Cell speed as phenotypic signature in drug discovery

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Abstract

Single-cell tracking throughout several cell cycles allows to trace kinships of cells in lineage trees and find correlations among phenotypes. It allows to utilize the fact that related cells bear information on the underlying mechanisms behind single cell phenotypes. Combined or contextual analyses can therefore help to extract more information from noisy data on cells as compared to independent analyses for each cell. Cell speed is so far poorly analysed, however, it gives information on inherent properties of cells since they move with various speed and sister cells tend to move similarly. Cell speed therefore deserves to be called a phenotype. The present results are produced using the software KoBio Celltrack (`https://korsnesbiocomputing.no/`). It is under active development as a robust and lightweight software to visualize and track cells from label-free long term recordings produced by various instruments. The purpose is to provide data of direct biological interest as well as ground-truth for BIG data analyses.

Introduction

Single and collective cell motility modes have been conserved through evolution and they depend on active reorganization of the cytoskeleton [1]. Cell motility plays a crucial role in many vital processes as well as in cancer invasion and metastasis. A metastatasic cascade can enable some initial cells to migrate and create their own migration tracks [2].

Quantitative analysis of migration tracks can help to discover biological functions or processes involved in diseases. This can be useful in evaluation of drug treatment, detection of rare sub-populations and discovery of drug-tolerant persister states. Two-dimensional (2D) *in vitro* experiments including single-cell tracking can provide data on individual cell motility behaviour under drug treatment. Data from such experiments often show individual variation in motility in the same clonal population.

The following factors can affect cell motility:

- States/conditions of the cytoskeleton.
- Interplay between cell adhesion and contractility.
- Confinement.
- Proliferation.
- Cell shape.
- Deformability of the nucleus.
- Plasticity of migration mechanisms.
- Stress/mechanical stress.
- Differentiation.
- Intracellular cascade of signalling events.

Cell speed variation in clonal populations allows to correlate speed of sister cells. Such covariation can indicate that cell speed is an inherent property.

Aim of study

The aim of this study is to explore cell speed to obtain biological relevant information from cells and their response to drug exposure. Cell speed estimates are one of the easiest available parameters to derive from single-cell tracking. Speed is therefore a natural candidate to explore cell inherent properties. Uncovering the utility of speed data will therefore presumably have a significant impact on phenotypic screening for drug discovery.

Materials and Methods

A549 cell lines were cultured in RPMI 1640 (Lonza, Norway), supplemented with $9\,\%$ heat inactivated fetal calf serum (FCS, Bionordika, Norway), 0.02 Hepes buffer 1M in $0.85\,\%$ NaCl (Cambrex no 0750, #BE17-737G) and $10\,\mathrm{ml}$ 1X Glutamax (100X, Gibco #35050-038), $5\,\mathrm{ml}$ in $500\,\mathrm{ml}$ medium. Cells were maintained at $37\,^{\circ}\mathrm{C}$ in a humidified $5\,\%$ CO_2 atmosphere.

Time-lapse microscopy

A549 cells were plated onto 96 multiwell black microplates (Greiner Bio-One GmbH, Germany) for time-lapse imaging. Cells were imaged into Cytation 5 Cell Imaging Reader (Biotek, USA) with temperature and gas control set to $37\,^{\circ}\mathrm{C}$ and $5\,\%$ CO_2 atmosphere, respectively. Sequential imaging of each well was taken using 10X objective. The bright and the phase contrast imaging channel was used for image recording. Two times two partly overlapping images were stitched together to form images of appropriate size. A continuous kinetic procedure was chosen where imaging was carried out with each designated well within an interval of $6\,\mathrm{min}$ for a $94\,\mathrm{h}$ incubation period. Exposed cells were recorded simultaneously subject to three different YTX concentrations (200; 500; 1000 nM).

Results

The following results give arguments that cell speed can be considered as a phenotype to help characterize cells. Figure 1 shows two examples of tracks of sister cells with similar speeds after their birth. A simple way to quantify similarity between speed of different cells is to compare their track lengths (or average speed) for given time periods. This parameter significantly varies among cells. Figure 2 illustrates this variation showing distributions of track length of the speed of the first generations sister cells in the present recordings using this simple concept of similarity where the actual time period is between $5\,\mathrm{h}$ and $15\,\mathrm{h}$ after birth.

Figure 3 displays joint distributions between these parameters for the actual sister cells. Note the general variation of speed (cf Figure 2) and the correlation between the speed of the sister cells. This is an argument for considering cell speed as a proper phenotype. Korsnes and Korsnes [3] similarly used max speed as definition of "speed" where track length is defined as length of track subject to a smoothing operation. This work applies similar smooting of thrack to define track length or speed.

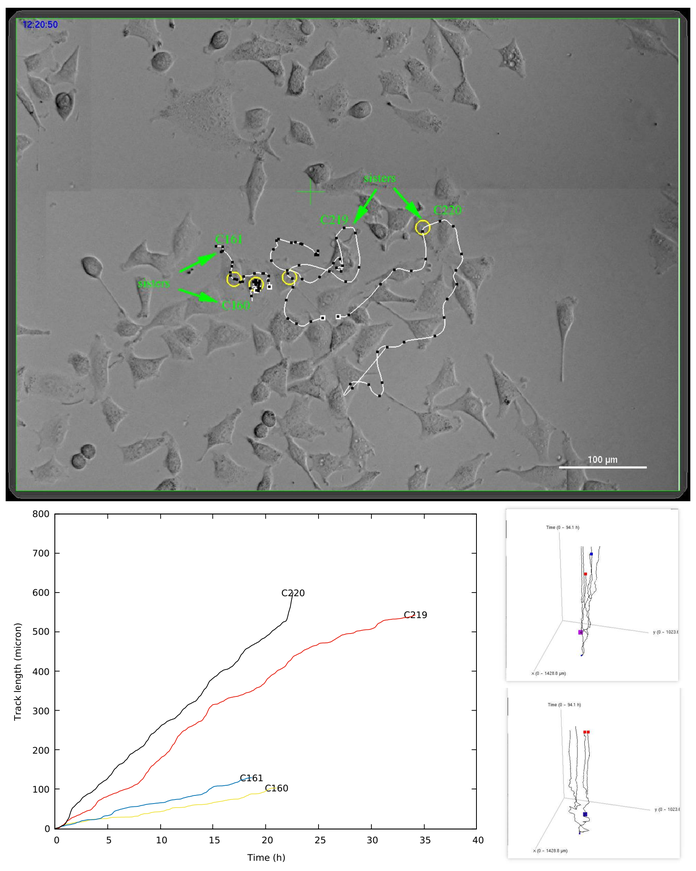

Figure 1: Examples of tracks for two couples of sister cells. The top figure shows their tracks superimposed on an image from the actual video recording. The lower left part shows graphs for the corresponding track lengths as function of time after their birth. The lower right part shows the actual 3D tracks (position versus time) for the whole pedigree tree.

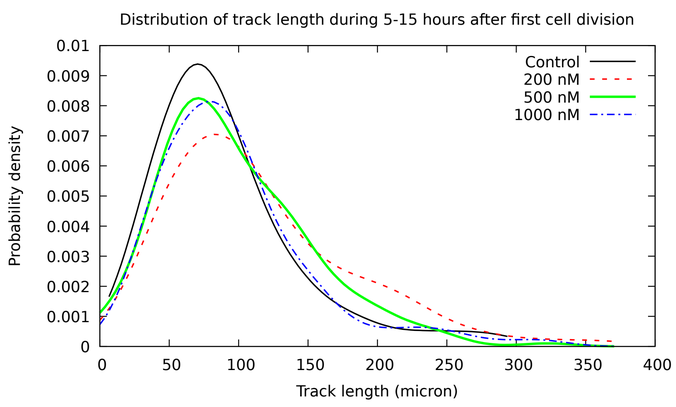

Figure 2: Distribution of track length 5-15 h after first cell division of recording. The graphs illustrate increased variation for exposed cells as compared to the control.

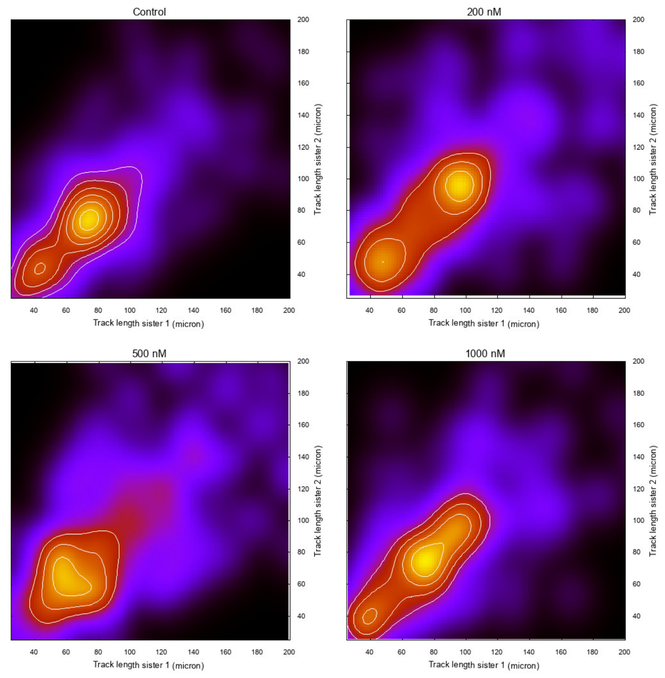

Figure 3: Joint distribution of track length for first generation sister cells during 5-15 hours after cell division. The cells were subject to YTX exposure at concentrations $200\,\mathrm{nM}$, $500\,\mathrm{nM}$ and $1000\,\mathrm{nM}$ as well as control (no exposure). Each distribution results from observations of 100 initial cells normally dividing the first day of recording. Note the rich structure and change in distributions depending on exposure.

Conclusions

Sister cells tend to move more similarly as compared to random cells in a clonal population. This strongly indicates that cell speed reflects varying inherent properties. Speed therefore deserves to be called a phenotype. This understanding conforms with the idea of stereotypical behaviour as explored by Luke *et al.* [4] as well as Mencattini *et al.* [2].

The Figures 2 and 3 apply track length during $5\,\mathrm{h}$ - $15\,\mathrm{h}$ after the actual cell division. This is an arbitrary choice. The graph of Figure 1 (lower left part) can indicate that speed similarity may be formalized in more complex ways which more precisely reflect innate properties. Such alternative definitions may assume to include analyses of data from all cells in the whole available pedigree tree in a combined analysis to define and identify distinct inheritable phenotypes of a cell [5]. This idea has conceptual similarities to using data from relatives when making diagnosis in cancer research.

Data from trajectories of single cells contain information that help to predict behavior in heterogeneous cell populations. Observations of heritable traits are currently difficult to perform using single cell tracking based on 3D platforms. Trajectory data from 2D *in vitro* experiments may therefore give proxy data for 3D situations since the physical underlying mechanisms for movements in 2D and 3D are presumably much the same.

This presentation elaborates speed data to illustrate its potential as a phenotype reflecting varying inherit properties among cells. Data on velocity (vector) certainly provides additional information reflecting coordination, persistence and duration of processes. However, focusing on speed only in the 2D situation may have an advantage as proxy data for 3D as compared to the vectorial quantity *velocity*.

References

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