Abstract:

Background: In recent years, high-throughput microscopy has emerged as a powerful tool to analyze cellular dynamics in an unprecedentedly high resolved manner. The amount of data that is generated, for example in long-term time-lapse microscopy experiments, requires automated methods for processing and analysis. Available software frameworks are well suited for high-throughput processing of fluorescence images, but they often do not perform well on bright field image data that varies considerably between laboratories, setups, and even single experiments.

Results: In this contribution, we present a fully automated image processing pipeline that is able to robustly segment and analyze cells with ellipsoid morphology from bright field microscopy in a high-throughput, yet time efficient manner. The pipeline comprises two steps: (i) Image acquisition is adjusted to obtain optimal bright field image quality for automatic processing. (ii) A concatenation of fast performing image processing algorithms robustly identifies single cells in each image.

We applied the method to a time-lapse movie consisting of 315,000 images of differentiating hematopoietic stem cells over 6 days. We evaluated the accuracy of our method by comparing the number of identified cells with manual counts. Our method is able to segment images with varying cell density and...
different cell types without parameter adjustment and clearly outperforms a standard approach. By computing population doubling times, we were able to identify three growth phases in the stem cell population throughout the whole movie, and validated our result with cell cycle times from single cell tracking. Conclusions; Our method allows fully automated processing and analysis of high-throughput bright field microscopy data. The robustness of cell detection and fast computation time will support the analysis of high-content screening experiments, on-line analysis of time-lapse experiments as well as development of methods to automatically track single-cell genealogies.