Aspects of the analysis of cell imagery: from shape to understanding

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Summary

Microscopy supports us in observing small objects like cells, tissues, and micro-organisms. In Bio-Imaging images are captured to study biological phenomena. Often large collections of images are captured so that these images can be analysed in order to understand a phenomenon. In order to do these analyses, we need computational methods so that we can generate knowledge from these images. In this manner, imaging can accelerate studies in the biomedical domain. In this thesis, we have studied cell images from which analysis gives insight in the understanding of the content of these images. These are pollen grains and immune cells. For these cells the image analysis comprised analysis of 2D and 3D images. Moreover, the dynamics is considered in time-lapse sequences. The analysis of the pollen images focusses on the classification of the pollen whereas the analysis of immune cells focusses on the dynamics of neutrophils through tracking in time-lapse sequences. The approach that is required for these analyses is based on techniques from artificial intelligence in which computers are trained to learn recognizing specific patterns.

Pollen images are often made from airborne samples. From these samples different types of pollen can be recognised by a specialist. Sometimes it is very difficult, if not impossible to tell different species apart. In our study, we address a problem where this is typically the case and attempt to distinguish the allergenic pollen *Parietaria* from the non-allergenic pollen *Urtica*, and *Urtica membranacea* in the same Urticaceae family. We use images from pollen to see if we can train a computer to tell these species apart. In order to have sufficient detail of the pollen we capture a pollen grain in 20 optical slices and use a projection method from these images. For each pollen image, we compute three different projections to obtain images as input for a classification model. In this manner, we aim to integrate as many representative features as possible. Moreover, with these three projections as input images, the performance of the classification has proven to increase.

The amount of images was sufficient to probe deep learning-based classification models. Initially, we have trained three of these models to categorize the three pollen species. These are VGG16, MobileNet V1 and MobileNet V2. To further explore possibilities for deep learning, we extended our studies with extra three deep-learning models. These are AlexNet,

VGG19 and ResNet50. In daily practice, it is not always guaranteed that an abundant amount of samples is available. Therefore, in addition to the deep learning methods, we investigated the performance of traditional machine learning-based classification models such as Support Vector Machine (SVM) and Random Forest (RF). In convolutional neural networks (CNN), features are automatically extracted and used in the training of the model. For traditional machine learning we have to design a set of handcrafted features to train a model. Feature selection methods have been adopted to reduce the unrelated features. After feature selection, different flat classification models were constructed, respectively, to identify each category. In addition, a hierarchical strategy was conducted by categorizing all classes into subgroups. For each subgroup, different classifiers were trained separately. We have demonstrated that this hierarchical strategy improves the performance compared to flat models.

From the implementation of different classification models for both deep learning-based and traditional machine learning-based methods, we conclude that: compared to machine learning-based methods, deep learning-based models achieve higher classification accuracy, in particular, i.e. ResNet50. The experimental results on both large and small datasets have proven the robustness of deep learning models. An ablation study explains the success of deep learning models by integrating different techniques, i.e. data augmentation, transfer learning and hard voting. We have elaborated and implemented a workflow to find a solution to accurately classify those high-similar pollen categories. This study provides a good insight in complex classification of pollen thereby allowing healthcare professionals to better advise hay fever patients with their treatments and outdoor activity planning.

Next to pollen imagery, we studied a completely different domain, in which we can also easily obtain images in different dimensions. We address the monitoring of immune cells in response to a possible infection. So, here we study life-images of cells while they are moving in a body; i.e. neutrophils in a zebrafish larvae. We monitor these neutrophils by tracking them in time-lapse sequences. Neutrophils are involved in the first line of defense against pathogens to protect the organism, in our case a tail wound in a zebrafish larvae. As part of the understanding of the cell behavior of the immune system, the monitoring of the migration of neutrophils *in vivo* is important. Neutrophil tracking, however, is a challenging problem. In our studies, we have analysed time-lapse sequences of neutrophils that have been imaged with a confocal microscope. From the time-lapse sequences computational methodologies and algorithms are designed with the aim to solve the tracking problems in both 2D and 3D spatial domains.

For the neutrophil tracking in a 2D time-lapse sequence, we propose a pipeline involving cell segmentation, cell motion tracking between adjacent frames, and trajectory linkage. The neutrophils are fluorescently labelled and a first step for the identification is a segmentation

of these cells. For segmentation of cells we use three U-Net-based segmentation models and a rule-based Watershed Masked Clustering (WMC) segmentation algorithm. The models are constructed and compared for performance with respect to accurate cell location for the tracking procedure. We intend to select the best performing segmentation model. Subsequently, from the segmentation, the cell migration motion needs to be understood. Therefore, we construct an additional U-Net model that is trained to learn the cell migration motion from annotated ground truth data. In this manner, we can predict the cell position in the next frame. In order to obtain cell trajectories, we need to solve the challenging cell merging/splitting problems that arise from the sequences. To that end, we have made a key contribution in that we designed an extension to the Viterbi linkage algorithm to be able to objectively solve this. Our pipeline has achieved satisfying performance on the neutrophil tracking task.

After our accomplishments in 2D time-lapse sequences, we investigated neutrophil tracking in 3D time-lapse sequences. We designed and implemented a pipeline including 3D cell segmentation, a feature-weighted similarity estimation and trajectory linkage. We select a 3D U-Net deep learning model to segment 3D cell images at each time point. A similarity estimation is done by extracting cell features, i.e. cell travel distance, direction of cell movement and average cell travel distance over trajectory. Next, a similarity score is calculated by weighting these features at different scales. Based on the similarity score, we adapted the Hungarian algorithm to link the cell trajectories. Overall, compared to the existing state-of-the-art methods, our method demonstrates a good performance according to our experimental results.

In this thesis, we have demonstrated the use of deep learning methods for different kinds of cell imagery and shown the potential for future use whist illustrating pitfalls, but also presenting efficient new algorithmic approaches.