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Live Single Cell Imaging and Analysis Using Microfluidic Devices

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Doctoral thesis

Akademisk avhandling

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Abstract

Today many cell biological techniques study large cell populations where an average estimate of individual cells' behavior is observed. On the other hand, single cell analysis is required for studying functional heterogeneities between cells within populations. This thesis presents work that combines the use of microfluidic devices, optical microscopy and automated image analysis to design various cell biological assays with single cell resolution including cell proliferation, clonal expansion, cell migration, cell-cell interaction and cell viability tracking. In fact, automated high throughput single cell techniques enable new studies in cell biology which are not possible with conventional techniques.

In order to automatically track dynamic behavior of single cells, we developed a microwell based device as well as a droplet microfluidic platform. These high throughput microfluidic assays allow automated time-lapse imaging of encapsulated single cells in micro droplets or confined cells inside microwells. Algorithms for automatic quantification of cells in individual microwells and micro droplets are developed and used for the analysis of cell viability and clonal expansion. The automatic counting protocols include several image analysis steps, e.g. segmentation, feature extraction and classification. The automatic quantification results were evaluated by comparing with manual counting and revealed a high success rate. In combination these automatic cell counting protocols and our microfluidic platforms can provide statistical information to better understand behavior of cells at the individual level under various conditions or treatments *in vitro* exemplified by the analysis of function and regulation of immune cells. Thus, together these tools can be used for developing new cellular imaging assays with resolution at the single cell level.

To automatically characterize transient migration behavior of natural killer (NK) cells compartmentalized in microwells, we developed a method for single cell tracking. Time-lapse imaging showed that the NK cells often exhibited periods of high motility, interrupted with periods of slow migration or complete arrest. These transient migration arrest periods (TMAPs) often overlapped with periods of conjugations between NK cells and target cells. Such conjugation periods sometimes led to cell-mediated killing of target cells. Analysis of cytotoxic response of NK cells revealed that a small sub-class of NK cells called serial killers was able to kill several target cells. In order to determine a starting time point for cell-cell interaction, a novel technique based on ultrasound was developed to aggregate NK and target cells into the center of the microwells. Therefore, these assays can be used to automatically and rapidly assess functional and migration behavior of cells to detect differences between health and disease or the influence of drugs.

The work presented in this thesis gives good examples of how microfluidic devices combined with automated imaging and image analysis can be helpful to address cell biological questions where single cell resolution is necessary.

Keywords: Single cell analysis, time-lapse fluorescence imaging, automated image analysis, microwell, droplet microfluidics, NK cells, single cell tracking, migration behavior analysis, cell-cell interaction.

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