Automated cell tracking using StarDist and TrackMate

Elnaz Fazeli¹, Nathan H. Roy², Gautier Follain³,⁴, Romain F. Laine⁵,⁶, Lucas von Chamier⁵, Pekka E. Hänninen¹, John E. Eriksson³,⁴, Jean-Yves Tinevez⁷, Guillaume Jacquemet³,⁴

¹Laboratory of Biophysics, Institute of Biomedicine, Faculty of Medicine, University of Turku, Turku, Finland
²Department of Pathology and Laboratory Medicine, Children’s Hospital of Philadelphia Research Institute, Philadelphia, PA 19104, USA
³Turku Bioscience Centre, University of Turku and Åbo Akademi University, Turku, Finland
⁴Cell Biology, Faculty of Science and Engineering, Åbo Akademi University, Turku, Finland
⁵MRC-Laboratory for Molecular Cell Biology, University College London, London, UK
⁶The Francis Crick Institute, London, UK
⁷Image Analysis Hub, Institut Pasteur, Paris, France

Abstract

The ability of cells to migrate is a fundamental physiological process involved in embryonic development, tissue homeostasis, immune surveillance, and wound healing. Therefore, the mechanisms governing cellular locomotion have been under intense scrutiny over the last 50 years. One of the main tools of this scrutiny is live-cell quantitative imaging, where researchers image cells over time to study their migration and quantitatively analyze their dynamics by tracking them using the recorded images. Despite the availability of computational tools, manual tracking remains widely used among researchers due to the difficulty setting up robust automated cell tracking and large-scale analysis. Here we provide a detailed analysis pipeline illustrating how the deep learning network StarDist can be combined with the popular tracking software TrackMate to perform 2D automated cell tracking and provide fully quantitative readouts. Our proposed protocol is compatible with both fluorescent and widefield images. It only requires freely available and open-source software (ZeroCostDL4Mic and Fiji), and does not require any coding knowledge from the users, making it a versatile and powerful tool for the field. We demonstrate this pipeline's usability by automatically tracking cancer cells and T cells using fluorescent and brightfield images. Importantly, we provide, as supplementary information, a detailed step-by-step protocol to allow researchers to implement it with their images.

Keywords

Cell migration, Image analysis, StarDist, TrackMate, Deep-learning, Automated tracking
This article is included in the NEUBIAS - the Bioimage Analysts Network gateway.

Corresponding author: Guillaume Jacquemet (guillaume.jacquemet@abo.fi)

Author roles: Fazeli E: Formal Analysis, Investigation, Software, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; Roy NH: Data Curation, Investigation, Writing – Review & Editing; Follain G: Data Curation, Investigation, Writing – Review & Editing; Laine RF: Methodology, Software, Writing – Review & Editing; von Chamier L: Methodology, Software, Writing – Review & Editing; Hänninen PE: Writing – Review & Editing; Eriksson JE: Writing – Review & Editing; Tinevez JY: Methodology, Software, Writing – Review & Editing; Jacquemet G: Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Copyright: © 2020 Fazeli E et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Grant information: This work was supported by grants awarded by the Academy of Finland (G.J.), the Sigrid Juselius Foundation (G.J.), Åbo Akademi University Research Foundation (CoE CellMech; G.J.) and by Drug Discovery and Diagnostics strategic funding to Åbo Akademi University (G.J.). G.F. was supported by National Cancer Center Finland (FICAN). NHR acknowledges support from the Cancer Research Institute and NIH T32 AR007442. R.F.L. acknowledges the support of the MRC Skills development fellowship (MR/T027924/1). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

How to cite this article: Fazeli E, Roy NH, Follain G et al. Automated cell tracking using StarDist and TrackMate [version 1; peer review: awaiting peer review] F1000Research 2020, 9:1279 https://doi.org/10.12688/f1000research.27019.1

First published: 28 Oct 2020, 9:1279 https://doi.org/10.12688/f1000research.27019.1
Introduction
The study of cell motility typically involves recording cell behavior, using live-cell imaging, and tracking their movement over time. To enable the analysis of such data, various software solutions have been developed. However, despite the availability of these computational tools, manual tracking remains widely used among researchers due to the difficulty in setting up fully automated cell tracking analysis pipelines. Automated tracking pipelines share a typical workflow that starts with a segmentation strategy that identifies the objects to track in each image. Tracking algorithms are then used to link these objects between frames. One challenging aspect of an automated tracking pipeline is often achieving an accurate segmentation of the objects to track. One option to facilitate cell segmentation is to label their nuclei, using fluorescent dyes or protein markers. Nuclei can then be automatically segmented using intensity-based thresholding. However, this approach tends to become inaccurate when images are noisy or when the cells to track are very crowded. Deep-Learning approaches have demonstrated their robustness against these two issues. In this work, we present a new analysis workflow that builds upon a Deep-Learning segmentation tool and a cell tracking tool to achieve robust cell tracking in cell migration assays. We combine StarDist, a powerful deep learning-based segmentation tool, and TrackMate, a user-friendly tracking tool, into a tracking pipeline that can be used without requiring expertise in or specialized hardware for computing.

Methods
Pipeline
The use of deep learning networks, such as StarDist, often requires the user to train or retrain a model using their images. While high-quality StarDist pre-trained models are readily available, they are likely to underperform when used on different data with, e.g., different staining, noise, and microscope type. To train StarDist models, we took advantage of the ZeroCostDL4Mic platform, allowing researchers to train (and retrain), validate, and use deep learning networks. Importantly, the ZeroCostDL4Mic StarDist 2D notebook can directly output a file containing all the nuclei’s geometric center coordinates (named tracking files), that can be used as input for TrackMate. Therefore, our proposed pipeline can be divided into three parts.

1. **Train a StarDist model**
   - **A. Create a training dataset**
     - Manual annotations
     - Semi-automated annotations
   - **B. Train and validate a StarDist model using ZeroCostDL4Mic**

2. **Generate "Tracking files" using a StarDist model**
   - Movies to analyze
   - StarDist predictions
   - Tracking File
   - Each detected object is replaced by a pixel

3. **Tracking the "Tracking files" using TrackMate**
   - Tracking File
   - TrackMate (Fiji)
     - Analyze files one by one using the graphical interface
     - Batch Analysis
   - Cell Tracks
   - Quantitative results
     - Track information
     - Track duration
     - Track speed

![Figure 1. Workflow depicting how StarDist and TrackMate can be combined to track cells automatically.](image)
for each type of data. 2) Second, the trained StarDist model is used to segment the object to track and generate Tracking files. 3) Finally, the tracking files can be used in TrackMate to track the identified objects.

Training a StarDist model requires a set of images and their corresponding masks (Figure 1 and Figure 2). Generating a training dataset is by far the most time-consuming part of the analysis pipeline presented here as it requires the manual

**Figure 2. Example of datasets analyzed using StarDist and TrackMate.** (A, B) Migration of MCF10DCIS.com, labeled with Sir-DNA, recorded using a spinning disk confocal microscope and automatically tracked. Examples of images used to train StarDist (A), and an example of results obtained using automated tracking are displayed (B, Video 1). The yellow square indicates a magnified ROI, where the local track of each nucleus is displayed. The full cell tracks are displayed on the left. Tracks are color-coded as a function of their maximum instantaneous velocity (blue slow, red fast tracks). (C–E) Migration of activated T cell plated on VCAM-1 or ICAM-1, recorded using a brightfield microscope and automatically tracked. Examples of images used to train StarDist (C) and an example of results obtained using automated tracking are displayed (D, Video 2). (E) Comparison of the migration of activated T cells on VCAM-1 or ICAM-1. Track mean speed and track straightness were quantified. Data are displayed as boxplots. *** p-value = <0.001, p-T cells migrating on ICAM-1 or VCAM-1. (F, G) Cancer cells flowing in a microfluidic chamber, recorded live using a brightfield microscope and automatically tracked (Video 3). Examples of images used to train StarDist (F), and an example of results obtained using automated tracking are displayed (G). The full tracks shown here were color-coded as a function of their x coordinate.
annotations of the images to analyze (Extended data: Supplementary protocol\textsuperscript{16}). For instance, to generate the training datasets presented in Figure 2, each cell/nuclei contour was drawn manually using the freehands selection tool in Fiji. The creation of a high-quality training dataset is a critical part of the process as it will impact the specificity and performance of the StarDist model. However, the generation of a training dataset is only required once per dataset type. If a StarDist model already exists for similar images it can be used to significantly accelerate the creation of the training dataset via semi-automated annotation (see Extended data: Supplementary protocol\textsuperscript{16}).

One of our analysis pipeline’s key features is that, once a StarDist model has been satisfactorily trained, movies of migrating cells can efficiently be processed in batch. Indeed, while individual tracking files can be analyzed one by one using the TrackMate graphical interface, we also provide a Fiji macro to analyze a folder containing multiple tracking files. Our batch processing macro will provide basic quantitative information for each track, including median and maximal speeds. If more information is needed, the tracking results generated by our script are directly compatible with the Motility lab website, where they can be further processed\textsuperscript{17}.

Implementation and operation

The described image analysis pipeline is composed of a Jupiter notebook optimized to run in Google Colab (ZeroCostDL4Mic framework\textsuperscript{18}) and a Python script that can run in Fiji\textsuperscript{19}. A step-by-step protocol describing how to use our analysis pipeline is provided as Extended data\textsuperscript{20}.

Use case

To illustrate our analysis pipeline’s functionality and flexibility, we first trained a StarDist model to analyze the behavior of breast cancer cells migrating collectively (Figure 2A; Extended data: Video 1\textsuperscript{16}). The cancer cell’s nuclei were fluorescently labeled, and the cells imaged using fluorescence-based microscopy. The creation of the training dataset used in this example was greatly facilitated by the availability of a StarDist model, released by the StarDist creators, capable of segmenting fluorescent nuclei. In this case, the StarDist Fiji plugin was used to segment the location of nuclei in the training images, and all miss-annotations were manually corrected (Extended data: Supplementary protocol\textsuperscript{16}).

Finally, we used our pipeline to automatically track non-adherent cancer cells flowing in a microfluidic chamber (Figure 2F and G; Extended data: Video 3\textsuperscript{20}). In this case, automated tracking is especially useful due to the very high number of frames to analyze. For the last two examples, no suitable pre-trained StarDist models were available. Therefore, to generate the training datasets, we manually annotated 20 images and trained a first StarDist model. This model was then used to accelerate the annotation of the rest of the training images.

Use case dataset creation

**Breast cancer cell dataset.** MCF10DCIS.com cells were described previously\textsuperscript{15,22}. DCIS.COM lifeact-RFP cells were incubated for 2h with 0.5 µM SiR-DNA (SiR-Hoechst, Tetu-bio, Cat Number: SC007) before being imaged live for 14 h using a spinning-disk confocal microscope (1 picture every 10 min). The spinning-disk confocal microscope used was a Marianas spinning disk imaging system with a Yokogawa CSU-W1 scanning unit on an inverted Zeiss Axio Observer Z1 microscope (Intelligent Imaging Innovations, Inc.) equipped with a 20x (NA 0.8) air, Plan Apochromat objective (Zeiss).

**T cell dataset.** Lab-Tek 8 chamber slides (ThermoFisher) were coated with 2 µg/mL ICAM-1 or VCAM-1 overnight at 4°C\textsuperscript{19}. Activated primary mouse CD4+ T cells were washed and resuspended in L-15 media containing 2 mg/mL D-glucose. T cells were then added to the chambers, incubated 20 min, gently washed to remove all unbound cells, and imaged. Imaging was done using a 10x phase contrast objective at 37°C on a Zeiss Axiovert 200M microscope equipped with an automated X-Y stage and a Roper EMCCD camera. Time-lapse images were

To highlight that our pipeline can also be used to analyze brightfield images, we generated a StarDist model to track T cells migrating on ICAM-1 or VCAM-1 (Figure 2C–E; Extended data: Video 2\textsuperscript{16}). Importantly, automated analysis of these data could reproduce the results obtained via manual tracking\textsuperscript{11}.

---

**Underlying data. Video 2: Automated tracking of T cell migrating on ICAM-1**

1 video file

https://doi.org/10.6084/m9.figshare.13122755.v1\textsuperscript{20}

Activated T cell plated ICAM-1 were recorded using a brightfield microscope and automatically tracked using StarDist and TrackMate. Local tracks are displayed.

---

**Underlying data. Video 3: Automated tracking of cancer cells flowing in a microfluidic chamber**

1 video file

https://doi.org/10.6084/m9.figshare.13122764.v1\textsuperscript{21}

AsPC1 pancreatic cancer cells flowing in a microfluidic chamber were recorded live using a brightfield microscope and automatically tracked using StarDist and TrackMate. Local tracks are displayed.

---

**Underlying data. Video 1: Automated tracking of breast cancer cell migrating collectively**

1 video file

https://doi.org/10.6084/m9.figshare.13122635.v1\textsuperscript{16}

MCF10DCIS.com cells, labeled with Sir-DNA; were recorded using a spinning disk confocal microscope and automatically tracked using StarDist and TrackMate. Local tracks are displayed.
collected every 30 sec for 10 min using SlideBook 6 software (Intelligent Imaging Innovations).

**Flow chamber dataset.** Cancer cells (500,000 cells/ml in PBS) were perfused at a speed of 300 µm/sec using a peristaltic pump (ISMATEC MS12/4 analonic) and a homemade tubing system (Ismatek 3-Stop tubes and Ibidi® tubings and connectors) in a microchannel (Ibidi® µ-slides400 LUER). Images were acquired with a brightfield microscope (Zeiss Laser-TIRF 3 Imaging System, Carl Zeiss) and a 10X objective.

**Data display and statistical analyses**
Box plots were generated using PlotsOfDat23. Randomization tests were performed using the online tool PlotsOfDifferences24.

**Conclusions**
Here we show that StarDist and TrackMate can be integrated seamlessly and robustly to automate cell tracking in fluorescence and brightfield images. We envision that this pipeline can also be applied to any circular or oval-shaped objects. However, we acknowledge that using brightfield images may not always work directly with our pipeline, especially if cells display complex and interchanging shapes, since StarDist is mostly designed to detect round or compact shapes. In this case, other tools, such as Usigaci, could also be considered1. Still, brightfield images could also be artificially labeled using deep learning, transforming the brightfield dataset into a pseudo-fluorescence one, as can be done with ZeroCostDL4Mic already45. The pipeline described here is currently limited to the tracking of objects in 2D. However, a similar workflow can be applied to 3D datasets as both StarDist and TrackMate can accommodate 3D images12,13,25.

**Data availability**

**Underlying data**
Zenodo: Combining StarDist and TrackMate example 1 - Breast cancer cell dataset, http://doi.org/10.5281/zenodo.403497626

Zenodo: Combining StarDist and TrackMate example 2 - T cell dataset, http://doi.org/10.5281/zenodo.403492927

Zenodo: Combining StarDist and TrackMate example 3 - Flow chamber dataset, http://doi.org/10.5281/zenodo.403493928

**Extended data**
Zenodo: Combining StarDist and TrackMate - Extended data, http://doi.org/10.5281/zenodo.409146716.

This project contains the following extended data:

- Supplementary protocol
- Video 1: Automated tracking of breast cancer cell migrating collectively. MCF10DCIS.com cells, labeled with Sir-DNA, were recorded using a spinning disk confocal microscope and automatically tracked using StarDist and TrackMate. Local tracks are displayed.
- Video 2: Automated tracking of T cell migrating on ICAM-1. Activated T cell plated ICAM-1 were recorded using a brightfield microscope and automatically tracked using StarDist and TrackMate. Local tracks are displayed.
- Video 3: Automated tracking of cancer cells flowing in a microfluidic chamber. AsPC1 pancreatic cancer cells flowing in a microfluidic chamber were recorded live using a brightfield microscope and automatically tracked using StarDist and TrackMate. Local tracks are displayed.

Data are available under the terms of the Creative Commons Attribution 4.0 International license (CC-BY 4.0).

**Software availability**
Source code available from: https://github.com/HenriquesLab/ZeroCostDL4Mic

Archived source code at time of publication: http://doi.org/10.5281/zenodo.409147426

License: MIT license.

**Acknowledgments**
The Cell Imaging and Cytometry Core facility (Turku Bioscience, University of Turku, Åbo Akademi University, and Biocenter Finland), the Finnish Euro-Bioimaging Node, and Turku Bioimaging are acknowledged for services, instrumentation and expertise.

This publication was supported by COST Action NEUBIAS (CA15124), funded by COST (European Cooperation in Science and Technology).

**References**

1. Jacquemet G, Baghirov H, Georgiadou M, et al.: L-type calcium channels regulate filopodia stability and cancer cell invasion downstream of integrin signalling. Nat Commun. 2016; 7: 13297. PubMed Abstract | Publisher Full Text | Free Full Text

2. Jacquemet G, Morgan MR, Byron A, et al.: Rac1 is deactivated at integrin activation sites through an IQGAP1-filamin-A-RacGAP1 pathway. J Cell Sci. 2013; 126(Pt 18): 4121–4135. PubMed Abstract | Publisher Full Text | Free Full Text
3. DuChez BJ: Automated Tracking of Cell Migration with Rapid Data Analysis. Curr Protoc Cell Biol. 2017; 76: 12.12.3–12.12.16. PubMed Abstract | Publisher Full Text | Free Full Text

4. Cordelieres FP, Petit V, Kumasaki M, et al.: Automated Cell Tracking and Analysis in Phase-Contrast Videos (iTrack4U): Development of Java Software Based on Combined Mean-Shift Processes. PLoS One. 2013; 8(11): e71266. PubMed Abstract | Publisher Full Text | Free Full Text

5. McQuin C, Goodman A, Chernyshev V, et al.: CellProfiler 3.0: Next-generation image processing for biology. PLoS Biol. 2018; 16(7): e2005970. PubMed Abstract | Publisher Full Text | Free Full Text

6. Piccinini F, Kiss A, Horvath P: CellTracker (not only) for dummies. Bioinformatics. 2016; 32(6): 955–957. PubMed Abstract | Publisher Full Text

7. Barry DJ, Durkin CH, Abella JV, et al.: Open source software for quantification of cell migration, protrusions, and fluorescence intensities. J Cell Biol. 2015; 209(1): 163–180. PubMed Abstract | Publisher Full Text | Free Full Text

8. Usigaci: Instance-aware cell tracking in stain-free phase contrast microscopy enabled by machine learning. SoftwX. 2019; 9: 230–237. Publisher Full Text

9. Pijuan J, Barceló C, Moreno DF, et al.: In vitro Cell Migration, Invasion, and Adhesion Assays: From Cell Imaging to Data Analysis. Front Cell Dev Biol. 2019; 7: 107. PubMed Abstract | Publisher Full Text | Free Full Text

10. Chenouard N, Small D, de Chaumont F, et al.: Objective comparison of particle tracking methods. Nat Methods. 2014; 11(3): 281–289. PubMed Abstract | Publisher Full Text | Free Full Text

11. Caicedo JC, Roth J, Goodman A, et al.: Evaluation of Deep Learning Strategies for Nucleus Segmentation in Fluorescence Images. Cytometry A. 2019; 95(9): 922–935. PubMed Abstract | Publisher Full Text | Free Full Text

12. Tinevez JY, Perry N, Schindelin J, et al.: TrackMate: An open and extensible platform for single-particle tracking. Methods. 2017; 115: 80–90. PubMed Abstract | Publisher Full Text

13. Schmidt U, Weigert M, Broadus C, et al.: Cell Detection with Star-Convex Polygons. In: Medical Image Computing and Computer Assisted Intervention – MICCAI 2018 (eds. Frangi, A., F., Schnabel, J. A., Davatzikos, C., Alberola-López, C. & Fichtinger, G.) (Springer International Publishing, 2018), 11071: 265–273. Publisher Full Text

14. Schindelin J, Arganda-Carreras I, Frise E, et al.: Fiji: an open-source platform for biological-image analysis. Nat Methods. 2012; 9(7): 676–682. PubMed Abstract | Publisher Full Text | Free Full Text

15. Chamier LV, Laine RF, Jukkala J, et al.: ZeroCostDL4Mic: an open platform to use Deep-Learning in Microscopy. bioRxiv. 2020; 2020.03.20.000133. Publisher Full Text

16. Jacquemet G, Roy NH, Follain G, et al.: Combining StarDist and TrackMate - Extended data. Zenodo. 2020. http://www.doi.org/10.5281/zenodo.4091467

17. Wortel IMN, Dannenberg K, Berry JC, et al.: CelltrackR: an R package for fast and flexible analysis of immune cell migration data. 2019. Publisher Full Text

18. Jacquemet G: Video 1: Automated tracking of breast cancer cell migrating collectively. f1000research.com. Media. 2020. http://www.doi.org/10.6084/m9.figshare.13122635.v1

19. Roy NH, Kim SJ, Buffone A J, et al.: LFA-1 signals to promote actin polymerization and upstream migration in T cells. J Cell Sci. 2020; 133(17): jcs248328. PubMed Abstract | Publisher Full Text | Free Full Text

20. Jacquemet G: Video 2: Automated tracking of T cell migrating on ICAM-1. f1000research.com. Media. 2020. http://www.doi.org/10.6084/m9.figshare.13122755.v1

21. Jacquemet G: Video 3: Automated tracking of cancer cells flowing in a microfluidic chamber. f1000research.com. Media. 2020. http://www.doi.org/10.6084/m9.figshare.13122764.v1

22. Jacquemet G, Paatero I, Carisey AF, et al.: FiloQuant reveals increased filopodia density during breast cancer progression. J Cell Biol. 2017; 216(10): 3387–3403. PubMed Abstract | Publisher Full Text | Free Full Text

23. Postma M, Goedhart J: PlotsOfData—A web app for visualizing data together with their summaries. PLoS Biol. 2019; 17(3): e3000202. PubMed Abstract | Publisher Full Text | Free Full Text

24. Goedhart J: PlotsOfDifferences – a web app for the quantitative comparison of unaired data. bioRxiv. 2019; 578575. Publisher Full Text

25. Weigert M, Schmidt U, Haase R, et al.: Star-convex Polyhedra for 3D Object Detection and Segmentation in Microscopy. 2020; 8. Publisher Full Text

26. Jacquemet G: Combining StarDist and TrackMate example 1 - Breast cancer cell dataset (Version 1) [Data set]. Zenodo. 2020. http://www.doi.org/10.5281/zenodo.4034976

27. Roy NH, Jacquemet G: Combining StarDist and TrackMate example 2 - T cell dataset (Version 1) [Data set]. Zenodo. 2020. http://www.doi.org/10.5281/zenodo.4034929

28. Follain G, Jacquemet G: Combining StarDist and TrackMate example 3 - Flow chamber dataset (Version 1) [Data set]. Zenodo. 2020. http://www.doi.org/10.5281/zenodo.4034939

29. Jacquemet G, Fazeli E, Laine RF, et al.: Combining StarDist and TrackMate - Archived source code (Version v1). Zenodo. 2020. http://www.doi.org/10.5281/zenodo.4091474
The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com