Image Analysis and Platform Development for Automated Phenotyping in Cytomics

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Chapter 7

Conclusion and Discussion

7.1. Conclusions

This thesis explored solutions for image segmentation and object tracking for high-throughput/high-content (HT/HC) screens in cytomics studies. To demonstrate the usefulness of the solution, two case studies of cancer migration were discussed to elaborate the efficiency and limitations of several generic image and data analysis approaches frequently observed in HT/HC screen studies. From an analysis of these limitations, a number of dedicated algorithms are developed and evaluated. The evaluations show that the dedicated algorithms, namely watershed masked clustering (WMC) segmentation [62][29][15][10], kernel density estimation (KDE) with mean shift tracking algorithm [15][10][9], and energy-driven linear (EDL) model tracking algorithm, provide a more robust and accurate performance. From these dedicated algorithms, our case studies further demonstrate the possibility to produce an objective understanding of each unique phenotypic characteristic using morphology and motility measurements from both cellular and subcellular level.

With a good automated data management in place [145][146], the analysis pipeline can be further performed in a more efficient manner. The data management system shields end-users from the underlying complexity of the computational approaches in the pipeline by providing an integrated high-end GUI. The automation beneath the GUI will enable to scale to a higher volume of image data which is custom to HT/HC screen, i.e. terabyte level. It may further increase the data accessibility and interdisciplinary data conformity by standardizing different HT/HC image formats and measurement structures. The interdisciplinary data conformity is an important feature since the ultimate goal of cytomics and quantitative microscopy is to integrate all –omics data to provide a numeric modeling of genotype-to-phenotype mechanism.

Chapter 2 Robust Image Segmentation for Cytomics

The Chapter 2 has illustrated a dedicated segmentation algorithm, namely **Watershed Masked Clustering (WMC) algorithm**, for high-throughput cytomic studies. When compared to other segmentation algorithms, the WMC is capable of producing an accurate segmentation results when image contains objects with nonlinear intensity and morphology variation. Such a trait is particularly useful in HT/HC screen studies since the responses of treatments are often unclear prior to the experiment.

Chapter 3 Robust Object Tracking for Cytomics

The Chapter 3 has illustrated two dedicated object tracking algorithms, namely the **Kernel Density Estimation (KDE) with Mean Shift tracking algorithm** and the **Energy Driven Linear (EDL) model tracking algorithm**. Compared to other tracking algorithms, these two algorithms show a robust tracking performance for cellular and subcellular objects without manual intervention. Moreover, they can be extended to other application domains if new motion models can be built.

Chapter 4 A Study to Cell Migration Analysis

In this case study, we have numerically extracted the morphology and motility characteristics of random cancer migration under the influence of different growth factor treatments using image and data analysis solutions described in Chapter 2 and Chapter 3. Compared to manual analysis, it is clear that an automated image and data analysis solution can provide a more

objective and reproducible understanding of a cell biology experiment. It is an important trait in cytomics studies since it allows the experiment to scale to a larger volume of data without scarifying the quality of analysis while it is impossible with manual analysis.

Chapter 5 A Study to Dynamic Matrix Adhesion Analysis

In this case study, we have demonstrated an integrated solution to quantify the morphology and dynamics of both matrix adhesions and cells using the automated image analysis solution described in Chapter 2 and Chapter 3. From the measurements, we further confirm several correlations between matrix adhesion dynamics and cell migration. This case study [16] is one of the few attempting to reveal the subcellular control mechanism behind random cell migration using an automated method.

Chapter 6 Reasoning over Data in HT/HC Management

In Chapter 6, we have demonstrated the design and implementation of a dedicated data management system. In our study (Ch. 4 & 5) the data management system serves as both the starting and the end point of the analysis by shielding the end-user from underlying complexity with a high-end integrated GUI [146][145]. Thus, it allows end-user to perform sophisticated HT/HC screen studies without an extended knowledge of image and data analysis.

7.2. Discussion

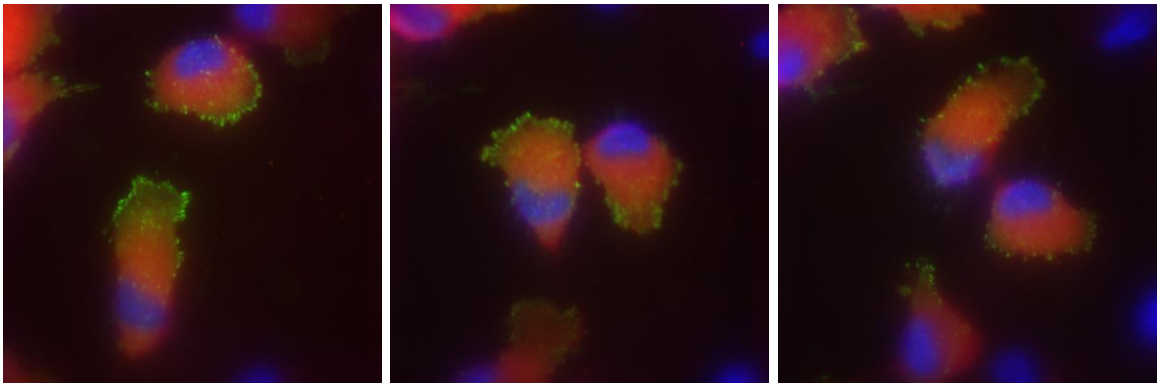
This thesis has explored the image and data analysis solutions for empirical HT/HC screen study. In the future, we will focus on making improvements in the following aspects.

1. Experiment Preparation in HT/HC Screening

Although, experiment preparation is beyond the scope of this thesis, in the two case studies (cf. Chapter 4 and Chapter 5) it has demonstrated how image quality is affected by the experiment preparation. At current stage, the cell-to-cell variability [19] is a typical challenge frequently encountered in our research. Often cell behavior is subjected to number of factors such as cell age, local cell density, individual mutation, etc. It is possible to employ a normalization strategy similar to microarray data analysis [155][156][157], but it would reveal more information if the true mechanism behind the cell-to-cell variability can be understood. Dedicated research should be conducted to gain understanding of these phenomena.

2. Image Acquisition

Modern microscopes are frequently equipped with additional functional controllers allowing an on-the-fly adaptation of the microscope settings such as focus and position of specimens. However, these adaptation mechanisms are subjected to the heuristics from which the adaptation algorithm is built. For example, in the experiments underlying the work in Chapter 5, frequently out-of-focus errors were encountered (cf. Figure 7-1). In addition, in live cell migration or subcellular dynamics study the temporal-resolution of image sequences is often sacrificed in exchange of a better image quality. Both issues are hardware related that can be significantly improved by new developments in the microscope technology.



Frame 1 focused | Frame 50 out of focus | Frame 100 slightly out of focus

Figure 7-1 out-of-focus error during live cell imaging

3. Image Analysis

Tuning of Segmentation

We believe that a mechanism of robust self-adaptation is the key to a successful image segmentation solution in HT/HC screen studies. For this adaptation mechanism, a robust representation of image heuristics [62][62] is crucial for its success. The sophistication of the self-adaptation mechanism can benefit most from new progress in machine vision [158].

Phenotypic Measurement

The phenotypic measurements used in this thesis are capable of extracting each unique phenotypic profile of objects. However, they are often not scale-free [33][32][31] and cannot be compared across different microscopy settings. Moreover, for some cell phenotype, the current measurement is not optimized. For example, the velocity is often measured from the difference between objects in consecutive frames, but the difference is not necessarily only associated with motility (cf. Figure 7-2) [140][159]. A local regression approach [126] is frequently used to extract the majority trend of migration while a more sophisticated motion modeling solution [140][159] is preferred for the measuring of motility.

(a) shifting of mass center is correlated to motion **(b) shifting of mass center is correlated to deformation**

Figure 7-2 a Z-projection plot of cell body contours during migration

4. Data Analysis

In the case studies of Chapter 4 and Chapter 5, we use low-order statistics to provide some characterizations of cellular and subcellular dynamics; previously unknown or unverified. However, by losing the temporal dimension, it is very easy to overlook particular dynamic behavior behind control mechanism of cell migration. Therefore, the study of expanding temporal statistical analysis into temporal-spatial data is required.

The HT/HC screen study is an emerging field in cytomics research. Together with image and data analysis it provides an efficient analysis tool for studies in functional genomics and cell

biology. There are challenges to be met in image and data analysis of HT/HC screen studies. However, with new approaches developed, it will be eventually possible to accomplish the genotype-to-phenotype modeling. This requires combining data from different aspects of the same study-object. Cytomics can provide accurate and well annotated data for such boarder view.