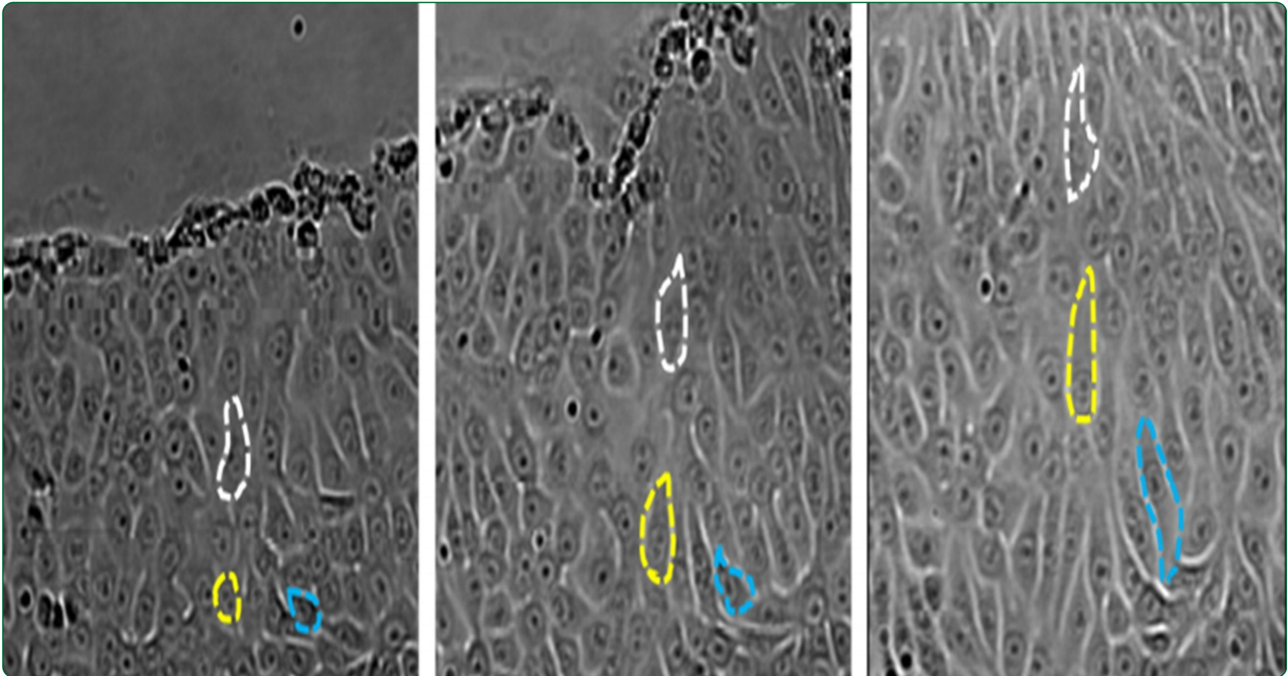
(GIGA)^n SCIENCE



Live time-lapse dataset of *in vitro* wound healing experiments

Zaritsky *et al.*

BioMed Central

Zaritsky *et al. GigaScience* (2015) 4:8
DOI 10.1186/s13742-015-0049-6

Zaritsky et al. GigaScience (2015) 4:8
DOI 10.1186/s13742-015-0049-6

(GIGA)n SCIENCE

DATA NOTE Open Access

Live time-lapse dataset of *in vitro* wound healing experiments

Assaf Zaritsky[1*], Sari Natan[2], Doron Kaplan[3], Eshel Ben-Jacob[4,5,6] and Ilan Tsarfaty[2*]

Abstract

Background: The wound healing assay is the common method to study collective cell migration *in vitro*. Computational analyses of live imaging exploit the rich temporal information and significantly improve understanding of complex phenomena that emerge during this mode of collective motility. Publicly available experimental data can allow application of new analyses to promote new discoveries, and assess algorithms' capabilities to distinguish between different experimental conditions.

Findings: A freely-available dataset of 31 time-lapse *in vitro* wound healing experiments of two cell lines is presented. It consists of six different experimental conditions with 4–6 replicates each, gathered to study the effects of a growth factor on collective cell migration. The raw data is available at 'The Cell: an Image Library' repository. This Data Note provides detailed description of the data, intermediately processed data, scripts and experimental validations that have not been reported before and are currently available at GigaDB. This is the first publicly available repository of live collective cell migration data that includes independent replicates for each set of conditions.

Conclusions: This dataset has the potential for extensive reuse. Some aspects in the data remain unexplored and can be exploited extensively to reveal new insight. The dataset could also be used to assess the performance of available and new quantification methods by demonstrating phenotypic discriminatory capabilities between the different experimental conditions. It may allow faster and more elaborated, reproducible and effective analyses, which will likely lead to new biological and biophysical discoveries.

Keywords: Collective cell migration, Wound healing assay, HGF/SF-Met, Live cell imaging, Image analysis

Data description

The wound healing assay, the traditional method to study collective cell motility and migration in medicine and biology, is performed by following the closure of a wound formed by scratching a confluent cell culture. The scratch is imaged and measured periodically during the healing process, and the rate of change in the wound's area is recorded and compared with those in other cells and treatments. Quantifying the change in wound area has been well established for over 20 years, but recent advances demonstrated the ability to use live time-lapse data to capture phenotypes that are invisible to the naked eye (e.g., refs [1-6]) and may have future implications in screening [1,3,7].

To our knowledge, the only public repository available for collective cell migration setting was produced by Simpson et al. [8], who tested how wound healing dynamics of MCF-10A breast epithelial cells alter in response to targeting by small interfering RNAs (siRNAs). This time-lapse data is severely limited in their statistics. First, a single time lapse for one gene knockdown does not allow significant statistical confidence (i.e., two conditions cannot be distinguished with N = 1). Second, the imaged field of view contains only a small (<200) number of cells limiting statistical sufficiency to quantify the collective behavior. These limitations are addressed here by presenting a dataset that includes 4–6 independent experiments for each condition, each with a large field of view with at least 1,000 cells.

Here we present a public dataset of live wound healing time-lapse data. The experiments were designed to study

* Correspondence: assafzar@gmail.com; ilants@post.tau.ac.il
[1]Department of Cell Biology, UT Southwestern Medical Center, Dallas, TX 75024, USA
[2]Department of Clinical Microbiology and Immunology, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel
Full list of author information is available at the end of the article

BioMed Central

© 2015 Zaritsky et al.; licensee BioMed Central. This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly credited. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.

Table 1 Summary of the raw data included in this Data Descriptor and their access number at 'The Cell: an Image Library' repository [15,16]

Cell line	Treatment	N	CIL numbers
DA3	Control	6	CIL43406-43411
DA3	+HGF/SF	5	CIL43401-43405
DA3	PHA	4	CIL45451-45454
DA3	PHA + HGF/SF	6	CIL43412-43417
MDCK	Control	5	CIL44501-44502
			CIL44506-44508
MDCK	+HGF/SF	5	CIL44503-44505
			CIL44509-44510

the spatiotemporal dynamics of *in vitro* wound healing, particularly the effects of Hepatocyte Growth Factor/ Scatter Factor (HGF/SF) [9,10], as a model system to determine the effects of a growth factor on collective cell migration [2,11]. Two cell lines were examined: Madin-Darby Canine Kidney epithelial cells (MDCK), the line commonly used for *in vitro* collective migration studies, and the mouse mammary adenocarcinoma line D1-DMBA-3 (DA3). DA3 cells were either left untreated or treated with HGF/SF ([9,12]) or the Met inhibitor PHA665752 [13] (PHA henceforth) with or without HGF/ SF. MDCK cells were either left untreated or treated with HGF/SF. All raw image data is publicly available at 'The Cell: an Image Library' [14-16] and detailed in Table 1. A total of 21 DA3 experiments (6 Control, 5 + HGF/SF, 4 PHA, 6 PHA + HGF/SF) and 10 MDCK experiments (5 Control, 5 + HGF/SF) were deposited. Intermediate processed data that includes the monolayer contours, the estimated motion-fields and scripts for spatiotemporal analysis are available via the GigaDB database [17] and detailed in Table 2.

The experimental methodology to generate this dataset is expanded from previous descriptions [2,11] and is detailed in Additional file 1. It has further been validated, curated and annotated as detailed below. DA3 and MDCK cell lines were selected because they are well characterized for their HGF/SF induced biological activities as well as their Met expression and signaling, the molecular focus of our studies (Additional file 2). HGF/SF concentration and PHA dosage was determined as detailed in Additional file 3, which also includes information regarding blindness, data curation and annotation. Additional file 4 details the intermediate processed data and the scripts available in the GigaDB database [17].

We previously used this data to propose a generalization scheme to quantify *in vitro* collective migration assays using classification for phenotypic discrimination between these different experimental conditions [2]. More recently, we used this data to describe the propagation of strain rate, directionality and coordination to cells located in deeper layer of the cell sheet [11].

The raw data presented here can be reused to (a) evaluate the classification-power of new quantitative measures on different experimental conditions, (b) reproduce our results or (c) find additional insights. Below we suggest several alternative uses.

In ref [2] we used spatiotemporal measures of speed or texture to distinguish between control, +HGF/SF-treated and PHA + HGF/SF-treated DA3 cells via classification. The updated dataset additionally includes DA3 cells treated with PHA and MDCK cells (Control, +HGF/SF). This data can be used to look for differences between more conditions or across the two cell lines. It would also be useful to investigate different measures to characterize changes between cell lines or different conditions (e.g., refs [1,3,18]).

Repetitions of control experiments (six for DA3 cells, five for MDCK cells) allow searching for intrinsic phenotypes that emerge during collective cell migration. The complete dataset allows investigation of the role of HGF/SF as a model growth factor in collective cell migration. We find the following open questions to be important and suggest that the presented dataset be used to study them:

Late stage of the wound healing process

We have previously focused on the initial stages of wound healing, from the initial scratch until first contact between cells from opposing borders of the wound [11].

Table 2 Summary of the raw and intermediately processed data included in this Data Descriptor and experiments name in GigaDB

Cell line	Treatment	N	Experiment names in GigaDB
DA3	Control	6	SN29_L1, SN29_L5, SN29_L6, SN77_L1, SN77_L2, SN77_L8
DA3	+HGF/SF	5	SN29_L8, SN29_L9, SN29_L10, SN29_L11, SN29_L12
DA3	PHA	4	SN77_L20, SN77_L28, SN77_L29, SN77_L30
DA3	PHA + HGF/SF	6	SN77_L33, SN77_L35, SN77_L36, SN77_L38, SN77_L40, SN77_L41
MDCK	Control	5	DKWH7_L1, DKWH7_L3, DKWH7_L10, DKWH7_L15, DKWH7_L16
MDCK	+HGF/SF	5	DKWH7_L4, DKWH7_L5, DKWH7_L6, DKWH7_L18, DKWH7_L19

Very limited analysis was dedicated to the later stages of the healing process [2], an open arena that can further be investigated using the presented dataset.

Single cell analysis

Density plays a major role in collective migration: denser cells move slower but more coordinately (e.g., in refs [4,5]. However, in the current dataset cells seem to become sparser and faster [2] but more coordinated [11] as response to HGF/SF. Cell density was estimated based only on a few cells that were manually annotated and no direct single cell analysis was performed because the measures were calculated for a grid of subcellular-sized local patches. New investigations can focus on the dynamics of cell density and their relation to different motility phenotypes for the two cell lines or under HGF/SF-Met signaling.

Characterizing cells in coordinately migrating clusters

We recently introduced a method to detect spatial clusters of cells that migrate coordinately within the monolayer [11]. How these cells' dynamics differ from less-coordinated cells remains an open question that may help understand intercellular coordination and can be addressed using the presented dataset.

Availability and requirements

- Project name: Time-lapse in vitro wound healing experiments
- Project home page: https://github.com/gigascience/paper-zaritsky2015
- Operating systems: Platform independent
- Programming language: Matlab
- Other requirements: Matlab
- License: GNU GPL v3
- Any restrictions to use by non-academics: none

Availability of supporting data

The datasets supporting the results of this article are available in the *GigaScience* repository, GigaDB [17]. The raw data is also available at 'The Cell: an Image Library' repository [15,16].

Additional files

Additional file 1: Supporting Methods.

Additional file 2: Literature-based Validations of Cell Line Selection.

Additional file 3: Technical Validation.

Additional file 4: Intermediately Processed Data available at GigaDB Database.

Abbreviations

DA3: Mouse mammary adenocarcinoma line D1-DMBA-3; HGF/SF: Hepatocyte Growth Factor / Scatter Factor; MDCK: Madin-Darby Canine Kidney epithelial cells.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

AZ conceived the study, curated and published the data and scripts, and drafted the manuscript. SN carried out the DA3 experiments, performed most of the technical validation and prepared Figure S1 (in Additional file 3). DK performed the MDCK experiments. IT managed all experiments. All authors read, revised and approved the final manuscript.

Acknowledgments

This work was supported in part by research grants from the Breast Cancer Research Foundation (to IT); the Federico Foundation Grants (to IT); the Israel Science Foundation (to IT); the Tauber Family Foundation at Tel Aviv University (to EBJ); the Center for Theoretical Biological Physics sponsored by the NSF (Grant PHY-0822283 to EBJ); by the Cancer Prevention and Research Institute of Texas (CPRIT) at Rice University (to EBJ) and by CPRIT R1255 at UT Southwestern Medical Center at Dallas (to Gaudenz Danuser supporting AZ). The funders had no role in study design, data collection and analysis, decision to publish or preparation of the manuscript. We thank Arieh Zaritsky for proofreading the manuscript. We are grateful to David Orloff and Willy Wong from 'The Cell: an Image Library' for uploading and annotating the dataset.

Author details

[1]Department of Cell Biology, UT Southwestern Medical Center, Dallas, TX 75024, USA. [2]Department of Clinical Microbiology and Immunology, Sackler School of Medicine, Tel Aviv University, Tel Aviv 69978, Israel. [3]Israel Institute for Biological Research, P.O.B. 19, Ness Ziona 74100, Israel. [4]School of Physics and Astronomy, The Raymond and Beverly Sackler Faculty of Exact Sciences, Tel-Aviv University, Tel-Aviv 69978, Israel. [5]Center for Theoretical Biological Physics, Rice University, Houston, TX 77005-1827, USA. [6]Research & Development Unit Assaf Harofeh Medical Center, Zerifin 70300, Israel.

Received: 3 November 2014 Accepted: 5 February 2015
Published online: 25 February 2015

References

1. Deforet M, Parrini MC, Petitjean L, Biondini M, Buguin A, Camonis J, et al. Automated velocity mapping of migrating cell populations (AVeMap). Nat Methods. 2012;9:1081–3.
2. Zaritsky A, Natan S, Ben-Jacob E, Tsarfaty I. Emergence of HGF/SF-induced coordinated cellular motility. PloS One. 2012;7:e44671.
3. Milde F, Franco D, Ferrari A, Kurtcuoglu V, Poulikakos D, Koumoutsakos P. Cell Image Velocimetry (CIV): boosting the automated quantification of cell migration in wound healing assays. Integr Biol. 2012;4:1437–47.
4. Petitjean L, Reffay M, Grasland-Mongrain E, Poujade M, Ladoux B, Buguin A, et al. Velocity fields in a collectively migrating epithelium. Biophys J. 2010;98:1790–800.
5. Angelini TE, Hannezo E, Trepat X, Fredberg JJ, Weitz DA. Cell migration driven by cooperative substrate deformation patterns. Phys Rev Lett. 2010;104:168104.
6. Ng MR, Besser A, Danuser G, Brugge JS. Substrate stiffness regulates cadherin-dependent collective migration through myosin-II contractility. J Cell Biol. 2012;199:545–63.
7. Hulkower KI, Herber RL. Cell migration and invasion assays as tools for drug discovery. Pharmaceutics. 2011;3:107–24.
8. Simpson KJ, Selfors LM, Bui J, Reynolds A, Leake D, Khvorova A, et al. Identification of genes that regulate epithelial cell migration using an siRNA screening approach. Nat Cell Biol. 200;10:1027–38.
9. Firon M, Shaharabany M, Altstock RT, Horev J, Abramovici A, Resau JH, et al. Dominant negative Met reduces tumorigenicity-metastasis and increases tubule formation in mammary cells. Oncogene. 2000;19:2386–97.
10. Gherardi E, Birchmeier W, Birchmeier C, Woude GV. Targeting MET in cancer: rationale and progress. Nat Rev Cancer. 2012;12:89–103.
11. Zaritsky A, Kaplan D, Hecht I, Natan S, Wolf L, Gov NS, et al. Propagating waves of directionality and coordination orchestrate collective cell migration. PLoS Comput Biol. 2014;10:e1003747.
12. Natan S, Tsarfaty G, Horev J, Haklai R, Kloog Y, Tsarfaty I. Interplay between HGF/SF–Met-Ras signaling, tumor metabolism and blood flow as a potential target for breast cancer therapy. Clin Trials. 2014;3:10.

13. Crosswell HE, Dasgupta A, Alvarado CS, Watt T, Christensen JG, De P, et al. PHA665752, a small-molecule inhibitor of c-Met, inhibits hepatocyte growth factor-stimulated migration and proliferation of c-Met-positive neuroblastoma cells. BMC Cancer. 2009;9:411.
14. Orloff DN, Iwasa JH, Martone ME, Ellisman MH, Kane CM. The cell: an image library-CCDB: a curated repository of microscopy data. Nucleic Acids Res. 2013;41:D1241–50.
15. Zaritsky A, Kaplan D, Hecht I, Natan S, Wolf L, Ben-Jacob E, et al. The cell: an image library. 2014; CIL:44501 - CIL:44510, http://www.cellimagelibrary.org/groups/44501.
16. Zaritsky A NS, Ben-Jacob E, Tsarfaty I. The cell: an image library. 2013; CIL:43401 - CIL:43417, CIL:45451 – CIL:45454, http://www.cellimagelibrary.org/groups/45451.
17. Zaritsky A, Natan S, Kaplan D, Ben-Jacob E, Tsarfaty I. GigaScience Database. 2015. Supporting material for: "Live time-lapse dataset of in vitro wound healing experiments". http://dx.doi.org/10.5524/100118. http://gigadb.org/dataset/view/id/100118/token/R65FQe8MBijD9Wks/.
18. Lee RM, Kelley DH, Nordstrom KN, Ouellette NT, Losert W. Quantifying stretching and rearrangement in epithelial sheet migration. New J Phys. 2013;15:25–36.

Submit your next manuscript to BioMed Central and take full advantage of:

- **Convenient online submission**
- **Thorough peer review**
- **No space constraints or color figure charges**
- **Immediate publication on acceptance**
- **Inclusion in PubMed, CAS, Scopus and Google Scholar**
- **Research which is freely available for redistribution**

Submit your manuscript at
www.biomedcentral.com/submit