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

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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 a novel image analysis pipeline that handles all necessary image processing steps within one open-source platform.

The result is TRACMIT(pipeline for TRACking and analyzing cells on micropatterns through MITosis), 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. 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.
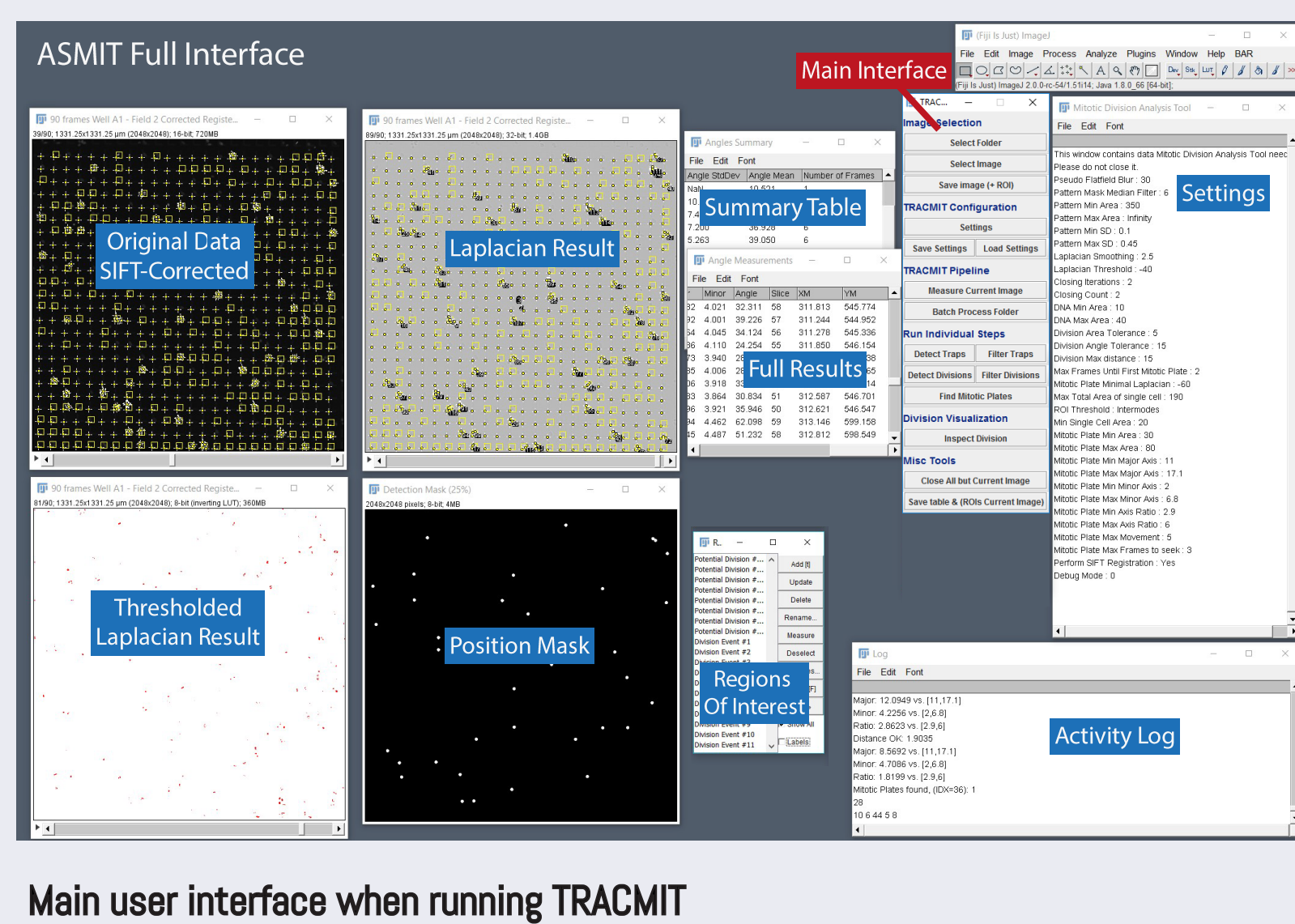- Backtrack the mitotic plate detection to get a time-dependedna angle value

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.
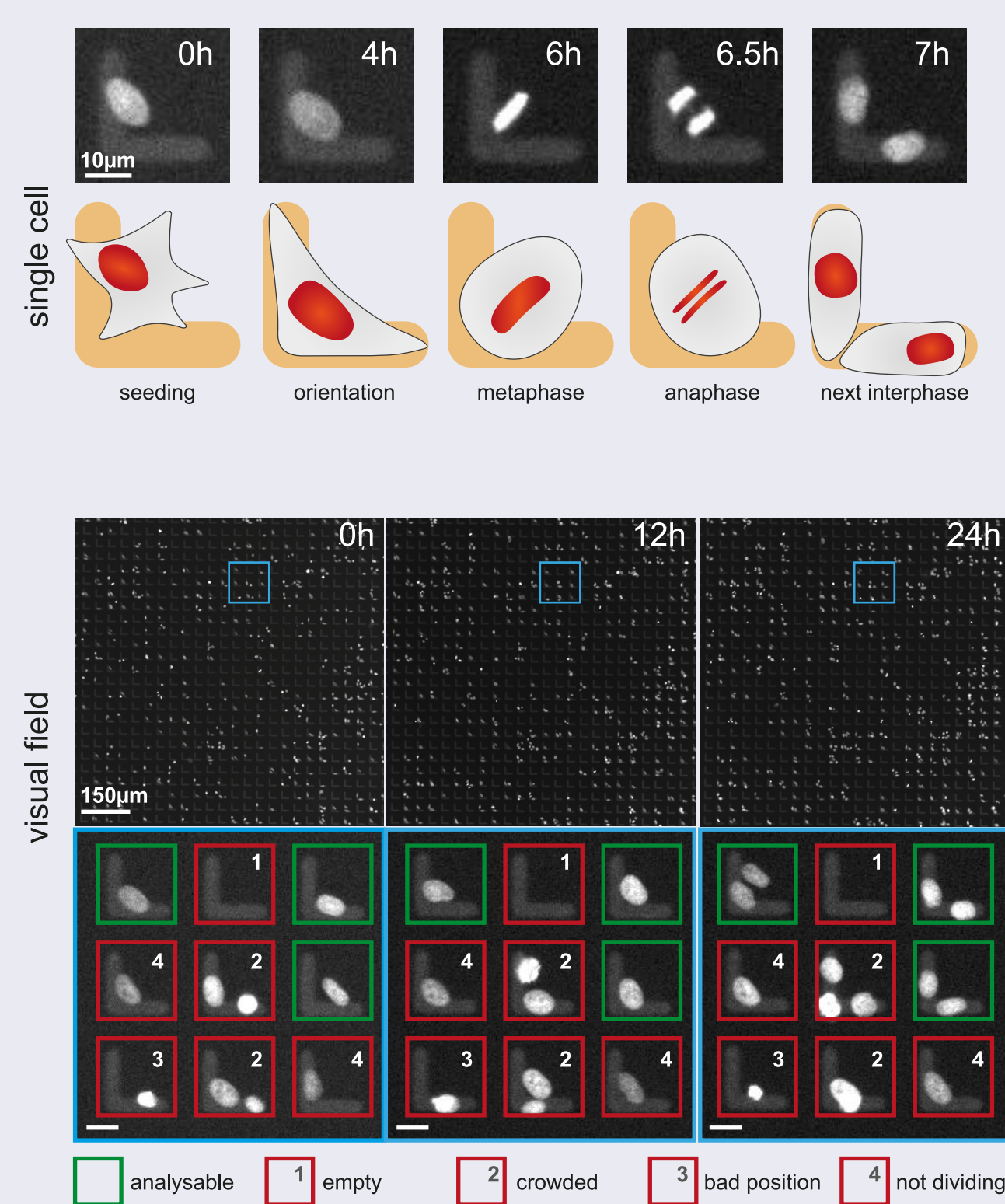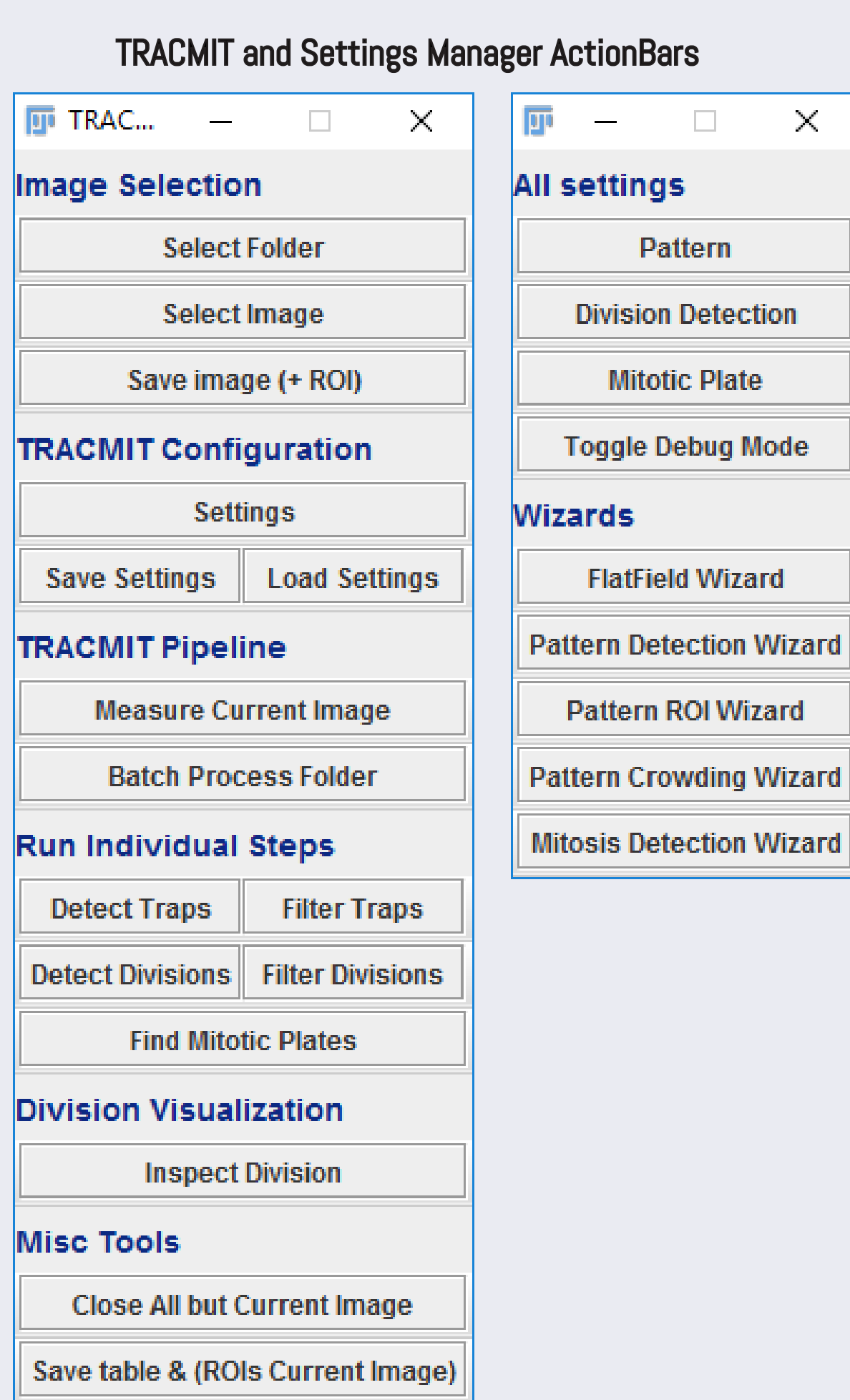

User Interface

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 imageJ1 macro, which makes debugging and testing code very fast and simple.
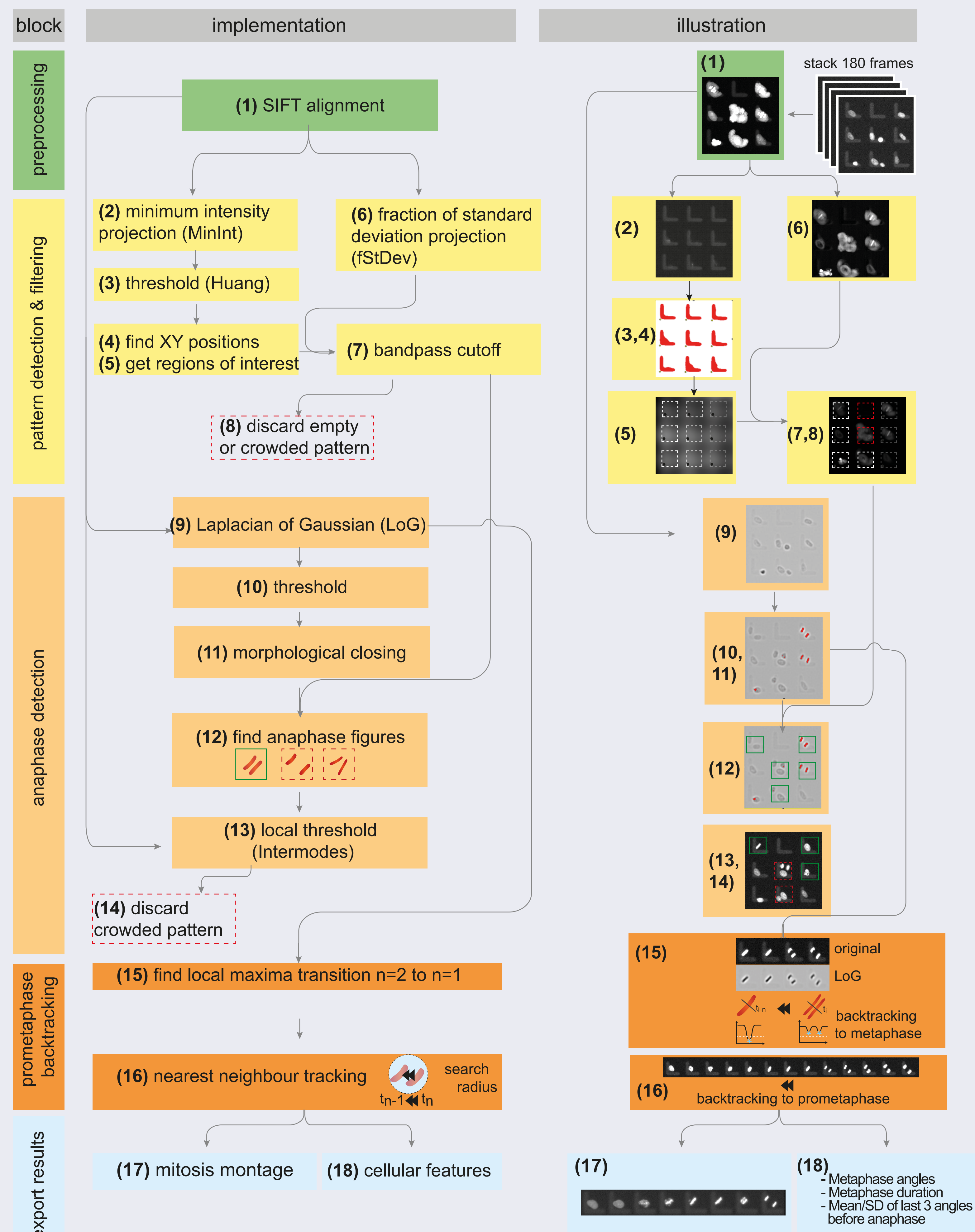
TRACMIT and Settings Manager ActionBars

Main user interface when running TRACMIT
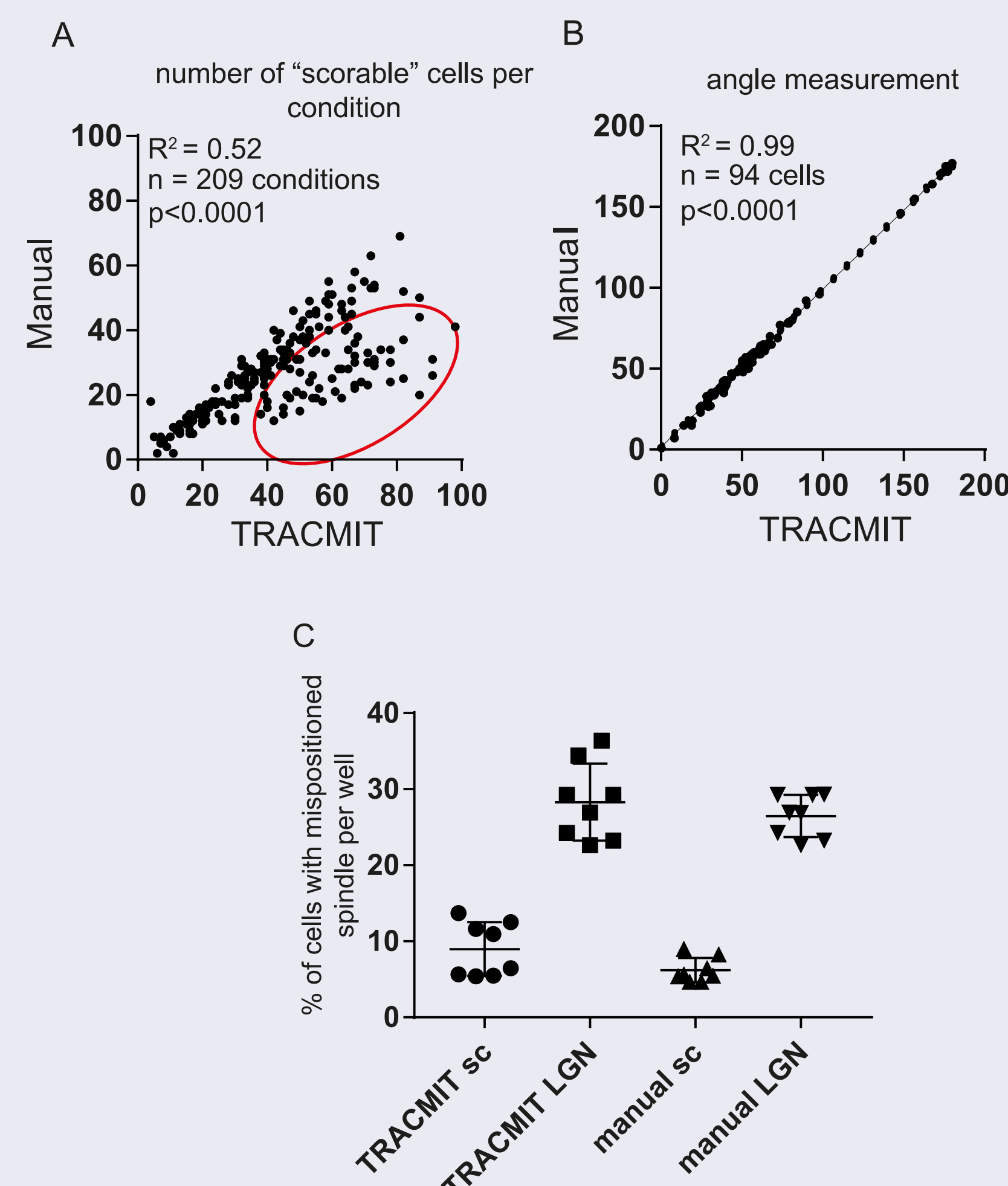
Algorithm Pipeline

Results

9045 cells from 209 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 yeilded an almost perfect correlation between the manual detection and TRACMIT (R2=0.99, n=94, See B).

Finally we checked whether depite 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.

References

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Software Repository

https://github.com/lacan/TRACMIT

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