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Expertomica Cells: analysis of cell monolayer development

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ABSTRACT

Summary: Expertomica Cells is a program for the creation and analysis of pedigree plots from time-lapse micrographs of cell monolayers. It enables recording the basic events in a cell cycle, cell neighbourhoods and spatial migration. The output is both numeric and graphical. The software helps to lower main hurdles in the manual analysis of cell monolayer development to practical limits; it reduces the operator processing time of typical experiment containing 5000 consecutive images from the usual 3 months to 3–10 h.

Availability and Implementation: Freely available on the web at http://www.expertomicacells.tk or http://www.expertomicacells.wu.cz. The source code is implemented in JAVA 6 and supported by Linux, Mac and MS Windows.

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Supplementary information: Supplementary data available at *Bioinformatics* online.

Cell monolayer development is a frequently used approximation to tissue and organ development. It is used in many applications in basic science and industrial development. There are surprisingly few detailed analyses of cell pedigrees (also referred to as cell genealogy) in these cultures (Glauche *et al.*, 2009). In most cases, the cell heritage is tracked with the use of fluorescence markers (Kaufmann *et al.*, 2007). For the cell development models, the utilized data come from primitive organism (Fisher *et al.*, 2008) or from stochastic models (Glauche *et al.*, 2009). Another proposition is that cell division is dependent on cell size and time (Tzur *et al.*, 2009). In our opinion, the problem is in the high intensity of manual analysis working with thousands of images. In this article, we describe a tool that enables manual analysis of a cell monolayer development and recording the basic neighbourhood relations in the culture. From that we can create basic or advanced plots and statistics.

The input data represents a series of consecutive microscopic images captured during the development of the cell culture. We chose the .jpeg data format but the nature of the task performed by the program does not depend on the data format. Any type of data transformed to .jpeg can be used. The size of datasets utilized in ordinary analyses is 3 GB; the size of a single time slide is ∼0.5 MB. In the computer memory, only one image is kept at a time. The record of a previous image can be seen on the neighbourhood network map (Figure 1).

The user has to analyse each consecutive image separated by a given time interval (1 min in the presented example). Cells are in the software classified into mitotic cells and cells in the interphase and the events into division and fusion. Also the immediate neighbourhood of cells is recorded. In the analysis, we observed cell movements and number of other distinct cell characteristics, namely structures and dynamics in the cell interior, but it is beyond the physical limit of the operator to monitor them. Currently, we are doing research on how to analyse images automatically, but the conclusion resulting from an extensive testing by five different operators is that due to the complexity of the problem the analysis is strongly dependent on the model we choose. The program does not restrict any pre-processing of the images that may be imported into the analysis. In our opinion, the manual analysis will always be a non-negligible part of the assessment of an experiment and the resulting data. Therefore, we propose the reported program to be a complete and stand-alone application.

From the recorded cell events, the operator may create cell genealogy or pedigree. The graphical output uses the free software Graphviz. The user may choose to export a complete graph but so far we have not found an application for that. The resulting graph structure is complex and difficult to analyse. By clicking an individual cell, the user gets a significant reduction of the complete graph, which is useful for the analysis (Supplementary Material 1). The original graph depicts the pedigree of the selected cell and the build-up of its neighbourhood. The resulting graph is significantly more complicated or, in other words, only shorter experiment time may be viewed.

The program automatically calculates the lengths of identifiable cell cycles. The output data may be exported in the text form, as a dataset readable in Graphviz. Among the experimental statistics, we can export the evolution of a number of cells in time and cell cycle times in the Matlab format. The Matlab file contains a commented script necessary for creating a graph of the cell growth and histograms of the cell cycle times. It may also be used as a standard comma separated text file, the parts of which may be imported into any table-processing software.

One typical experiment was the analysis of a cell pedigree (Supplementary Material 2–4). We analysed 5000 consecutive microscopic images, identified cell cycles, exported the sub-pedigree and analysed all the cell events in detail. A total of 39 cell cycles were identified, out of which seven were anomalous and were not included in the final analysis (Supplementary Material 5). We examined the hypothesis that the length of the cell cycle is dependent on the number and nature of events the cell undergoes, namely the number

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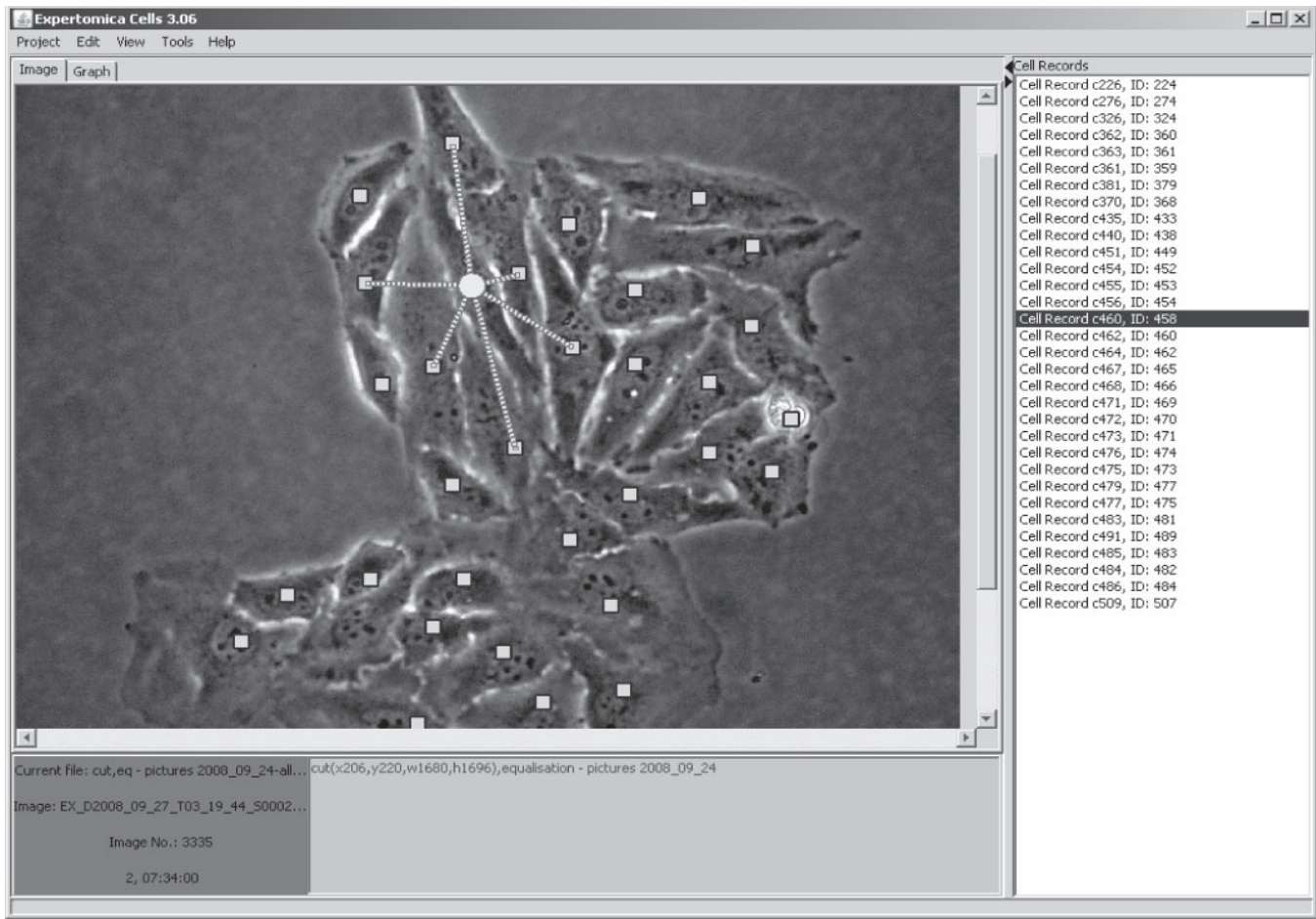

Fig. 1. The course of analysis in the Expertomica Cell program. The cells are identified manually by placing the identifier into the cell interior. The neighbourhood network is identified manually and indicated by dotted lines. The program recognizes only two types of cells, flat and round. This simplified identification was chosen when we found that there is no real consensus in which cell states should be sought and notified. Cell events or additional cell states may simply be identified through the creation of a daughter cell with a new identifier and comment. For example in case that the original identifier appears outside the cell borders, a new identifier is created and the event is notified as cell movement. This highly subjective analysis exceeds any automated method known to us.

of neighbours and the number of significant changes in their position. Although there was found no apparent, statistically significant correlation, the identification of individual cell cycles was a useful incentive for detailed examination of cell behaviour that enables us to distinguish individual cell state trajectories more closely. These may be classified in detail, selected for future in-depth inspection or sought in other pedigree analyses. Statistically significant results on a cell pedigree itself could only by obtained by numerous analyses of similar experiments which, however, may be prone to deviation caused by subtle changes in the experiment set-up.

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Conflict of Interest: none declared.

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