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

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Chapter 1

Introduction

Bioimaging is the study of making images from biological samples. The biological samples that are investigated basically vary from cells, tissues, organs, etc, with a resolution from micrometers to centimeters. To make images of those small samples, imaging instruments, i.e. microscopes, are required. A huge range of different microscopes and techniques have become available over time [1]. Hence, the selection of a microscope technique depends on the level of details that you want to obtain from small samples.

In this thesis, we intend to study cell image data with multi-dimensional characteristics. In order to guarantee an accurate analysis subsequently with computational tools, data acquisition techniques should be good enough to acquire as many details as possible. Several requirements need to be taken into account and for computational analysis, the volume of the data set is very important. A sufficient amount of data can provide diverse information that advances the computational procedures. Moreover, image resolution/quality determines if we can gain sufficient details from biological samples. With these requirements, we start our work.

The data we are considering is not just flat cells. Other than *in vitro* imaging, we look at *in vivo* cell data and try to find a microscopy technique that fits them to capture images. We have two particular classes of cell data. One is pollen grains, which are imaged with a brightfield microscope. The other one is the immune cells, in particular neutrophils, and these are imaged by a confocal microscope.

So we study a gamete cell and a cell that dynamically moves in the body. In order to analyse these cells we need 3D images or 3D images over time. So, the subjects of research are captured in multi-dimensional images.

Pollen types are as diverse as the plants that produce them. We specifically look at airborne pollen. Airborne pollen encompass the reproductive cells, gametes, generated from flowering plants. Some of these are causing issues, i.e. health problems for individuals

suffering from hay fever, leading to decreased productivity and increased healthcare costs [2]. In recent decades, the incidence of hay fever patients is rising worldwide, which has raised awareness of some institutions towards problems. Therefore, monitoring airborne pollen and identifying pollen taxa is becoming more and more important. For medical research in hay fever, this has quite an impact. It contributes in forecasting allergies and providing potential medicine treatments to alleviate allergy numbers. Pollen category identification based on distinguished family/genus levels is possible using microscopic methods. However, for pollen with quite similar morphology within the same species even genera, computational algorithms and strategies are essential, specifically to identify the allergenic pollen in a sample. In this thesis, we elaborate on a case study for a particular plant family. Pollen grains are such a nice object for study because they are isolated cells, one just has to identify each pollen grain from a sample.

Contrary to pollen grains, we study neutrophils *in vivo* in the body. In order to be able to do that we need a model system, and for that, zebrafish is very suitable. Neutrophils are a type of innate immune cell and play a role in the protection of organisms. They are involved in combating infections caused by bacteria, viruses, and other pathogens. The study of neutrophils in zebrafish aims to unravel the mechanisms behind various diseases, including autoimmune disorders, cancer, and infectious diseases [3][4][5]. We study the neutrophils as part of the innate immune system, therefore, we look at the early developmental stages of zebrafish; i.e. larvae stages. In this stage of development the zebrafish are transparent. This transparency allows for high-resolution imaging, providing detailed insights into the entire organism's internal structures, including the neutrophils. If this event that challenges the integrity of the organism occurs, then the neutrophils are triggered to play a role in maintaining the integrity. Thus neutrophils will move to where they are necessary to play a role in neutralizing intruders. In order to visualize this movement in the body, we need to capture 3D images over time. Subsequent to studying the behavior of neutrophils, cell tracking techniques, are required.

In this thesis, we intend to find the solutions for both problems. It holds that we are to find specific classes in the data. These are two typical cases, for which we acquire images with a microscope and get sufficient images to find the pattern that we are interested in.

1.1 Microscopy Imaging

Microscopes are the "eyes" that can observe the things that are not visible to the naked eye [6]. With the help of a microscope, we can see the structure of various proteins, viruses,

bacteria, cells, tissues and organs. It helps us to study the function of organisms so as to explore the mysteries in the world of living organisms.

In order to view the organisms at different scales, many types of microscopes are designed. The most widely used types of microscopes are optical microscopes and electron microscopes [7]. Each kind of these microscopes can see organisms in the range from nanometer to micrometer or millimeter [6][8], depending on the configurations being set. Fig. 1.1 shows the size scale of the different organisms and the corresponding applicable microscope.

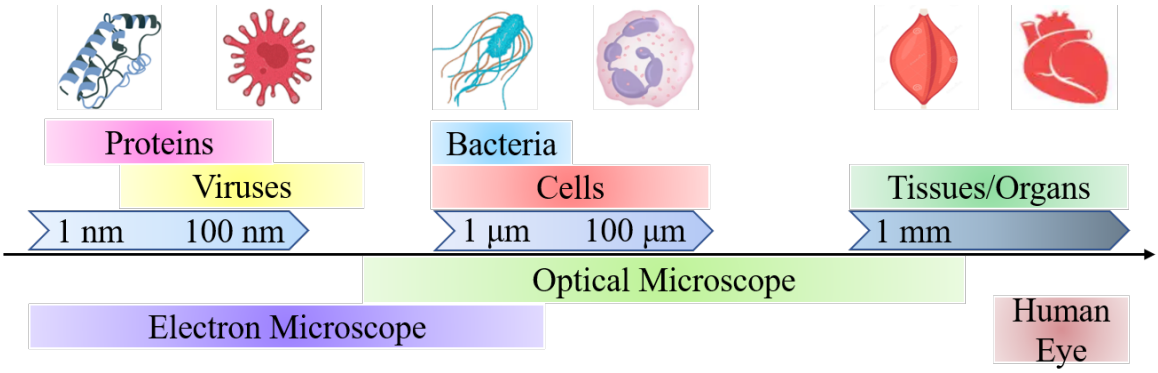

Fig. 1.1 The size scale of the organisms and the corresponding microscope applicable.

Optical microscopes, use visible light to illuminate the specimen and magnify the small samples. It is the most commonly used microscopy type due to the large observation range from nanometers to millimeters. Normally, optical microscopes are focused on studies of cellular-level samples. In the research mentioned in this thesis, we aim to seek computational approaches to analyze cellular-level microscopy images in multi-dimensions, from cells of pollen grain and neutrophil.

The isolated pollen cell is suitable to be imaged with a brightfield imaging technique using a light microscope. Because it uses visible light at wavelengths from approximately 400 to 700 nanometers and pollen can be seen exactly within this wavelength range. Fig. 1.2 (a) shows the light microscope system that was used in capturing our pollen images. For our research project, pollen grains are on a slide and that is scanned. On each slide, a list of XY positions of pollen is given, which is shown in Fig. 1.2 (b); it indicates where a pollen grain is on the slide. Given the positions with an automated stage, at each position a high-resolution image acquisition is realized. In our set-up this is done with the Zeiss software Zen BLUE. For each pollen grain, focusing through different focal levels automatically produces images per focal plane. In this manner, a Z-stack image representing the 3D structure is captured.

In contrast to a static pollen grain, neutrophils move through the zebrafish body. Moreover, neutrophils are fluorescently labeled through a green fluorescent protein (GFP). It needs to

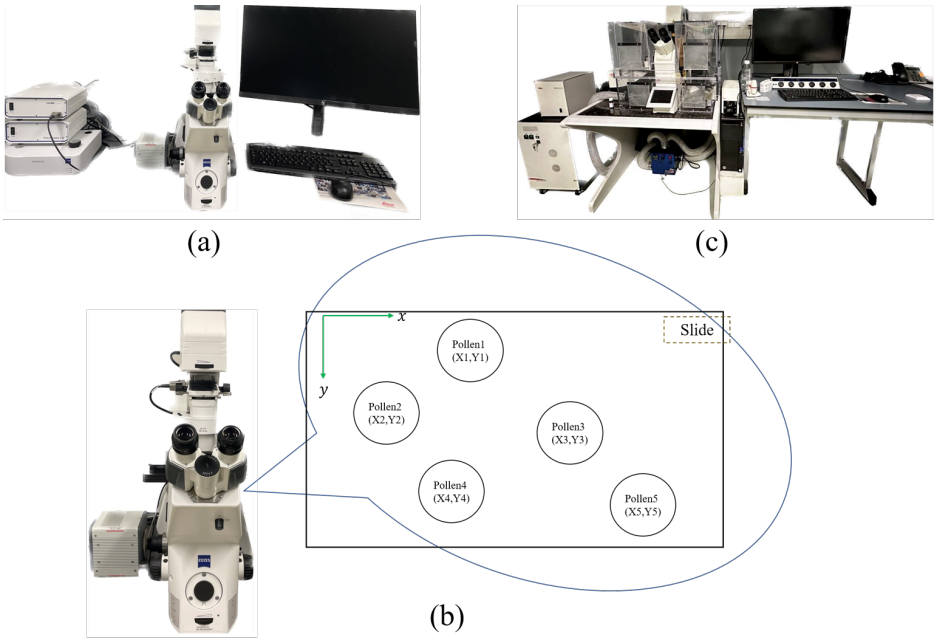

Fig. 1.2 Microscopes. (a) A light microscope system that is used to capture pollen images; (b) An example of the XY positions of each pollen grain is given on a slide with a light microscope; (c) A confocal microscope system that is used to capture neutrophil images.

be excited with blue light and emitted green because of the GFP. For this specific light data, a confocal microscope can utilize specific wavelengths to highlight GFP-labeled neutrophils in zebrafish. Other than a conventional microscope, confocal laser scanning microscopy (CLSM) is scanning a sample with a point source (a laser) that excites the sample locally and then registers the emitted light. The confocal principle realizes a thin optical section and, by moving a focal plane, a stack of optical sections is obtained. Therefore, in this thesis, we utilize a confocal microscope to capture 3D images of neutrophils in the zebrafish over time. The confocal microscope system is shown in Fig. 1.2 (c).

A high resolution/quality image ensures to provide sufficient detail and these selected microscopes fulfill the requirements to make these images. It subsequently helps us look for patterns in these collected image data.

1.2 Data Patterns

The pollen grains investigated in our studies are from a particular plant family named Urticaceae. They are typically small and spherical to ovoid in shape. The outer surface of the pollen grain, called the exine, can vary in texture and ornamentation between different genus/species within the Urticaceae family. This variation, however, is not easy to distinguish

even under a microscope due to its highly similar characteristics. In the research presented in this thesis, we intend to identify pollen patterns from pollen images, including their size, shape, and surface patterns, so that we can categorize each image into different classes accurately.

Neutrophils have irregular deformation and move in the body. To track the trajectory of each moving cell over time, the representative patterns that can distinguish the difference between a tracked cell and candidate cells should be identified, including cell morphological changes, cell migrated direction, cell moving speed, etc. Identifying these patterns of neutrophils from 3D images over time helps to localize the position of each cell and link them to a trajectory.

Based on the patterns of two classes of data, the design of machine learning techniques plays an important role to be able to correctly and automatically analyze, interpret and understand these cell data.

1.3 Microscope Image Analysis with Machine Learning

With respect to understanding the patterns from these cell images, not only qualitative but also quantitative measurements are essential. Just depending on human input to analyze images is not feasible due to large amounts of data, we have to develop techniques that can perform image analysis tasks automatically and very accurately [9]. The study of algorithms and methods to train computers to analyze, interpret, and understand visual data is referred to as computer vision. It aims to simulate part of the complexity of the human visual system and processes visual data in similar fashion to humans. Thanks to the progresses in the field of computer vision, the use of advanced computational methodologies to analyze and extract meaningful information from microscopy image data has been successfully applied [10][11][12][13][14]. In general, the main tasks that can be conducted with those data focus on the sub-domains that include, but are not restricted to, object detection, tracking, reconstruction, motion estimation, modeling, etc [15]. The field of bio-image analysis has been able to take big steps using wide-spread and common machine learning approaches; nowadays especially the innovations in deep learning [16][17].

Machine learning approaches include traditional machine learning techniques and deep learning convolutional neural networks (CNNs), [18] which is illustrated in Fig. 1.3 (a). Traditional machine learning techniques are algorithms that typically rely on handcrafted features and require extensive domain experience and expertise. These algorithms are based on explicit mathematical models so that they are better interpretable. They require a relatively small amounts of data and can perform well with those limited data. As a comparison, deep

learning CNNs consist of numerous interconnected layers with a huge number of parameters and are considered as "black boxes". From this structure, representative features can be directly learned from raw data during the training process, thus reducing the need for explicit feature extraction. However, the "black boxes" are less interpretable, and understanding the exact theoretical process can be challenging. Moreover, in order to be efficient, training deep learning CNNs requires huge amounts of data to achieve satisfactory performance, which has been demonstrated on various tasks [19]. Therefore, the selection of the two approaches often depends on the specific problem, available data, interpretability, and complexity requirements.

In this research, the applicability of machine learning strategies for bioimaging needs to be studied further; especially deep learning as compared to conventional machine learning. The most important applications we recognize are segmentation, basically a pixel classification problem, and classification over features obtained from images. In bio-imaging, images of cells are a dominant field for analysis. Therefore, investigating how machine learning can be integrated in workflows for analysis of these images is important for the understanding of analysis; this holds for both 2D and 3D image analysis.

In our studies we have chosen to work with images of cells that are known to be analyzed with 2D images whereas there is a 3D counterpart that can be addressed as well. Moreover, we might even consider images with a time-dimension for the analysis. As we will explain further, we have selected two classes of cells that can be studied and analyzed in 2D and 3D. These are pollen grains and immune cells.

In short, the methodology research in this thesis focuses on both traditional machine learning techniques and deep learning CNNs, and how CNNs can be efficiently applied to our kind of microscope images. The combination allows for a comprehensive analysis of the acquired cell data. Two applications are designed and implemented, they are the classification of pollen from images and neutrophil tracking (segmentation included) in time-lapse sequences. Fig. 1.3 (b) presents a brief diagram of the themes related to microscope image analysis that are addressed in this thesis. We expect to achieve accurate and reliable results, providing valuable insights into cell patterns, behaviors, and interactions.

1.3.1 Ground Truth Data

Ground truth data is essential for learning patterns using machine learning techniques as shown in Fig. 1.3 (b). It is defined as accurate and reliable information about the target variable or outcome that a model is trying to predict, which is considered to be real or true. It is often used to train, validate, and test a learning-based model, as well as evaluate the performance of a model [20]. In practice, the ground truth data is annotated manually controlled by experts based on their expertise. A convincing ground truth data is based on the

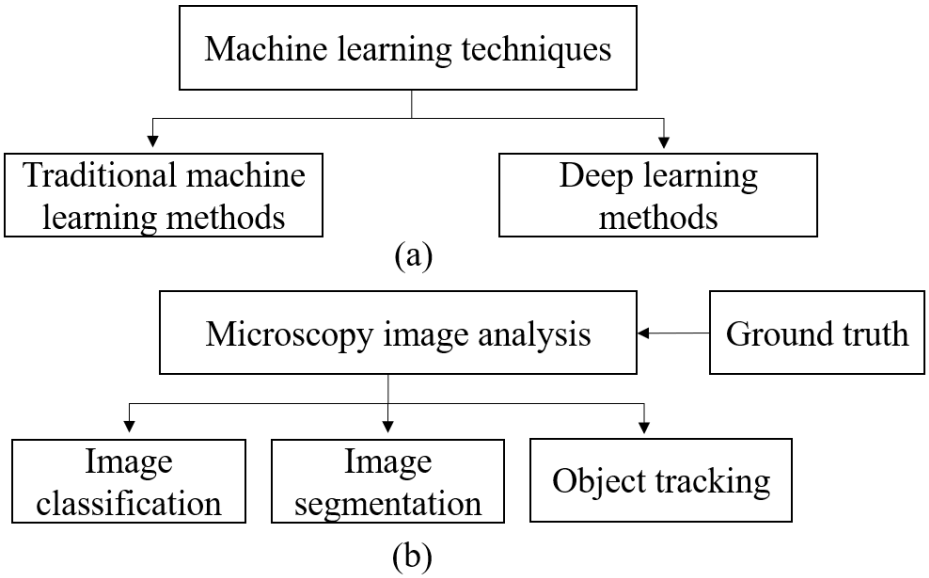

Fig. 1.3 The diagram of (a) components of machine learning techniques and (b) investigated themes in microscopy image analysis in this thesis.

annotation from different experts with several rounds of repetition. The annotation process is a laborious and time-consuming endeavor, especially for complex data. At present, some biomedical research work focuses on developing algorithms from public datasets which are already annotated and thus represent ground truth. These data sets are often employed in competitions [20][21][22]. For instance, the cell tracking challenge [23] which is held under the auspices of the IEEE International Symposium on Biomedical Imaging (ISBI), provides ground truth data for both segmentation and tracking of real cell data as well as synthetic data. It enables the evaluation of each state-of-art based on the same benchmark and allows the researchers to advance their studies conveniently and straightforwardly. However, in many cases, researchers are dedicated to designing a computational algorithm for a dataset with a special condition and experimental design. It requires additional labeling of ground truth data. In fact, the difficulties of annotating the ground truth data differ from various microscopy image analysis tasks. With respect to a classification task, the annotation is to decide which category each image or parts thereof belongs to. It is a relatively easy annotation. While a segmentation annotation is to identify the object(s) of interest and separately outline or mask each object in the image. For a complex study like 2D object tracking, a continuous trajectory of the object through a whole time-lapse sequence is required, and the positional information of each object on the trajectory needs to be recorded. It is particularly hard to conduct annotation in a 3D time-lapse sequence because annotating in the (X, Y, Z, T) axis requires visualization of 3D + Time space first, so as to localize the position of each

3D object through time frames. Due to the necessity of annotating the ground truth data, some annotation tools such as ImageJ/Fiji plugins [24] are developed as auxiliaries to reduce the burden and support labeling image segmentation masks or 2D tracking trajectories. To the best of our knowledge, there is still a lack of open-source tools to label 3D time-lapse data. Facing the potential challenge, we prepare the ground truth data in this thesis, with the assistance of different strategies and tools, to achieve an efficient annotation process. The details are given in each chapter.

1.3.2 Image Classification

Image classification aims to assign a label or category to an image based on its visual features [25]. Feature extraction is one of the essential steps to collect a pool of quantified features from the images, which are used as representations to distinguish between categories. Normally, the most relevant features are selected and the redundant features are removed, through a process of feature selection or dimensional reduction. A classifier is applied to calculate the probability that a certain image belongs to each class based on its representative features and determine the most likely category that an object from the image should be assigned. In the past years, both traditional machine learning methods and innovative deep learning methods have achieved great accomplishments in image classification [26][27]. The traditional machine learning methods treat the classification with feature selection and classifier parts separately. It extracts handcrafted features based on expertise first and chooses a good classifier in order to maximize the classification performance. In contrast, deep learning methods conduct the two parts sequentially and automatically through a structure of neural networks, which saves lots of human effort and often gains superior performance [28].

A special case of classification is addressed in this thesis on our microscopy images of pollen grains to explore the insights of the pollen categories.

1.3.3 Image Segmentation

The goal of image segmentation is to partition the image into multiple components, normally the regions of interest and background, which is referred to as binary classification. Microscopy images contain more fine-grained details and structures that need to be segmented accurately. Besides, the image-capturing process introduces noise to the images. This noise is caused by factors such as illumination, photon, and sensor noise [29]. Noise may influence segmentation accuracy, due to the pixel-wise-based prediction of segmentation [30]. Therefore, advanced image segmentation methods are needed for microscopy images that consider the diverse characteristics of these images. In particular, those cell segmentation methods

take various cell properties into account, such as size, and morphology, in order to recognize cellular processes and mechanisms. In the literature, the proposed approaches mostly fall into the following aspects which include thresholding, edge-based methods, region-based methods, and machine learning-based methods [31][32]. Thresholding requires a threshold value determined manually or automatically to segment foreground and background, while edge-based methods detect the edges or boundaries of cells to segment them. Region-based methods divide the image into regions based on their similarity and use these regions to represent cells. Machine learning-based methods especially deep learning convolutional neural networks are used to learn features from the input image and classify each pixel as belonging to a cell or not. The selection of a segmentation method is important to precisely localize the position of the cell and perform in a robust manner in the presence of noise.

1.3.4 Object Tracking

In conjunction with image segmentation, object tracking is used subsequently to identify and track the movement of objects across frames in a sequence. This typically involves using algorithms to identify features or key points of the object and match them across frames to determine the object's movement [33]. With respect to the biomedical field, cell tracking is the process of monitoring and tracing the movement and behavior of cells over time. Cell tracking uses various techniques such as microscopy, data processing, image analysis, and machine learning, to study the behavioral mechanisms of cells. At present, cell tracking is quite a challenging task due to factors such as morphological deformations and complex motion patterns, depending on the types of cells [34][35]. There is no single general tracking algorithm that can deal with diverse types of cell tracking tasks well. Therefore, designing a pipeline tailored for tracking specific cell types in biomedical research is essential. With the recent development of machine learning techniques, cell tracking tasks are transformed from manual annotation to automatic tracing. It tremendously diminishes the labor effort and achieves satisfactory accuracy. However, there is still room for improvement. Furthermore, for a learning-based method, annotating ground truth data is another challenge as we illustrated beforehand. Thus, in this thesis, we develop original strategies to perform cell tracking for a neutrophil migration study.

1.4 Research Questions and Main Contributions

In section 1.2, we mentioned that two classes of multidimensional microscopy image datasets are acquired: (1) 3D images of individual pollen; (2) 3D images of neutrophil moving

over time. The main purpose of our work is to explore machine learning so that it can help understanding these cell images. To fulfill this, we focus on two applications: the classification of pollen from images and neutrophil tracking in time-lapse sequences.

1.4.1 Classification of Pollen from Images

The pollen investigated in this work is from two genera of the Urticaceae family, named *Parietaria* and *Urtica*. Why Urticaceae was selected as the research type of pollen and what is the challenge of this family? Urticaceae is one of the most common pollen types encountered in the Netherlands. In recent years, pollen seasons are prolonged due to climate changes. Hay fever allergies have strongly affected people's health and daily activities. Furthermore, the two genera of Urticaceae are morphologically highly similar but induce allergy at a very different level, which leads to difficulties in medical treatments. The pollen of *Urtica membranacea* is the only species that can be recognized easily within this family. Therefore, our goal is to find an accurate classification method to identify the pollen within three categories: *Parietaria*, *Urtica*, and *Urtica membranacea*. With the popularity and success of deep learning neural networks in various similar classification applications in recent years, we come to our first Research Question:

RQ 1: Can the existing deep learning-based classification models work with images from morphologically similar pollen grain of related species and what is the performance of the different models?

To answer the **RQ 1**, we have conducted three commonly used deep learning classification models; i.e. VGG16, MobileNet V1, and MobileNet V2 in **Chapter 2**. Deep learning models learn the image features automatically using the structure of convolutional neural networks (CNNs). CNNs acertain sufficient distinguished features to identify each category. In order to guarantee the accuracy of the classification model further, the first contribution from this work is:

C. 1: The data quality is improved by pre-processing the raw 3D pollen images into different 2D projection images.

The three projections are Standard Deviation (STD), Minimum Intensity (MIN), and Extend Focus (EXT). It aims to integrate as many representative pollen images as possible and use them as the input of the classification model, so as to increase the performance of the model. Another innovation is that the pollen grains that we used are unacetolyzed. Compared to acetolyzed pollen grains, in which all pollen materials are destroyed by acetolysis with the exception of sporopollenin that forms the outer pollen wall (a.k.a the exine), unacetolyzed

pollen keeps their original organic features which are less apparent. It leads to the second contribution:

C. 2: This is the first work to apply deep learning classification models and compare them for the analysis of the unacetolyzed Urticaceae pollen grains.

Except for the three mentioned deep learning models, traditional machine learning classification models such as Support Vector Machine (SVM) and Random Forest (RF) have been proven to achieve great results in pollen classification tasks as well. Thus, we come to the second Research Question:

RQ 2: How does the performance of the traditional machine learning-based classification models compare to that of deep learning-based models?

Instead of extracting features automatically like a deep learning model, we design and extract the handcrafted features manually and different classifiers are selected to perform the classification tasks on the pollen images. We also investigate more deep learning models such as AlexNet, VGG19, and ResNet50 as a comparison to extend our research.

In addition, training a deep learning model requires a large amount of data. Even for the traditional machine learning-based methods, the more data collected, the more raw variables that can be used as features, so as to improve the performance of each model. However, in most cases, collecting sufficient data in practice is difficult, especially for bio-medical data. Thus, the third research question is considered:

RQ 3: To what extent is it possible that both the traditional machine learning-based and the deep learning-based classification models perform well on a relatively small amount of data?

We investigate this question by implementing our classification models on two small-scale datasets. We are curious about how much accuracy can be achieved by traditional machine learning-based and deep learning-based methods, respectively.

Investigating **RQ 2** and **RQ 3** in **Chapter 3** has led us to have a thorough understanding of the mechanisms of different classification models, as well as explore the insights of different machine learning methods. It has materialized into our third contribution:

C. 3: A comprehensive and deep exploration of different strategies for pollen classification.

1.4.2 Neutrophil Tracking in Time-lapse Sequences

Contrary to the analysis of individual-cell image research such as pollen grains, neutrophils need to be investigated with a form of multicellular movement in a spatial-temporal domain.

Neutrophils act as the first line of defense against invading pathogens and protect organisms accordingly. In order to learn the migration patterns and analyze the behaviours and functional mechanisms of neutrophils, we formulate the fourth question:

RQ 4: To what extent is it possible to develop an automated algorithm that provide accurate support in the tracking of neutrophils from time-lapse sequences in the 2D spatial domain?

Through the fourth question, in **Chapter 4**, we investigate the whole pipeline to solve a tracking problem that contains three parts: cell segmentation, cell tracking, and trajectory linkage. To that end, we train and analyze several segmentation models that include both rule-based and deep learning-based approaches to localize the position of neutrophils first and attempt to learn the migration behaviours with a U-Net deep-learning model. Subsequently, We compare different linkage methods and propose an improved algorithm that tailors for the specific movement patterns of neutrophils. Correspondingly, we substantiate the fourth contribution:

C. 4: A thorough pipeline for the tracking of neutrophils in 2D time-lapse sequences. It solves the complex problems that occur in the migration of neutrophils.

Except for the analysis of the movement of neutrophils in time-lapse sequences of the 2D spatial domain, we continue to solve the tracking task in the 3D spatial domain which is de facto the real situation. Therefore, we move our focus to the fifth research question in **Chapter 5**:

RQ 5: To what extent is it possible to develop an automated algorithm that provide accurate support in the track of neutrophils from time-lapse sequences in the 3D spatial domain?

Compared with **RQ 4**, elaborating **RQ 5** with a strategy of deep learning-based method is more difficult. This is because a deep learning model requires large amounts of annotations. However, labeling cell trajectory in 3D space along with a time axis is complex and laborious work. Thus, an effective and efficient tracking algorithm is still essential. It gives rise to our last contribution:

C. 5: A feature-weighted tracking method for tracking neutrophils' movement in 3D time-lapse sequences.

1.5 Thesis Structure

The thesis is structured based on the research question presented in the previous paragraphs. A series of publications that have been peer-reviewed and published are listed to support our work in each chapter, respectively.

Chapter 1: "Introduction" a brief introduction on the research background of bioimaging, and microscopy technology is given. Besides, the types of cellular images that would be investigated in this work are being discussed. Moreover, the discussion of different image analysis tasks for microscopy such as image classification, segmentation, and object tracking are presented. Subsequently, the research questions are proposed and the main contributions are highlighted.

Chapter 2: "Neural Networks for Increased Accuracy of Allergenic Pollen Monitoring" introduces the background and motivation for classifying the morphologically similar unacetolyzed pollen of two common genera and a species in the Urticaceae family that have highly differing allergenic properties. A new pollen image dataset is collected and three deep learning neural networks are utilized and compared to distinguish each category of the pollen images. The model with the best performance is taken into account as a case study for the unseen data from aerobiological samples. **Chapter 2** answer the research question **RQ 1** and give the contributions **C. 1**, **C. 2** based on a journal paper:

- M, Polling., **C, Li.**, L, Cao., F, J. Verbeek., L, de Weger., J, Belmonte., C, De Linares., J, Willemse., H, de Boer., B, Gravendeel., Neural Networks for Increased Accuracy of Allergenic Pollen Monitoring. *Scientific Reports*. 2021.

Chapter 3: "Analysis of Automatic Image Classification Methods for Urticaceae Pollen Classification" aims to extend the content of Chapter 2. We investigate three more deep learning-based classification models to compare the performance of differently structured CNNs. The traditional machine learning classification models are adopted and compared subsequently. We intend to evaluate the performance of different approaches and analyze the possible reasons leading to the differences. Finally, we find the most accurate and effective classifier for our pollen images among all models reviewed and probed. Aiming to explore deeper insights into how the generalization and robustness of the different approaches on small-size datasets, we conduct extra experiments with two small-scale datasets and evaluate the performance. **Chapter 3** answers the corresponding research question **RQ 2** and **RQ 3**. The contribution **C. 3** is published in a journal paper:

- **C, Li.**, M, Polling., L, Cao., B, Gravendeel., F, J. Verbeek., Analysis of Automatic Image Classification Methods for Urticaceae Pollen Classification. *Neurocomputing*. Vol. 522, 2023. pp. 181-193.

Both **Chapter 2** and **Chapter 3** focus on the pollen classification task, which is based on two publications. In each publication, evaluation criteria are required to evaluate the performance of each classification model. Therefore, in the two chapters, the same evaluation formulas are presented since they deal with the same classification tasks.

Chapter 4: "An Automated Cell Tracking Pipeline for the Analysis of Neutrophil Dynamics" focuses on finding a pipeline to accurately track the migration of neutrophils forward a tail wound in the zebrafish from 2D time-lapse sequences. We build and train the segmentation models to automatically detect the position of each cell frame by frame. Subsequently, the segmentation performances are compared and evaluated. The model with the best performance will be considered for the first part of this pipeline. In addition, we employ a deep learning-based tracking model to learn the movement pattern from the ground truth tracking trajectories, which is the second part of the pipeline. Lastly, an extended Viterbi linkage method is tailored for linking the trajectories of each cell through the whole time-lapse sequence. Experiments with the proposed pipeline indicate the effectiveness and improvement compared with the other state-of-art. **Chapter 4** give the answer of the corresponding research question **RQ 4**. The contributions **C. 4** is based on the following journal paper:

- **C, Li.**, W, W.C. Yiu., W, Hu., L, Cao., H, P. Spaink., F, J. Verbeek., An Automated Cell Tracking Pipeline for the Analysis of Neutrophil Dynamics. **Prepared for submission**.
- W, Hu., L, van Steijn., **C, Li.**, F, J. Verbeek., L, Cao., R, M.H. Merks., H, P. Spaink., A Novel Function of TLR2 and MyD88 in the Regulation of Leukocyte Cell Migration Behavior During Wounding in Zebrafish Larvae. *Frontiers in Cell and Developmental Biology*. Vol. 9, 2021.

Chapter 5: "A Feature Weighted Tracking Method for 3D Neutrophils in Time-lapse Microscopy" explores the possibility of tracking the cells' migration in 3D time-lapse sequences. We present a rule-based feature-weighted method that extracts cell features manually, instead of an automatic deep learning-based method. This is to overcome the laborious labeling of training data for a deep learning model, which is rather time-consuming. Thus, several tailored features for the migration of neutrophils are designed and extracted, so

as to link the cell trajectories further based on feature similarities. Nevertheless, tracking neutrophils in 3D time-lapse sequences using a deep learning method is still challenging and limited by data annotation. **Chapter 5** answers the final research question **RQ 5**. The contribution **C. 5** is based on the following conference paper:

- **C, Li.**, W, W.C. Yiu., W, Hu., L, Cao., F, J. Verbeek., A Feature Weighted Tracking Method for 3D Neutrophils in Time-lapse Microscopy. *2022 IEEE International Conference on Bioinformatics and Biomedicine (BIBM)*. 2022, pp. 2196-2202.

Both **Chapter 4** and **Chapter 5** deal with the cell tracking task, either in 2D or 3D. Similarly, in each publication, evaluation criteria are required. Thus, the same evaluation criteria are described which leads to part of content duplication.

Chapter 6: "Conclusion" summarizes the contribution of the presented methods for each application and elaborates the importance of our work. The limitations and possible solutions are illustrated in future work.