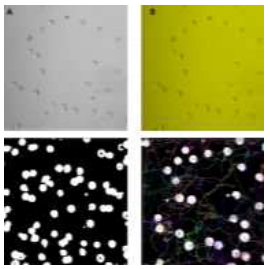
Sep 23, 2024

Easy tracking of unstained cells in ImageJ



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Protocol status: Working
We use this protocol and it's working

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Abstract

This protocol shows how stained and especially unstained cells can be tracked in ImageJ without much effort.

The aim was to establish a reproducible setup for electrical stimulation and to perform live tracking of the stimulation with a confocal laser microscope. The study also aimed to develop a methodology for a comprehensive and standardised assessment of all parameters independent of cell staining.
The underlying experiment aimed to develop methods for analysing cellular responses to electrical stimulation and to investigate the effects of electrical stimulation on the adhesion and migration behaviour of L929 and HGF-1 fibroblasts using an electrotaxis chamber. This method can be used for single cells and cell clusters and is not limited to fibroblasts. Scratch assays and other image series from the LSM can also be analysed.

For the experiment, time-lapse series were acquired every 5 minutes over 18 hours using an LSM 780 microscope (Carl Zeiss Microscopy GmbH, Germany; software: ZEN 2.3 SP1 FP3, black, Ver. 14.0.27.201). The images were exported to the ImageJ software (1.54f, Rasband, National Institute, USA) to analyse cell migration, which is presented here. Since a bicarbonate-free medium was used, additional CO_2 gasification was not necessary.

Slight deviations from the original setup can lead to different results, and insufficient parameter information makes reproducibility difficult. It is, therefore, essential to provide comprehensive information, including instrument specifications, sample preparation techniques and measurement conditions. Transparent reporting and open data practices improve the reproducibility and reliability of scientific studies.

Guidelines

N/A

Materials

N/A

Safety warnings

N/A

Ethics statement

N/A

Before start

Make sure following plugins are installed
- TrackMate-StarDist
- StarDist
- CSBDeep

1 Depending on whether images of stained or unstained cells were taken, proceed as follows:

STEP CASE

Image Processing 14 steps

2 **Process → Noise → 2x Despecle**

2.1 Select "Yes" to process the whole stack. Do this with every following prompt.

3 **Image → Adjust → Brightness/Contrast**

Move the sliders for Brightness and Contrast to the right until an evenly coloured background is created.

Brightness is set to around 80 % and Contrast to 100 %.

Tracking Presets

4 **Edit → Options → Memory & Threads → Parallel threads 1**

5 **Plugins → Tracking → TrackMate**

5.1 Specify frames (T)

6 **Select StarDist detector**

6.1 Continue till the detection took place and keep all spots at first.

7 **Now set the filters on spots**.

Quality	< 1,07
Perimeter	> 39,25
Perimeter	< 55,10
Shape Index	< 4,23
Signal/Noise-Ratio	> 1,74
Solidity	> 0,77
Std. intensity	> 7,50

Tracker

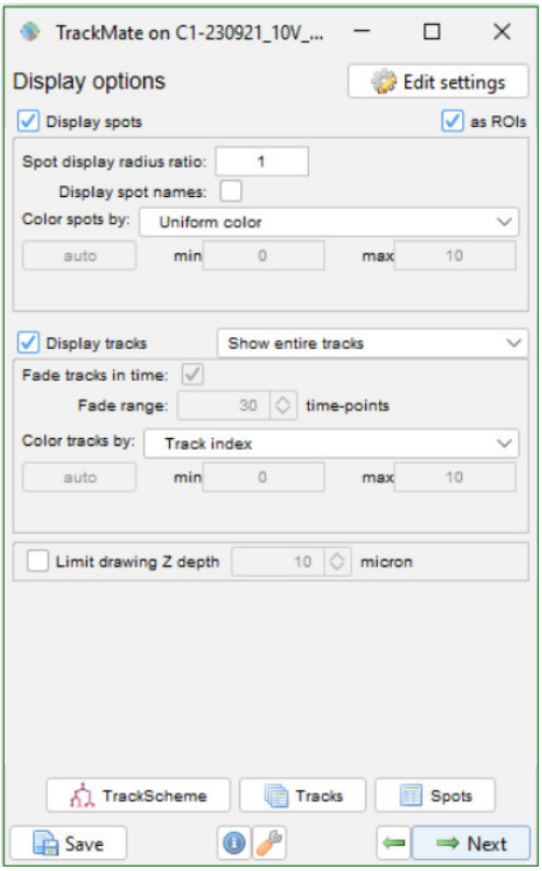
8 **Set the filters on tracks**.

The parameter settings must be compared with the image to remove nonsensical tracks immediately.

Confinement ratio	> -0,04
Linearity of forward progression	> -0,21
Longest gap	> 1,23
Max. distance travelled	> 14,58
Number of spots in track	> 62
Number of spots in track	< 72

9 In the Display Options window, tick Display Spots and Display Tracks. It is extremely important to save the required files for further work steps.

To do this, use the **Tracks** button to save the spots, edges and tracks (Export to CSV). The **Spots** (All Spots) can also be exported.



10 **Select LAP tracker and the parameters**

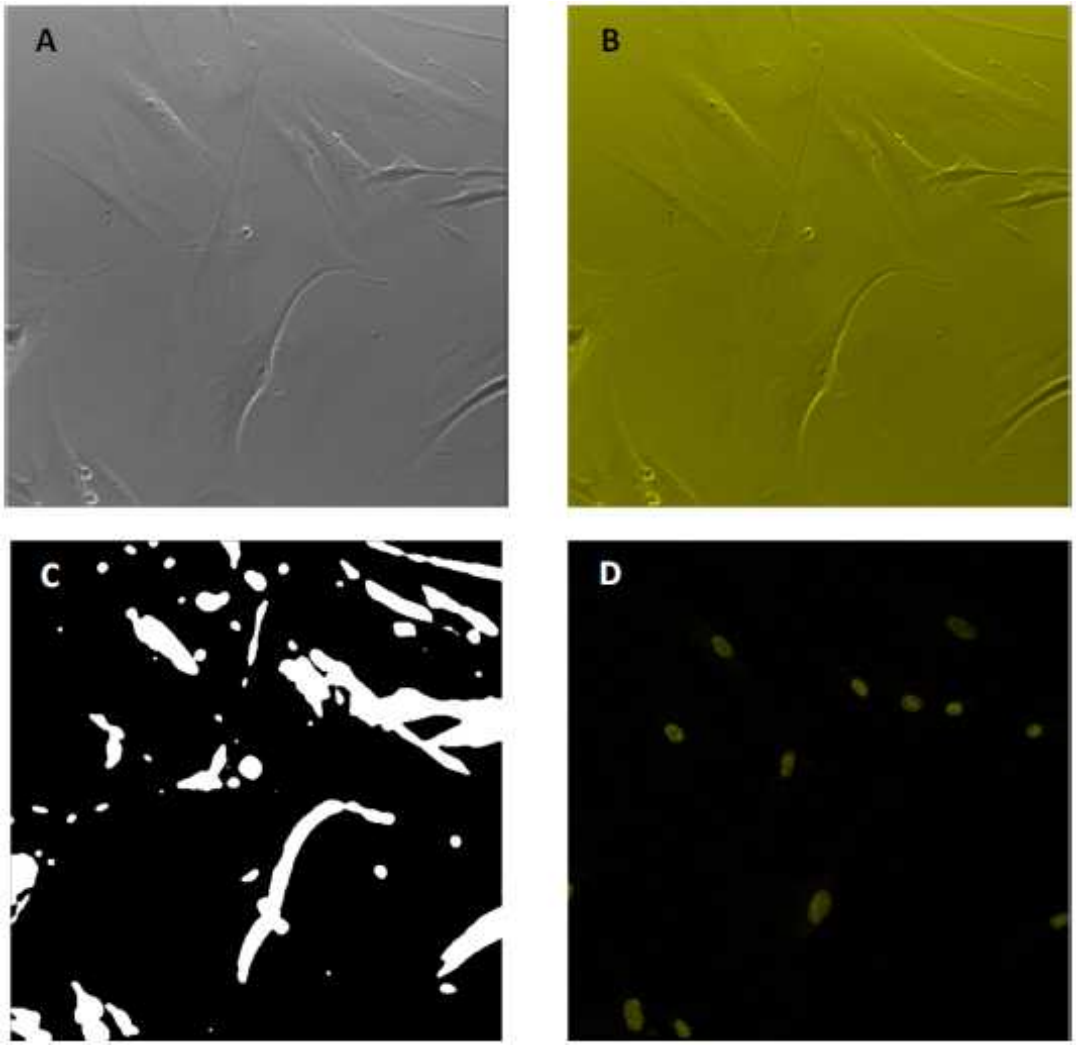
Frame to frame linking max. distance	45 µm
Track segment gap closing max. distance	40 µm
Max frame gap	15
Track segment merging	3 µm

11 Next, take care of the **TrackScheme**. Here, splits, fusions, and nonsensical tracks can be removed manually.

Expected results

12

Expected result



A picture of the HGF-1 was taken, raw and processed during the experiments. This example shows the first frame of the 20 V time series. A This image is a raw brightfield image captured using Ph3 phase contrast. No additional processing or adjustments have been applied to it. **B** In this image, a yellow Lookup Table (LUT) has been applied to enhance the visibility and recognition of the cells. This colour mapping helps distinguish the cells from the background. **C** Image processed with a Variance filter (pixel radius = 5) and Gaussian blur filter (radius = 2.00), as well as increased brightness and contrast. **D** The nuclei of the cells have been stained with Hoechst dye, which specifically binds to DNA. This staining allows for better visualisation and identification of the cell nuclei.

Protocol references

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Schmidt, U., Weigert, M., Broaddus, C. and Myers, G., 2018. Cell detection with star-convex polygons. In Medical Image Computing and Computer Assisted Interven on–MICCAI 2018: 21st International Conference, Granada, Spain, September 16-20, 2018, Proceedings, Part II 11 (pp. 265-273). Springer International Publishing.

Ershov, D., Phan, M.S., Pylvänäinen, J.W., Rigaud, S.U., Le Blanc, L., Charles-Orszag, A., Conway, J.R., Laine, R.F., Roy, N.H., Bonazzi, D. and Duménil, G., 2022. TrackMate 7: integrating state-of-the-art segmentation algorithms into tracking pipelines. Nature Methods, 19(7), pp.829-832.