TRACMIT: An Algorithm For Tracking Single Cells On Micropattern Through Mitosis

Olivier Buri*, Benita Wolf*, Arne Seitz*, Pierre Gönzy**

*EPFL SB Imaging & Optics Platform (BIOF), **Swiss Institute for Experimental Cancer Research (SICR), School of Life Sciences

Abstract

Mitotic division assessment is a rather common practice[1-4] in high throughput screening, and the tools are well suited to track cell division in classic multiwell plates. The analysis becomes difficult when combined with micropatterns where the cells can sit. In such cases, only patterns with the right cell geometry are to be considered for analysis. This has led us to develop a novel image analysis pipeline that handles all necessary image processing steps within one standalone platform. The result is TRACMIT(pipeline for TRACKing and analyzing cells on micropatterns through Mitoysis), a tool for Fiji to rapidly and accurately assess the orientation of the mitotic spindle during metaphase in time-lapse fluorescence microscopy of cells plated on micropatterns with an L-shape. The focus of the tool is to be able to always start from the raw data and avoid the need to save intermediate images, thereby decreasing data volume. ASMIT first selects micropatterns containing a single cell and then detects anaphase figures in the time-lapse recording. Next, the algorithm tracks back in time until metaphase, when the angle of the mitotic spindle with respect to the L-shaped micropattern is assessed. ASMIT was designed to allow manual validation of selected cells with a simple interactive interface. For ease of use, ASMIT is provided as a series of Fiji/ImageJ[5] macros, grouped into an ActionBar[6].

Rationale

The goal is to detect cells undergoing divisions on micropatterns which have been imaged in a single channel during a long timelapse with over 200 timepoints. Care must be given to
- Distinguish empty or crowded patterns to avoid needless computations.
- Detect mitosis events and mitotic plates accurately.
- Backtrack the mitotic plate detection to get a time-dependent angle value.
It was our goal to have it as an open-source tool in order to maximise the possibility of the code being reused by the scientific community.

User Interface

The user interface is presented as an ImageJ ActionBar and encompasses all steps from parameter selection to result checking.

The choice of ActionBar stems from the ease with which one can create interfaces where each button is comprised of an ImageJ macro, which makes debugging and testing code very fast and simple.

Results

9045 cells from 239 different siRNA conditions were manually assessed and compared with TRACMIT detection results. We found a Pearson correlation coefficient R2 of 0.52 between TRACMIT and manual inspection, primarily due to the pipeline not recognizing some micropatterns that had more than one cell (See A).

However spindle position accuracy yielded an almost perfect correlation between the manual detection and TRACMIT (R2=0.99, n=84, See B).

Finally we checked whether the relatively high rate of false positive detection TRACMIT could perform as accurately as an individual in discriminating between a control condition and a condition exhibiting a spindle positioning phenotype. We found that TRACMIT performed as well as a human being in recognizing wells with a high percentage of cells exhibiting a spindle positioning phenotype.

Software Repository

https://github.com/lacan/TRACMIT

References