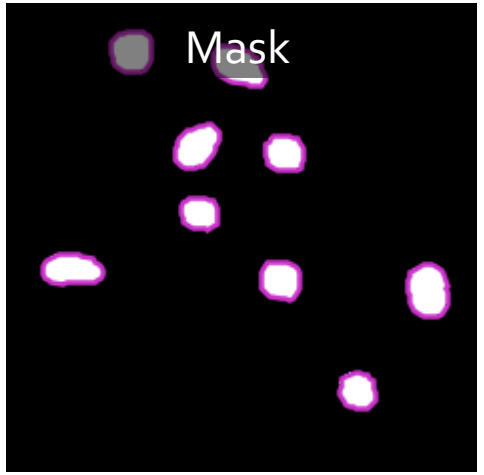




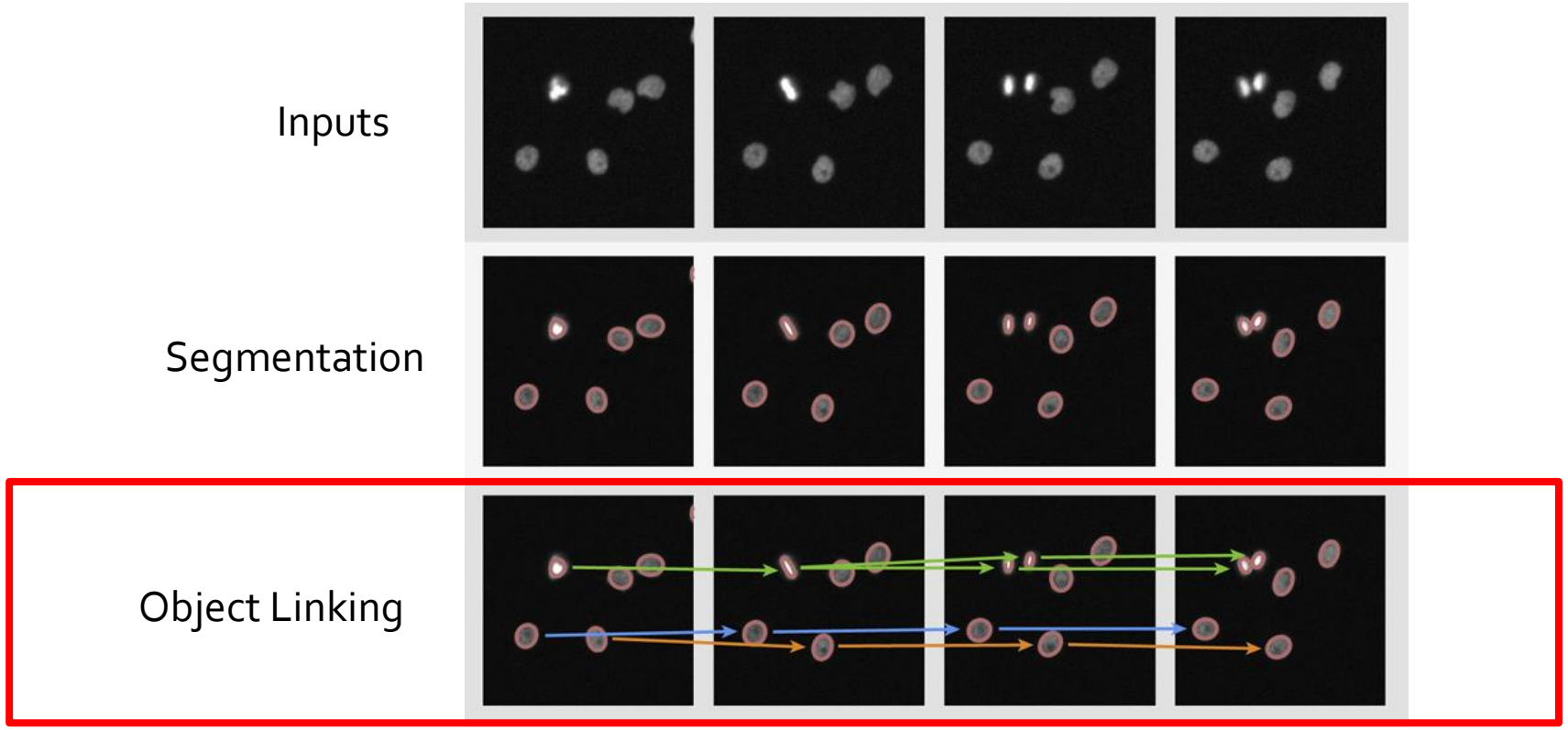








Tracking principle in TrackMate

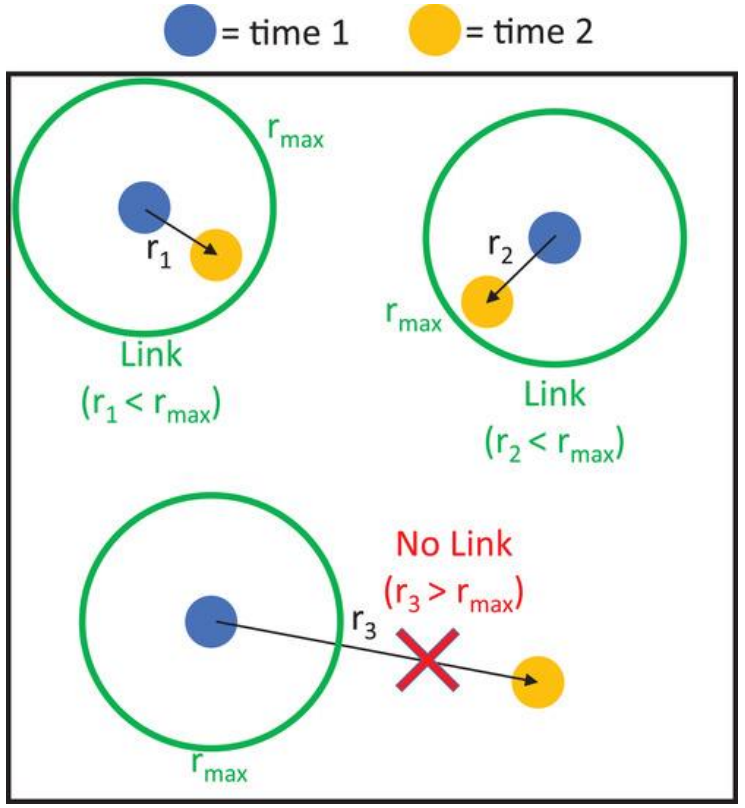


- **Trackers** are algorithms or tools used to follow the movement of objects over time in a series of images or videos.

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

Tracking algorithm example - LAP tracker

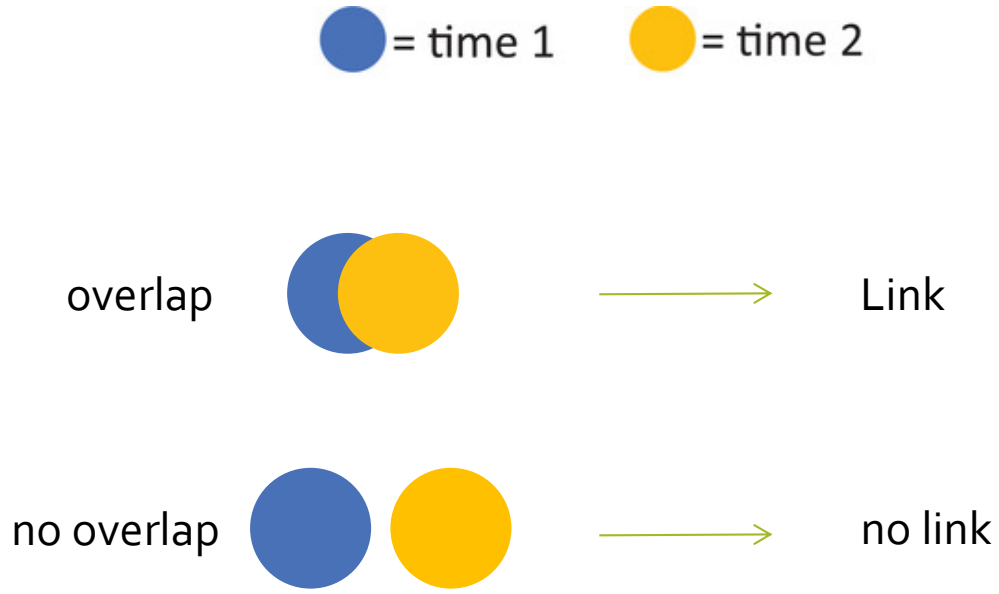
Object linking using a search radius (LAP tracker)



Aaron et al. (Current Protocols in Cell Biology, 2019)

Tracking algorithm example - overlap tracker

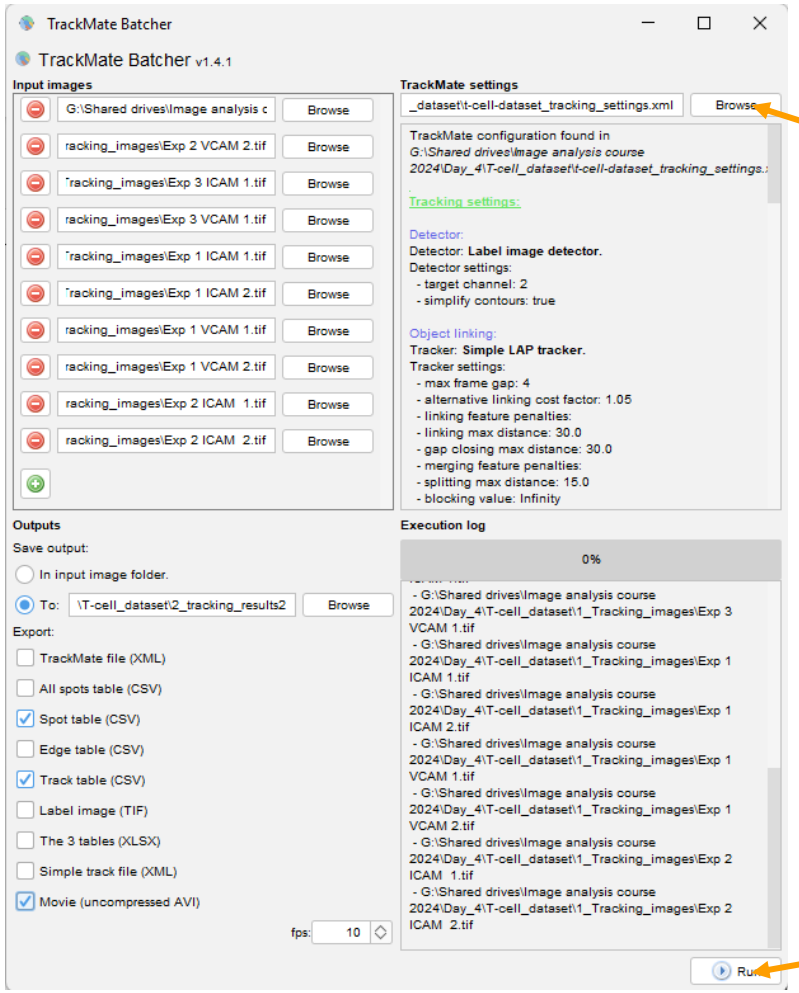
Object linking using an overlap (The overlap tracker)
The Overlap tracker is well suited for large objects that move by less than their diameter.



What if you have multiple movies to track?

Drag and drop the files to track

Export settings



Path to TrackMate settings file

t-cell-dataset_tracking_settings

Log window

Start!

Output:

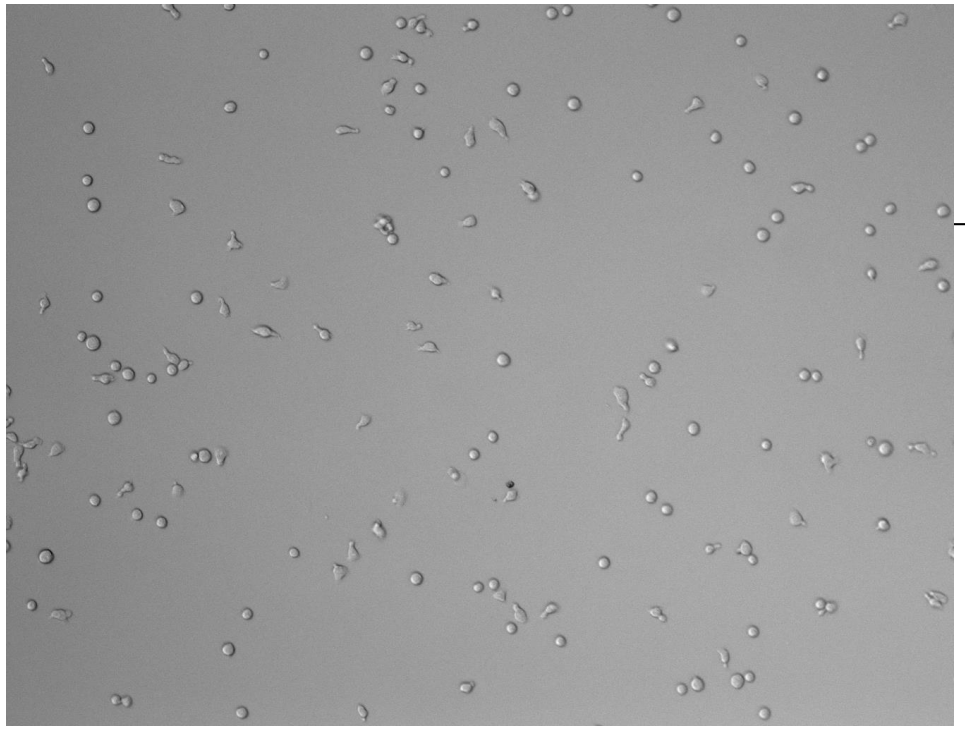
Name	Date modified	Type	Size
Exp 1 ICAM 1-movie	25/09/2024 15.06	AVI File	84 677 KB
Exp 1 ICAM 1-spots	25/09/2024 15.06	Microsoft Excel C...	1 653 KB
Exp 1 ICAM 1-tracks	25/09/2024 15.06	Microsoft Excel C...	66 KB
Exp 1 ICAM 2-movie	25/09/2024 15.06	AVI File	84 677 KB
Exp 1 ICAM 2-spots	25/09/2024 15.06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

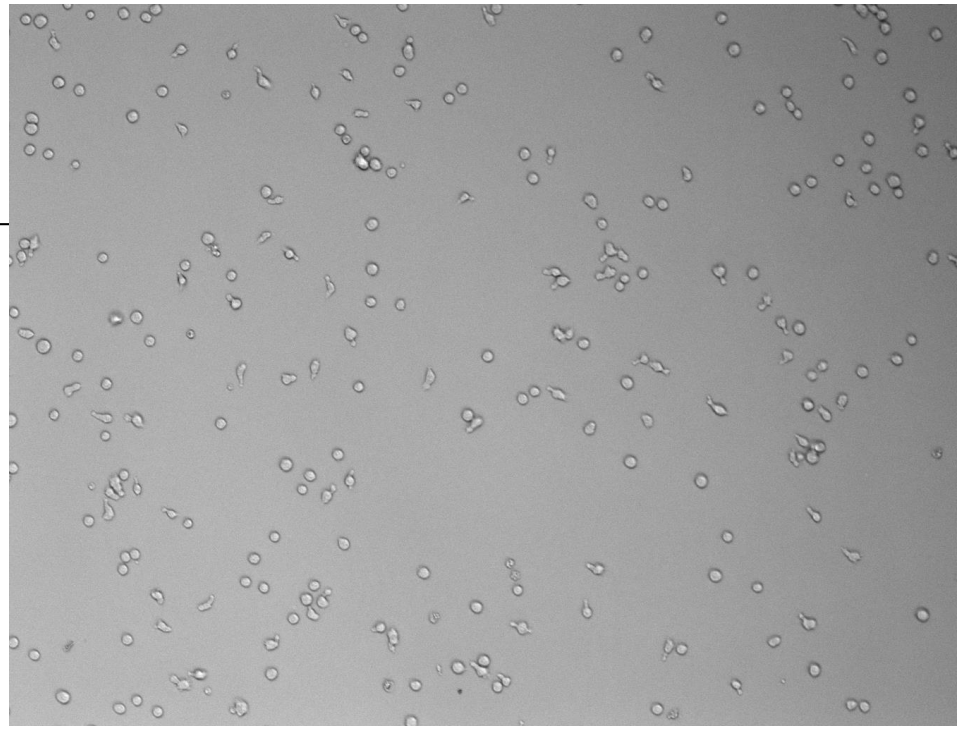
OUR TASK TODAY

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

ICAM



VCAM

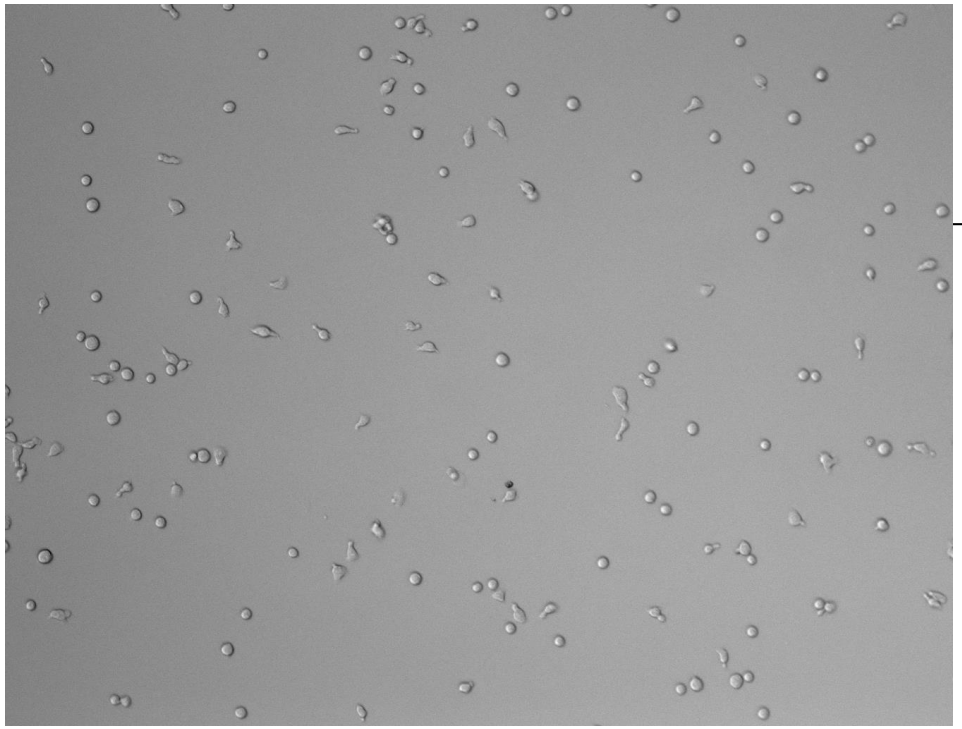


T-Cell Dataset Guillaume Jacquemet. (2023). **T cell dataset for CellTracksColab - 2** [Data set]. Zenodo. https://doi.org/10.5281/zenodo.8420011

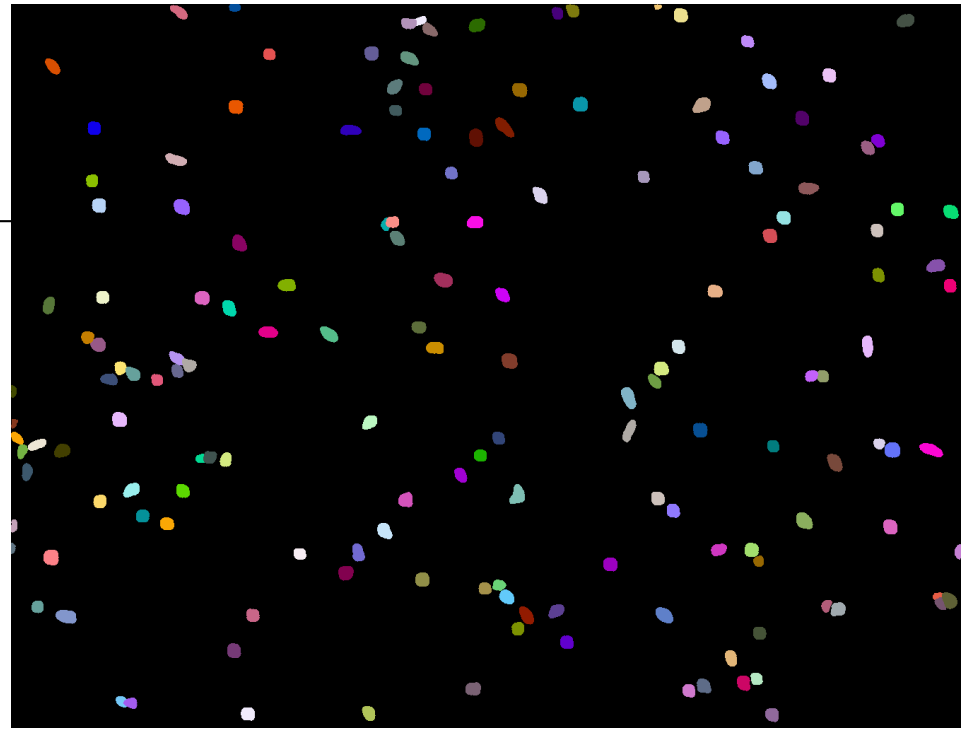
T-CELL DATASET

Your test dataset has two channels

Channel 1 – brightfield images



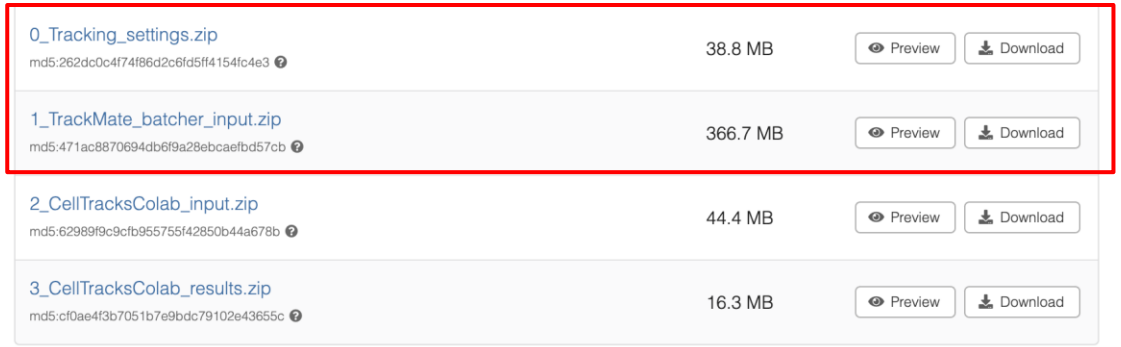
Channel 2 – cell labels



T-Cell Dataset Guillaume Jacquemet. (2023). **T cell dataset for CellTracksColab - 2** [Data set]. Zenodo. https://doi.org/10.5281/zenodo.8420011

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



https://zenodo.org/records/14645477

Track Analysis

Experiments

- Multiple conditions
- Multiple repeats
- Multiple FOVs

Datasets

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

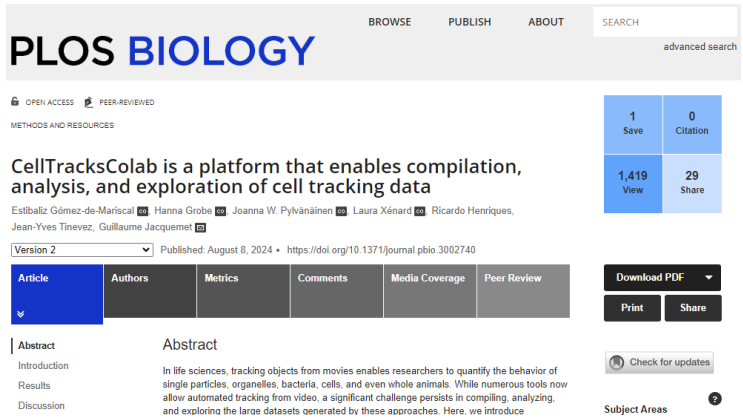
Analysis

HOW DO I ANALYZE ALL THIS DATA?

Cell Tracks COLAB

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



What is CellTracksColab?

ICY

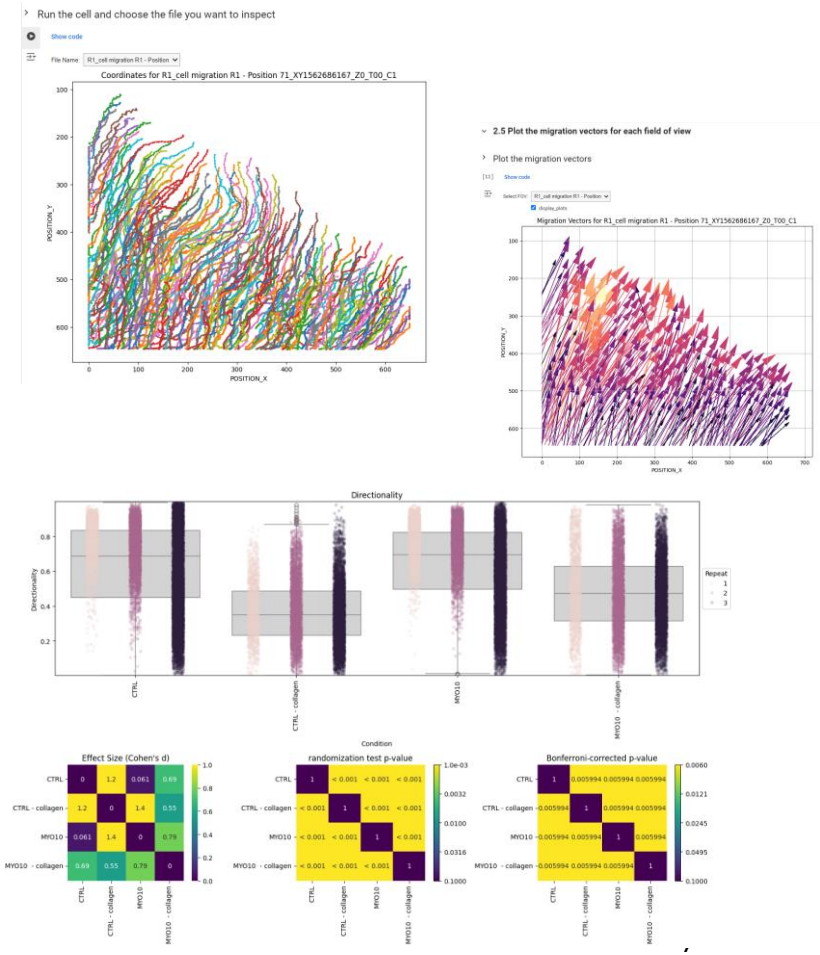
FIJI

Ilastik

CellProfiler

TrackMate

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

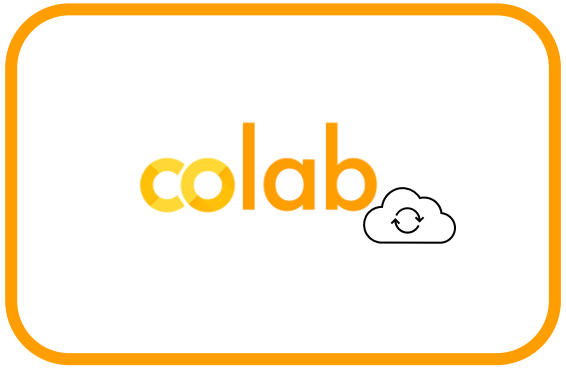
Simple analysis notebooks

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

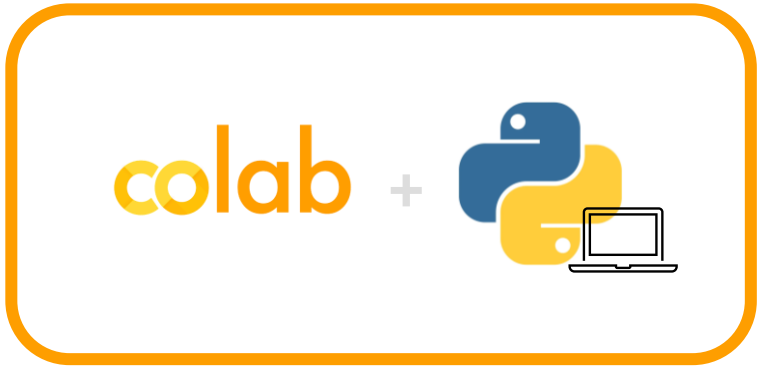
Advanced analysis notebooks

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

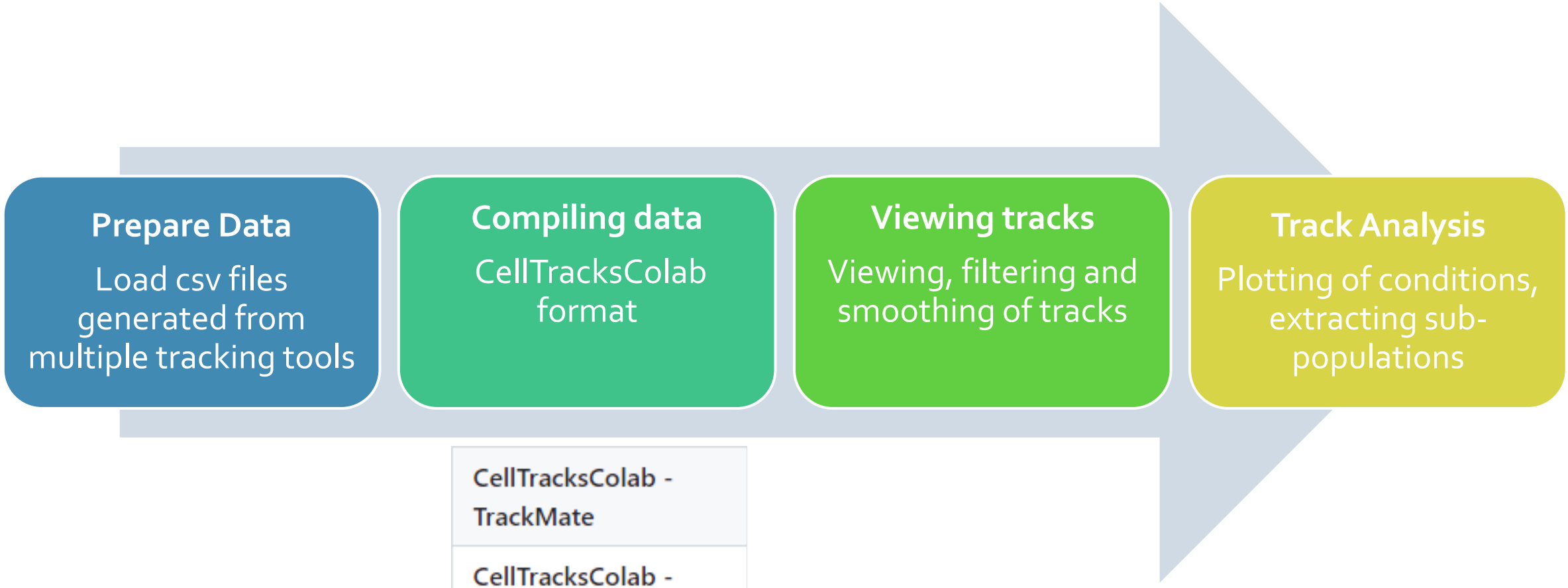


Locally using Google Colab

Locally using Jupyter

https://github.com/CellMigrationLab/CellTracksColab

CellTracksColab Workflow



Requirements for CellTracksColab

CellTracksColab - TrackMate

CellTracksColab - Custom

- 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

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

Minimum requirements for data

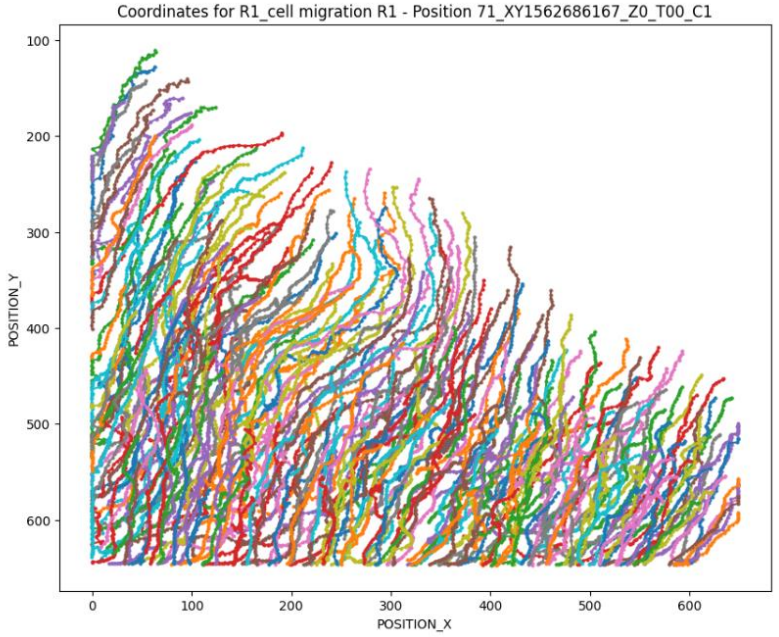
Track ID	X Coordinate	Y Coordinate	Z Coordinate	Time Point
0	687.991	150.047	0.0	0.0
1	1255.994	467.008	0.0	0.0
2	171.994	853.024	0.0	0.0
... (and so on)				

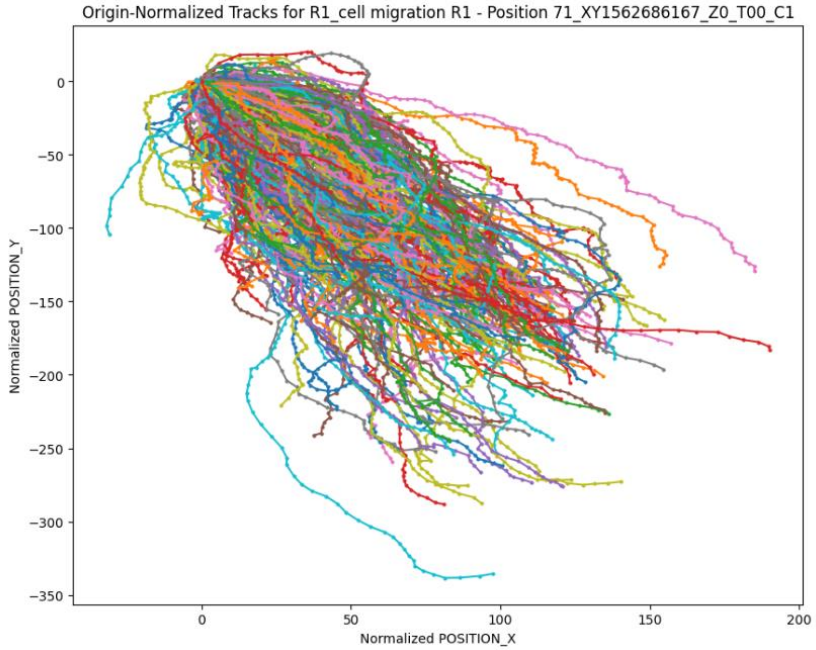
Track Visualization

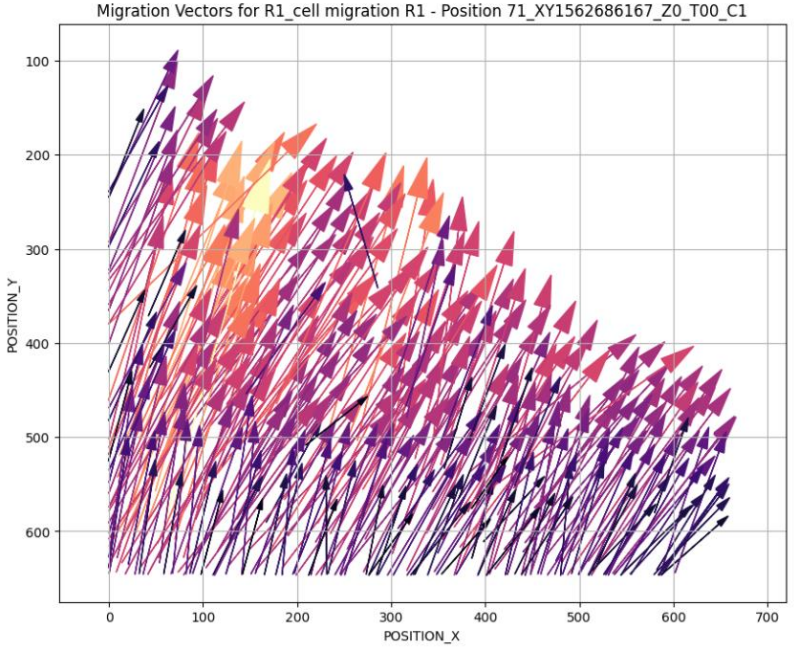
CellTracksColab - TrackMate

CellTracksColab - Custom

CellTracksColab - Viewer

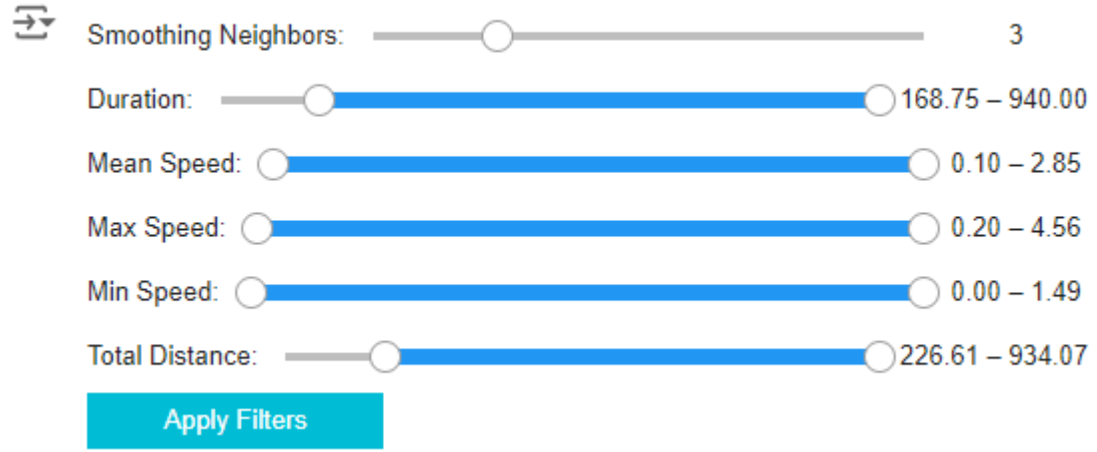


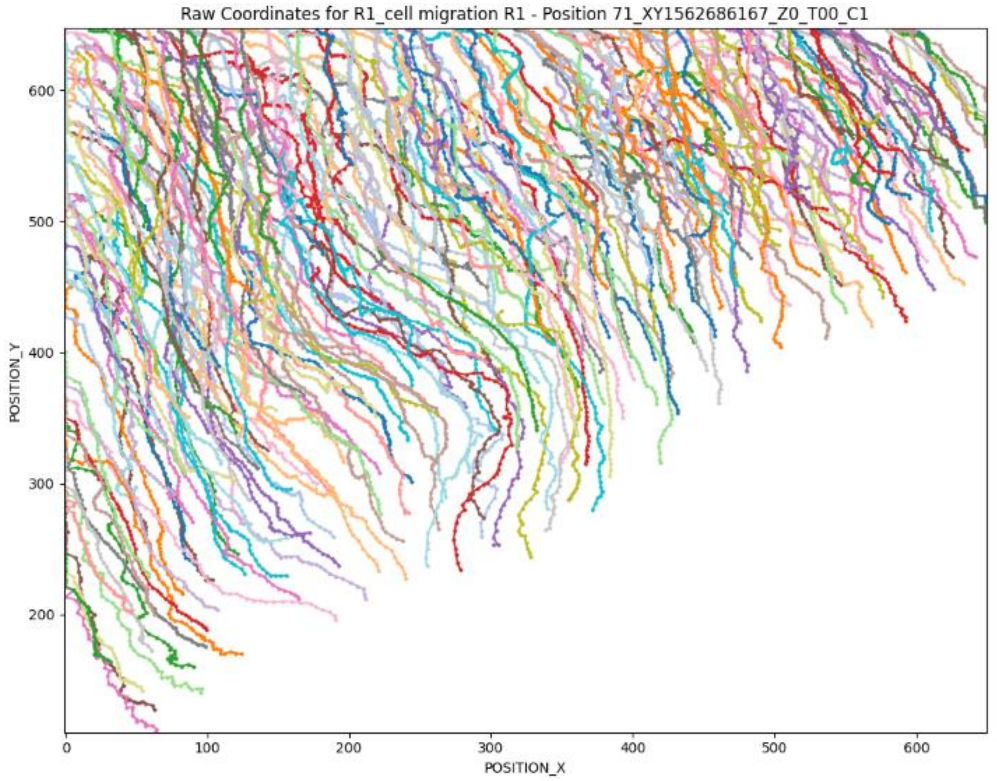


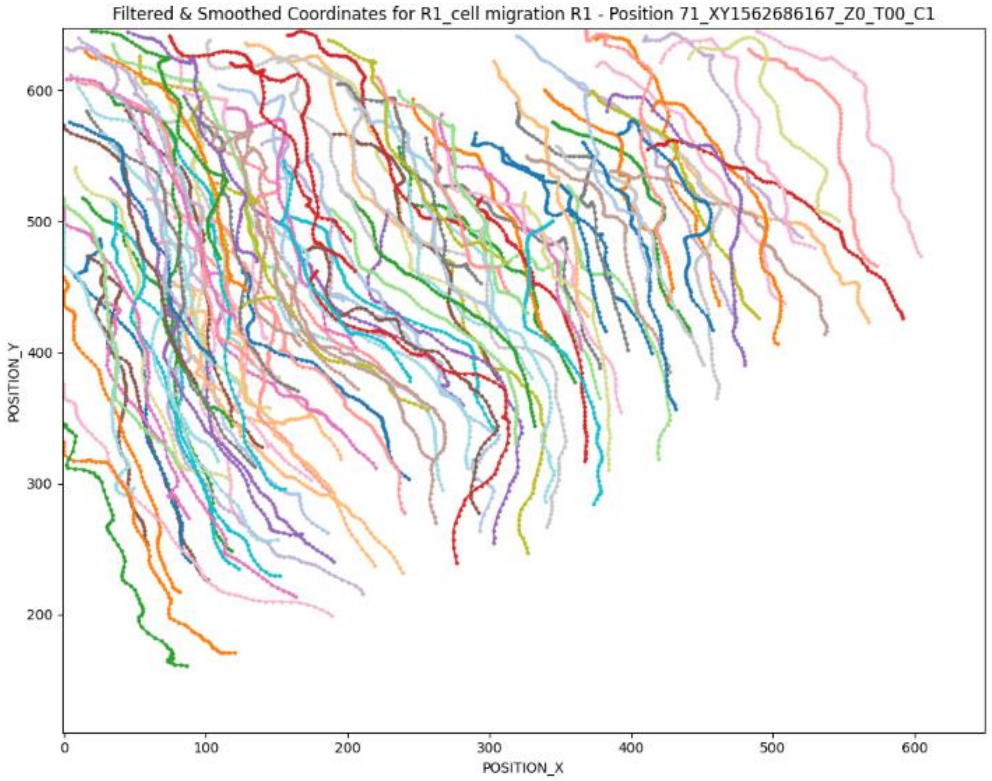


- By repeat
- By condition

Track filtering







CellTracksColab - TrackMate

CellTracksColab - Custom

CellTracksColab - Viewer

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`

CellTracksColab - TrackMate

CellTracksColab - Custom

CellTracksColab - Viewer

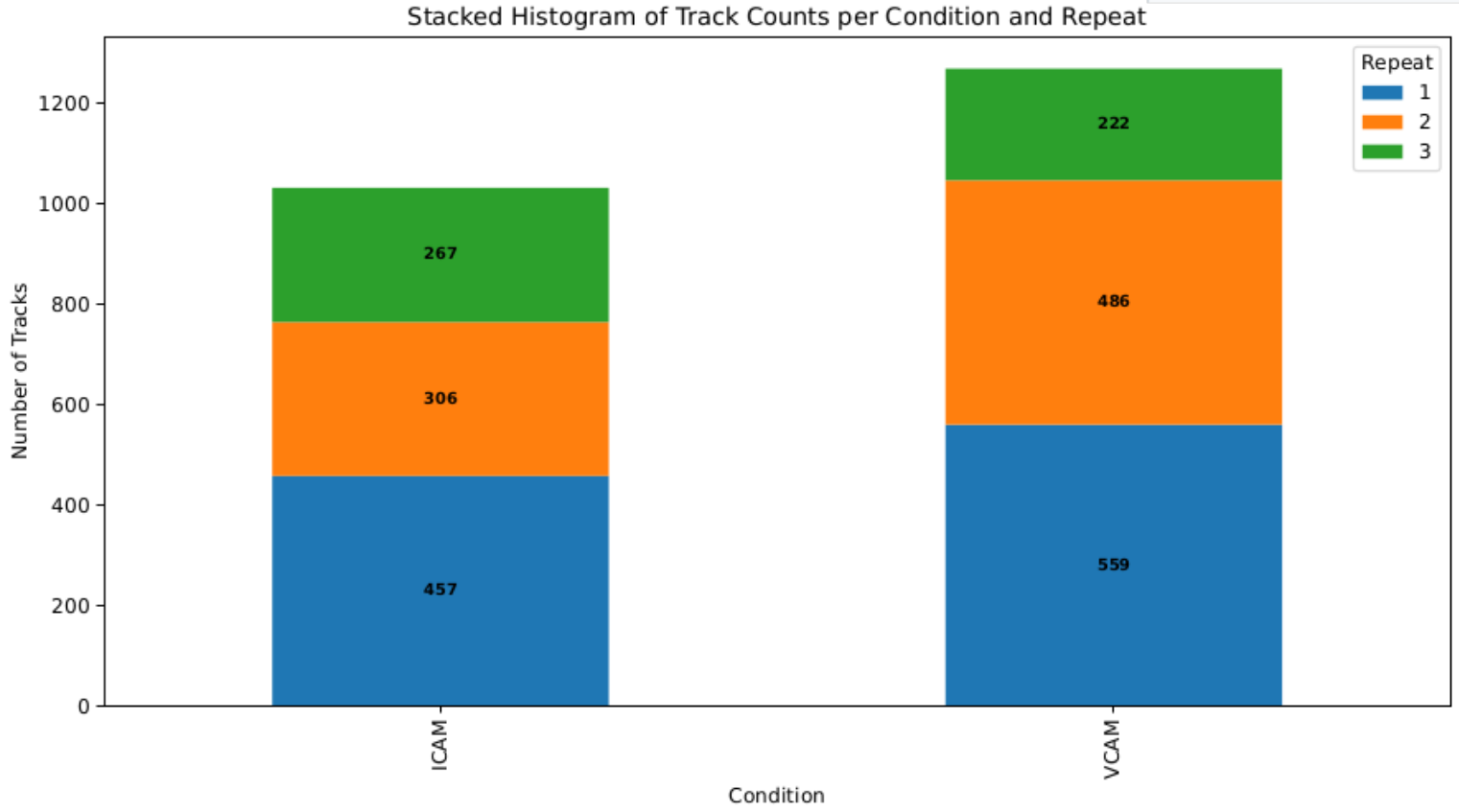
Quality Control - randomization

Purpose

- Each biological repeat should carry equal weight in the analysis
- Randomization is a method to check if an observed difference between two groups is **real** or just a **random chance**.

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.



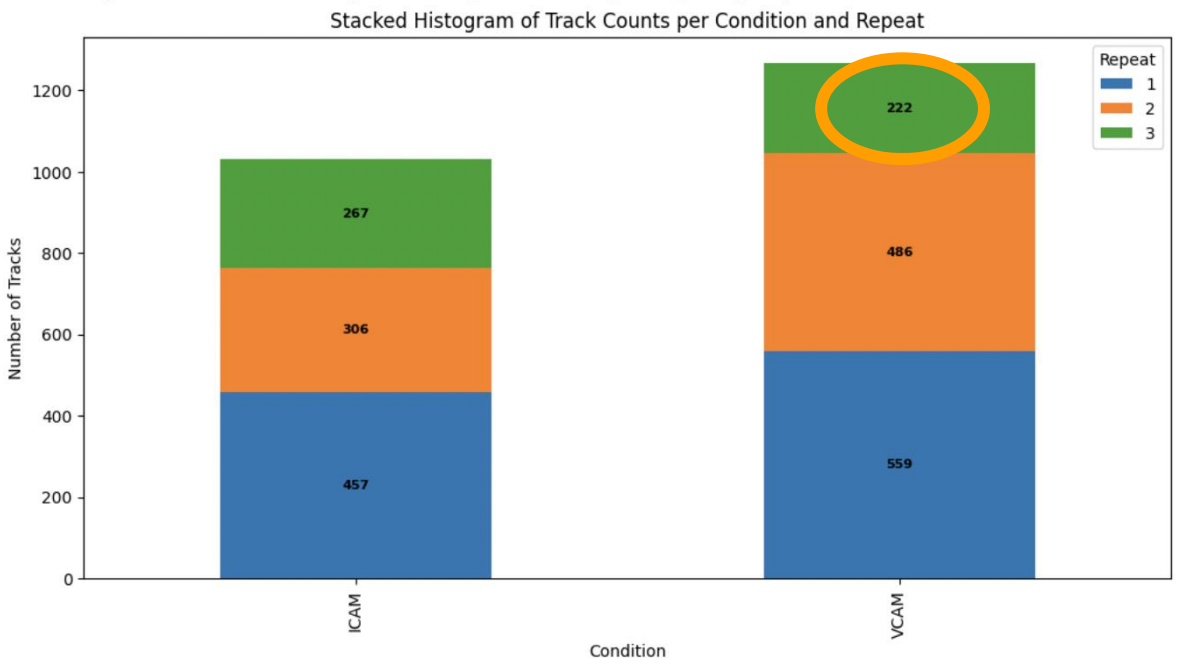
Note: If your data is imbalanced, consider balancing it to prevent skewing your results.

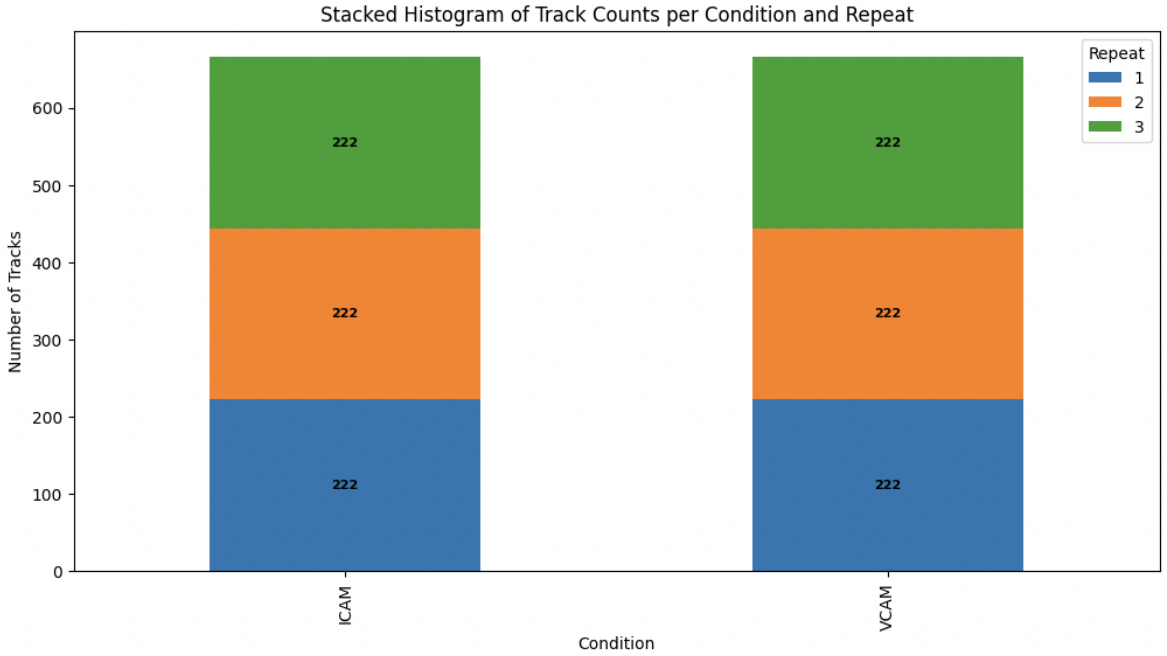
Data balancing - randomization

CellTracksColab - TrackMate

CellTracksColab - Custom

CellTracksColab - Viewer





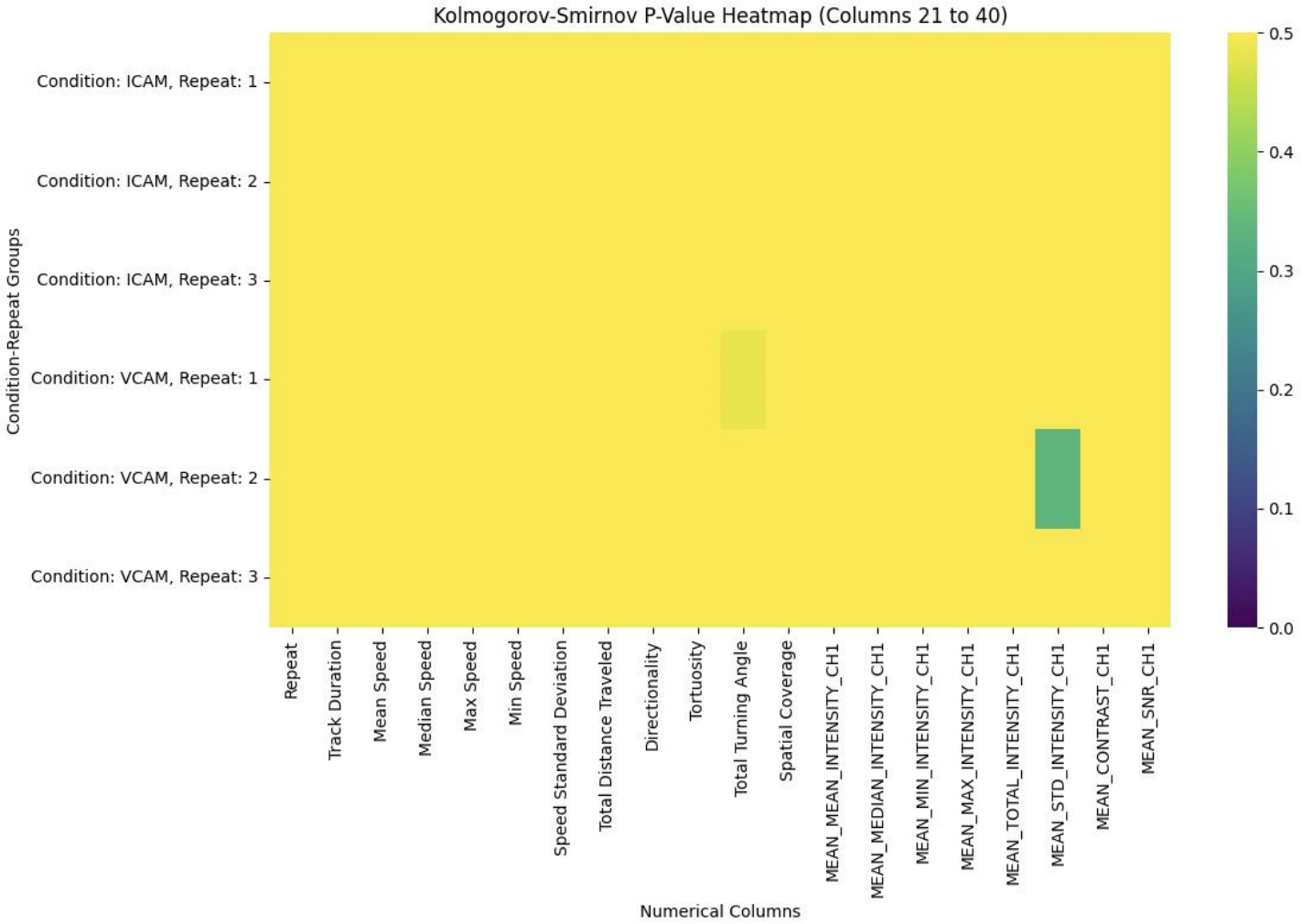
- Mix all data from all groups and randomly divide them into new groups.
- Calculate the effect size (difference) for the shuffled groups.
- Repeat x times to create a "random chance" baseline.

CellTracksColab - TrackMate

CellTracksColab - Custom

CellTracksColab - Viewer

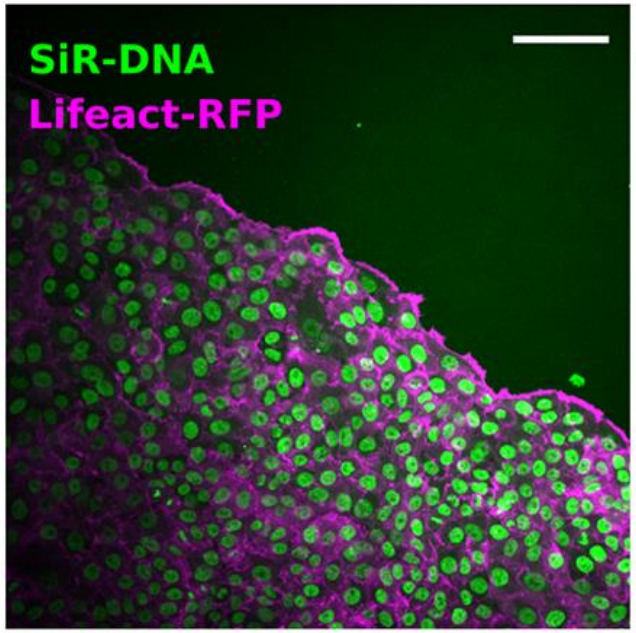
Effect of data balancing

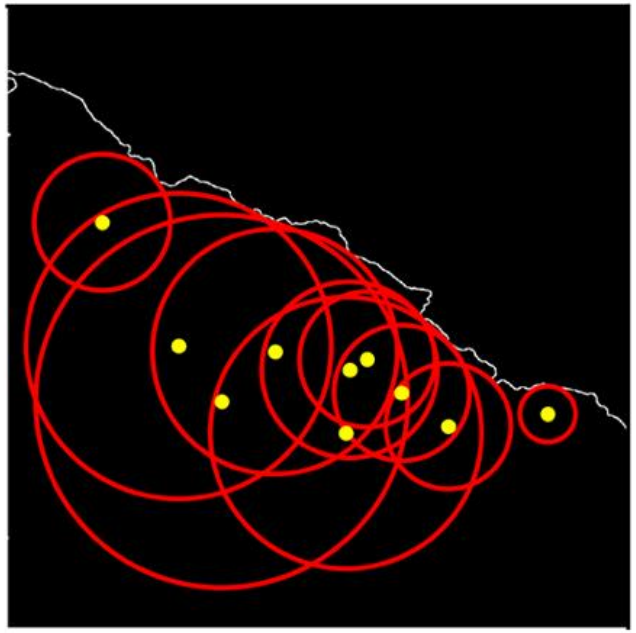


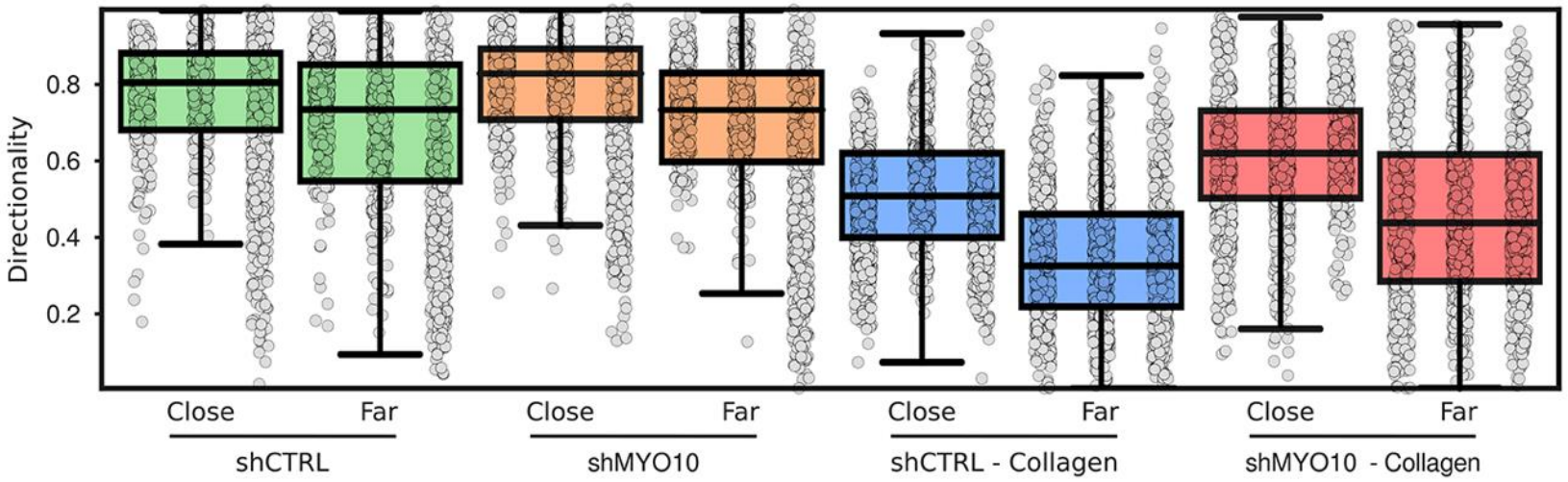
- Compares the distribution of each numerical column in the original and resampled datasets to assess if there is a significant change
- Presents p-values visually
- **High P-Values (Yellow):**
  - Down-sampling process likely did not significantly alter the distribution of that numerical column for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

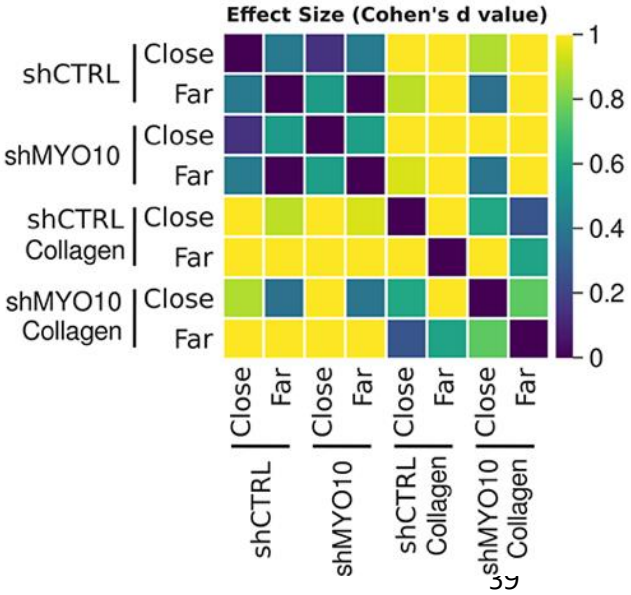
CellTracksColab - Distance to ROI

Distance to ROI







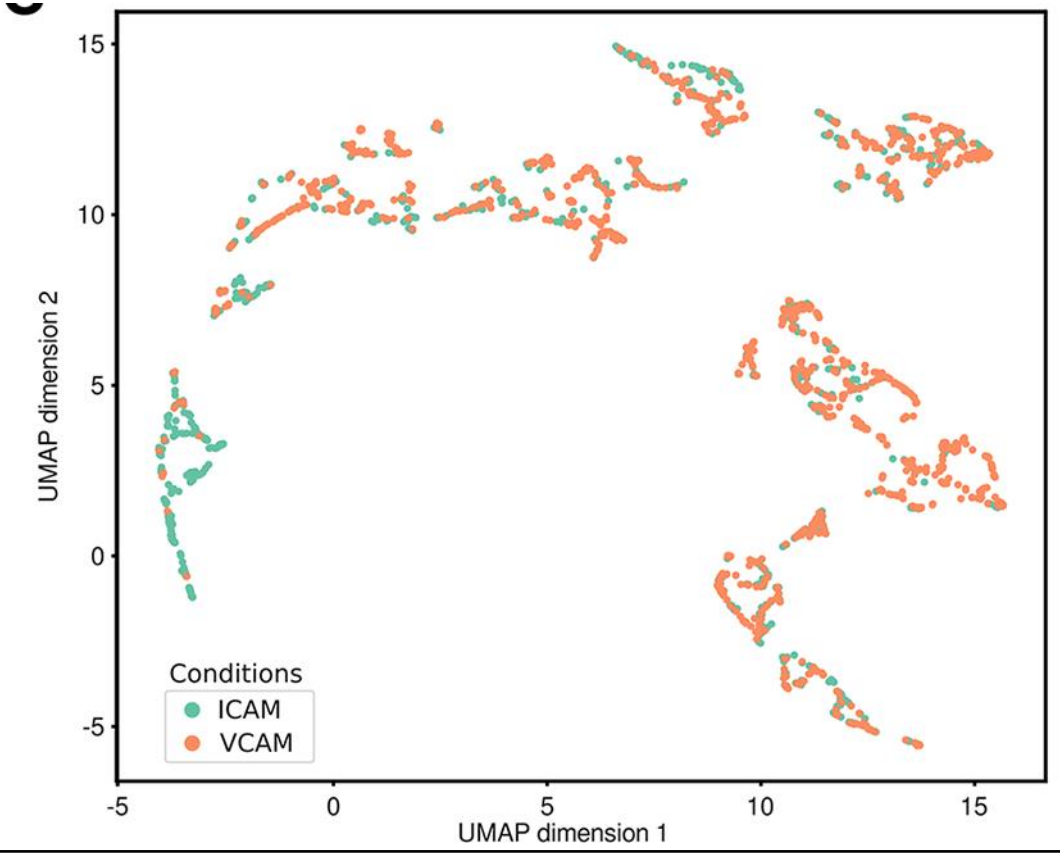


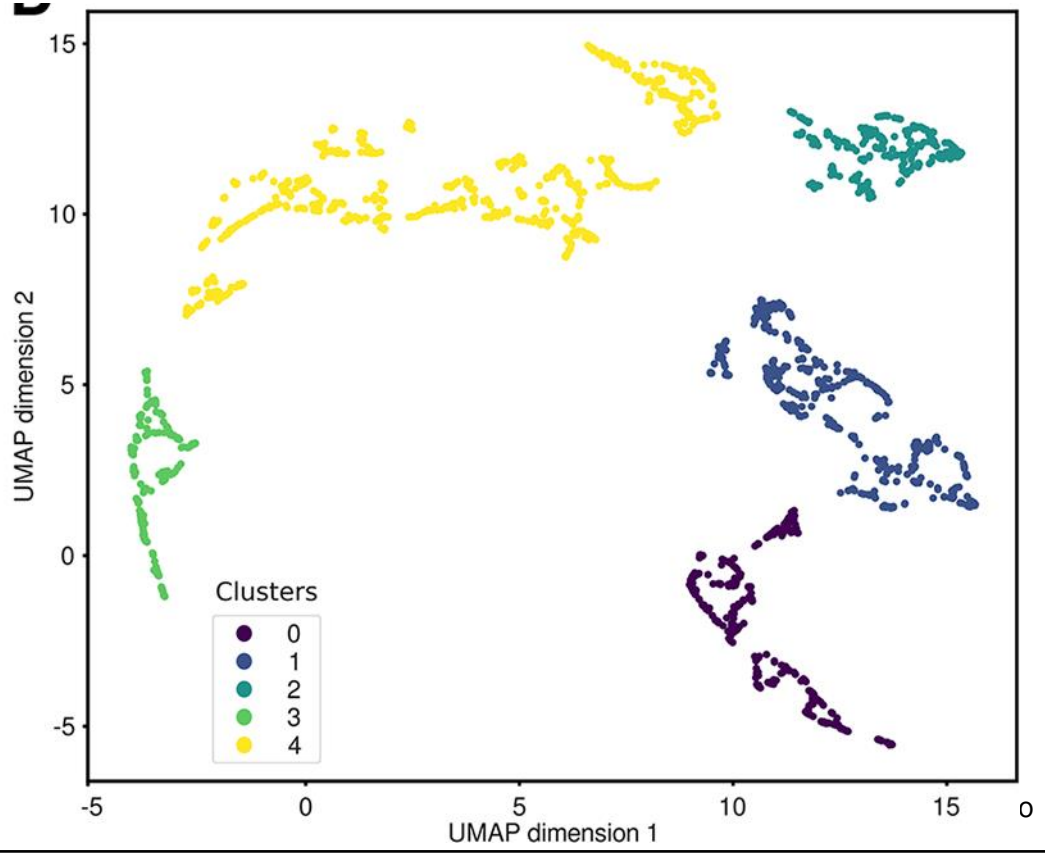
CellTracksColab - Dimensionality Reduction

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.

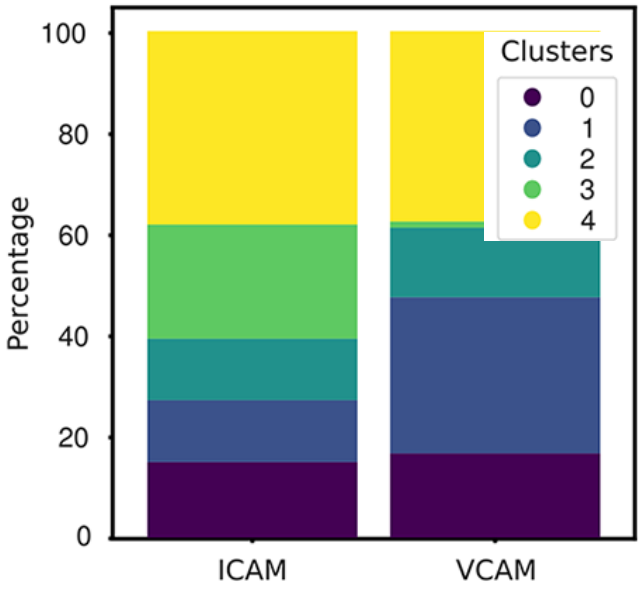




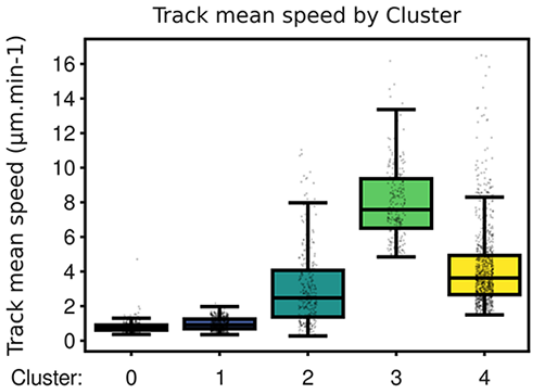
CellTracksColab - Dimensionality Reduction

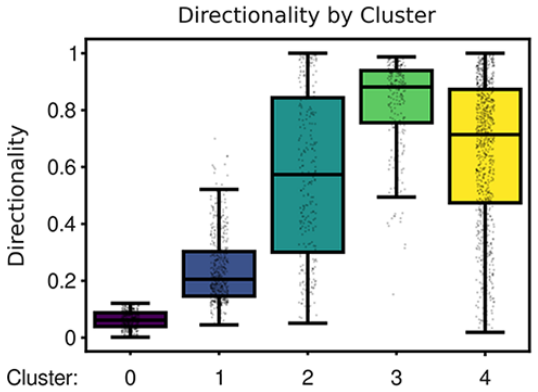
Data fingerprinting and sub-populations

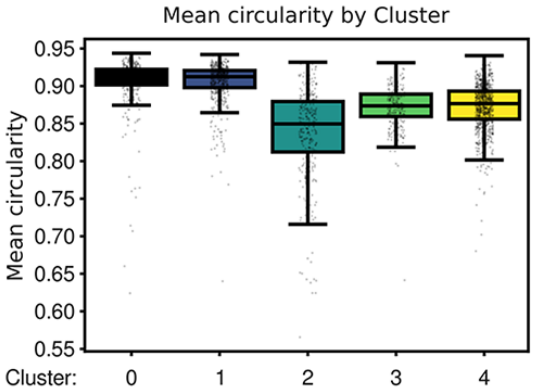
Fingerprinting:
Each cluster represents a group of similar cells or behaviours. Fingerprinting helps create a unique profile for each group.



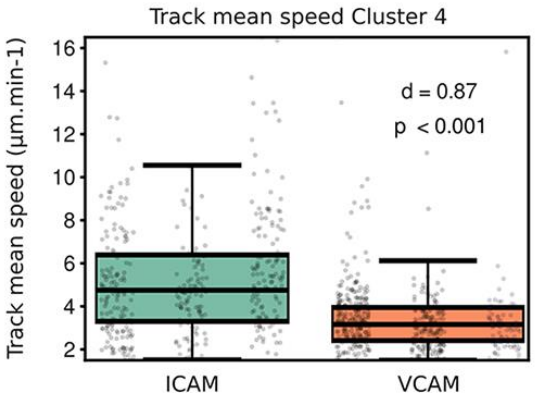
By cluster

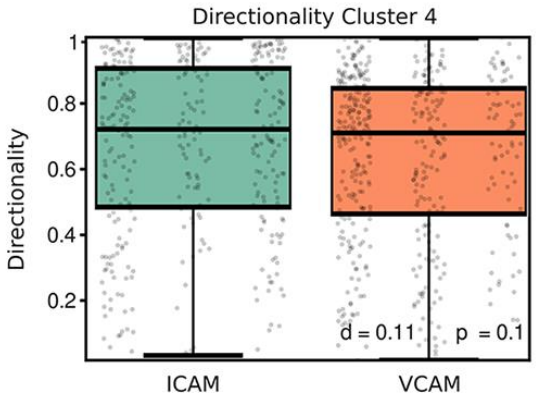


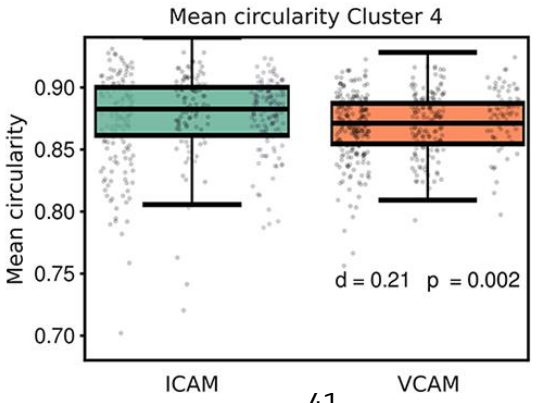




By condition







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.

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.