



PÁZMÁNY

Pázmány Péter Catholic University

Faculty of Information Technology and Bionics

Spatio-temporal event detection in medical image flows

Bendegúz H. Zováthi, Dóra Oláh, Gréta Pataki

Supervisor: CSEREY, György, GARAY Tamás

09 June 2023

Abstract

Cancer is one of the leading causes of death across the world, killing millions of people every year. There are multiple possible treatments including surgery and chemotherapy, however, none of them can guarantee the recovery of the patients. Moreover, while chemotherapy is a widely used treatment method, it has a lot of toxic side effects, so finding drugs that effect only cancer cells is in the focus of many researches. Examining the cellular processes of tumors introduces a powerful tool for cancer research in two ways: to access the grade of cancer that can define the method of treatment and also to measure the effectiveness of the different chemotherapy drugs.

Mitosis is the most important part of the cell cycle for these examinations, since the accelerated growth and division rate of the cells is one of the main characteristics of cancer. Multipolar mitosis is an atypical cell division into three or more daughter cells instead of two, which can lead to genome instability and tumor-specific apoptosis. One of the goals of target-specific chemotherapy is to induce these apoptotic events in cancer cells, so the calculation of the ratio of the atypical and normal mitosis events makes it possible to measure the efficiency of cancer treatments. This can be done manually by checking image sequences of cell cultures during the treatment, however, manual mitosis detection requires a copious amount of human resources.

Our goal was to implement an automatic detection algorithm to speed up the process of chemotherapy treatment evaluation. The provided dataset consisted of a large number of phase-contrast microscope video sequences of cancer cells, containing only non-annotated frames. We used a pretrained segmentation network called Cellpose and implemented a tracking algorithm on the output. The simple tracking algorithm was able to assign a unique ID to the individual cells and follow them across frames. Based on the segmentation and tracking we implemented a mitosis detection algorithm using handcrafted features which can predict mitosis events with a 0.70 F1 score, but at this phase can not differentiate between normal and multipolar mitosis yet. Furthermore, an application was developed that can help researchers examine image sequences of cells and annotate multipolar and normal mitosis events, so we could gather enough annotation of multipolar mitosis sequences to use as training data in the future for fully-automated multipolar mitosis detection.

Acknowledgements

The completion of this study could not have been possible without the expertise of Dr. Tamás Garay (Pázmány Péter Catholic University), who is the scientific supervisor of this research. We are grateful for all his professional support in describing the features of mitosis detection and for his cooperation as a biologist "end user" in our software development. We would also like to thank our three Tutored Research Project and Development supervisors, Dr. György Cserey (Pázmány Péter Catholic University), Pablo Carballeira López (Autonomous University of Madrid) and Jenny Benois-Pineau (University of Bordeaux) for their help during the previous semesters, and Fabien Baldacci whose course of IT management helped in the development of our desktop application.

Contents

Abstract	1
Acknowledgements	3
1 Introduction	6
2 Related Works	9
2.1 Existing methods for mitosis detection	9
2.1.1 Tracking-free methods	9
2.1.2 Tracking-based methods	11
2.1.3 Deep-learning based methods	13
2.1.4 Hybrid methods	14
2.2 Existing methods for cell segmentation	14
2.2.1 Usiigaci	14
2.2.2 Cellpose	15
2.3 Existing methods for cell tracking	17
3 Methods	19
3.1 Dataset	19
3.1.1 Phase contrast microscopy imaging	19
3.1.2 Data analysis	19
3.1.3 Data annotation	20
3.2 Cell segmentation	22
3.3 Cell tracking	23
3.4 Mitosis detection	24
3.5 Desktop application	27
3.6 Metrics	28
3.6.1 Region overlap	28
3.6.2 Recall, Precision, F1 score	28

4	Results	30
4.1	Semi-automated sequence analyser tool	30
4.2	Cell tracking results	33
4.3	Mitosis detection results	35
4.4	Conclusions	37
4.5	Future plans	37
5	Summary	38
	Bibliography	39

Chapter 1

Introduction

Cancer is one of the leading causes of death across the world, after only cardiovascular diseases. In 2020 there were more than 19 million new cancer cases, and while the survival rates are improving, around 10 million people still die of cancer every year [1]. The International Agency for Research on Cancer (IARC [2]) estimates that 1 out of 5 people develops some type of cancer during their life. While sometimes surgery is a possible treatment, it is only effective when the tumors can be localized, and they are in an accessible area. For most cases unfortunately other treatments are needed too, and chemotherapy is a widely used method for destroying cancer cells. However, chemotherapy drugs have a lot of toxic side effects, because they can damage healthy, non-cancer cells as well. For this reason, molecular-based treatments are becoming extremely valuable, to target specific molecules involved in the growth and spread of cancer cells. In this research field, examining the cellular processes of tumors introduces a powerful tool to measure the effectiveness of different treatments and the stage of cancer as well. One of the most important part of the cell cycle to examine in cancer research is mitosis.

Mitosis is a form of cell division, which normally results in two daughter cells with identical genetic information and the same number of chromosomes as the parent cell. The process can be divided into four or five stages: prophase, (prometaphase), metaphase, anaphase, and telophase. During prophase, the nucleus dissolves and the chromosomes begin to migrate to the midline of the cell. The cell also goes through some visually detectable changes at this stage: increases its brightness, shrinks its size, and becomes more globular. In metaphase, the chromosomes line up along the center, and the centrioles are at opposite poles of the cell with the mitotic spindle fibers extending from them, which are attached to each of the sister chromatids. Then in anaphase, the sister chromatids are pulled apart by the mitotic spindle, one chromatid goes to one pole, and the other chromatid to the opposite pole. Finally, during telophase a full set of chromosomes gather together at both poles of the cell, and a membrane forms around them to create two new

nuclei. The single cell pinches in the middle to form two separate daughter cells, but they are still attached together, and a specific form can be observed, which looks like the number “8”. Finally, the two daughter cells are separated from each other during telophase. The visual stages of the mitosis on phase-contrast microscopy can be seen in Figure 1.1.

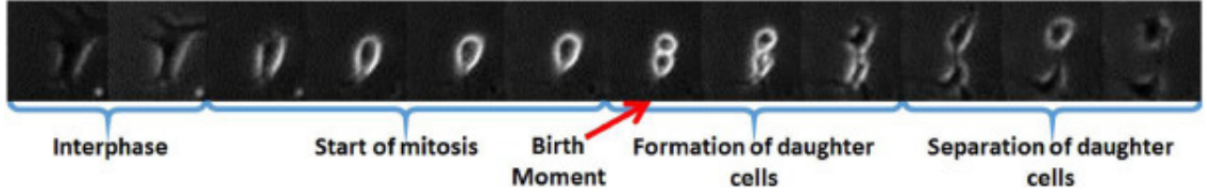


Figure 1.1: Phases of the cell cycle [3]

The number of mitotic events is an important factor in the calculation of the grade for breast cancer in the Nottingham Grading System [4], since accelerated growth and division rate is one of the main characteristics of cancer cells. This grade can describe the aggressiveness of a tumor, which determines the needed treatment methods, for example after surgery it can decide whether or not radiation or chemotherapy has to be used as well. Multipolar mitosis is an atypical cell division, where the chromosomal material is pulled to more than two poles, and the cell is divided into three or more daughter cells (Figure 1.2). This can lead to genome instability and tumor-specific apoptosis. However, this is only one of the possible outcomes: sometimes the daughter cells survive and they can later undergo further mitosis.

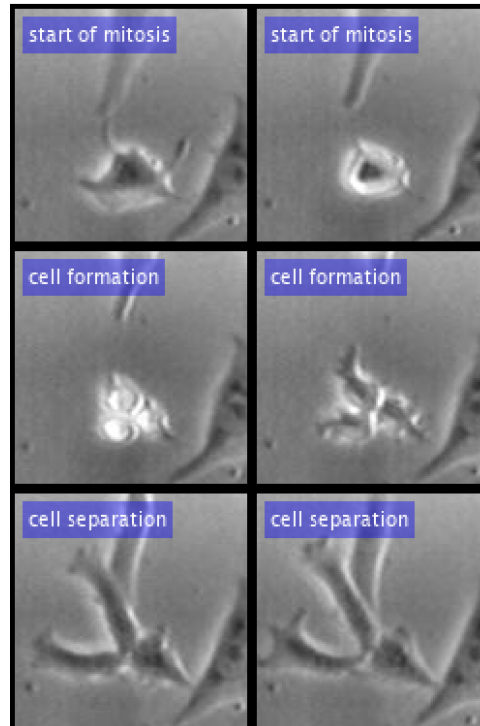


Figure 1.2: Multipolar mitoses on Phase Contrast Microscopy (image sequences from our dataset)

Cancer treatment aims to promote cancer-specific apoptosis, so the effectiveness of the treatment can be estimated by the number of multipolar mitosis events and the fate of the resulting daughter cells. This task can be done manually by experts, who can count the mitosis events by checking image sequences of cell cultures during the treatment. However, this manual mitosis detection requires a lot of time and human resources. Through automating the detection and counting of multipolar mitosis the chemotherapy treatment evaluation can be sped up significantly. To achieve this goal, we planned to implement the automatic detection algorithm in two phases. Since we decided to follow a track-based approach, the first phase includes segmentation of the images and cell tracking. After that, the second phase is a semi-automatic mitosis detection based on visual features and the tracking results, corrected by experts using our desktop application, which will lead to the creation of an annotated database. Finally, by using this annotated data, we would like to try deep-learning based methods to make the automatic detection more reliable. The main steps can be seen on Figure 1.3.

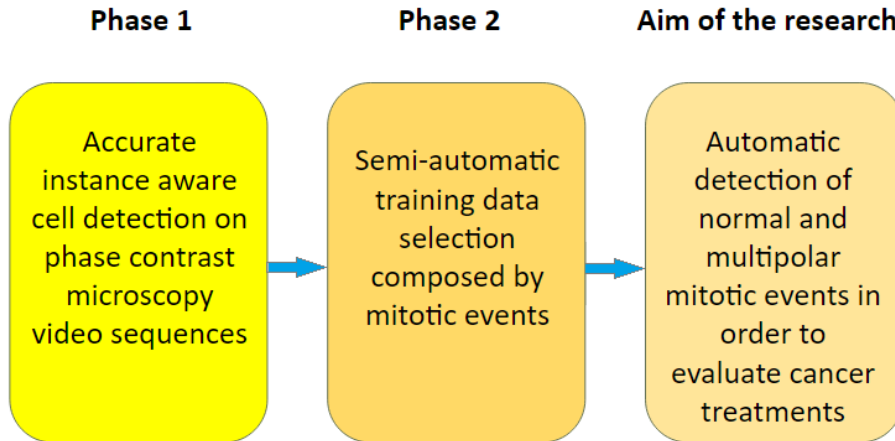


Figure 1.3: The planned phases of our project

In this work we review some previous researches in the field of mitosis detection and their results, then describe the data that we were working on and the methods that we used, present our evaluation of the results and conclude our findings. Finally, we outline some our future plans and ideas.

Chapter 2

Related Works

2.1. Existing methods for mitosis detection

We can divide the possible methods for mitosis detection into four categories: there are tracking-free and tracking-based methods, hybrid methods of the two approaches, and finally deep learning-based methods [5]. Deep learning-based methods have been widely used in many topics of computer vision in recent years. Multiple different types of architectures have been applied to various kinds of biological tasks as well, one of these being cell event detection, and there are also several researches about mitosis detection with the use of neural networks. However, to our knowledge, there is no research in automatic multipolar mitosis detection. In this section we present some of the existing methods and show some examples of previous researches for mitosis detection, since our goal is to detect not only multipolar, but both normal and multipolar mitosis events, we will use some similar methods.

2.1.1 Tracking-free methods

Tracking-free methods are mostly used when long-term tracking of the cells wouldn't be possible, for example in the case of histopathology images. In this scenario, the visual features of the mitotic cells are really important. For example, in 2013 Irshad et al. [6] used morphological and statistical features combined with a decision tree for classification on two Mitosis Detection in Breast Cancer Histological Images (MITOS) datasets. The mitosis events were annotated on the images by two senior pathologists, and the resulting ground truth consisted of 326 mitosis events that were split into training set and testing set, containing 226 and 100 mitosis respectively. The framework of their automatic mitosis detection can be seen on Figure 2.1. After a preprocessing of the input images, the detection of mitosis events was implemented in two main steps: candidate detection stage and candidate classification stage. Since their images were dyed with hematoxylin

and eosin, which results in the extracellular matrix having a pink shade, the cytoplasm is darker pink / purple, while the nucleus is blue, first they transformed the RGB images into so called blue ratio (BR) images [7].

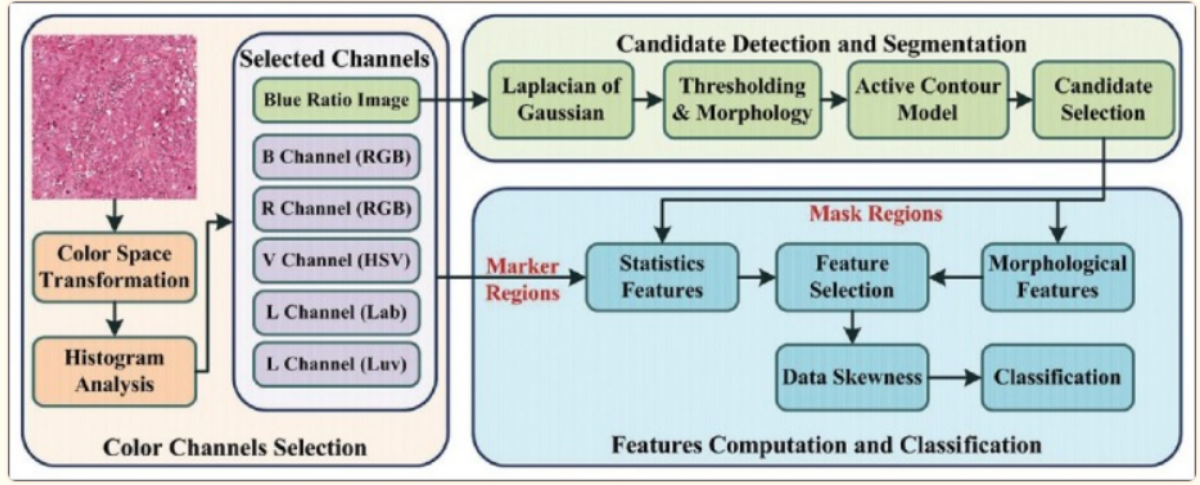


Figure 2.1: The framework of the automatic mitosis detection of Irshad et al. [6]

In the classification stage they used five morphological features that can be computed from the segmentation mask of each candidate:

- area of the cell
- roundness
- elongation
- perimeter
- equivalent spherical parameter

These features are all related to the fact that in the first stage of mitosis, the size of the cell decreases and it takes up a circular shape. The statistical parameters were related to the brightness of the cell, which also changes during the cell cycle, the mitotic form is usually brighter than the other phases. They used the pixel intensity of selected color channels to compute first order statistical features, for example mean, median, variance, kurtosis and skewness.

While the first stage had a detection rate of 88% and 81% for the two datasets, meaning that the detected candidates contained 88 and 81 mitosis events from the 100-100 ground-truth mitosis, they mostly consisted of non-mitotic events (6238 and 5720 candidates). The classification step could successfully eliminate most of the false positive candidates, but when using all the possible features, it also reduced the number of true positive mitosis detections. They

improved this by selecting only the most important features, removing irrelevant and redundant ones, and with this modification their method achieved 74% and 71% detection rate, 70% and 56% precision and 72% and 63% F-Measure on the 2 different datasets (Figure 2.1).

	annotation	detection	TP	FN	FP	precision	recall	F1
1, all features	100	72	52	48	20	0,72	0,52	0,6
1, selected features	100	106	74	26	32	0,7	0,74	0,72
2, all features	100	69	44	56	25	0,64	0,44	0,52
2, selected features	100	127	71	29	56	0,559	0,71	0,63

Table 2.1: Results of the automatic mitosis detection of Irshad et al. [6] for dataset 1 and 2

2.1.2 Tracking-based methods

If we can follow the cells for a longer period, we can get better results with tracking-based methods. Huh et al. [8] implemented a tracking-based automatic mitosis detection method for hematopoietic stem cell populations in 2012. Their algorithm consisted of three steps:

1. cell region detection and tracking
2. candidate detection and feature extraction
3. candidate validation

For cell detection they used a template matching method with ring filters [9], which can be very efficient for hematopoietic stem cells since their appearance is circular and surrounded by bright halos. The cell tracking was based on a frame-by-frame association method, calculating the likelihood that a track is associated with a cell for each possible track and cell with a global nearest neighbor (GNN) scheme that uses an extension of the Munkres algorithm [10].

In the second step they used cell size as an indicator of a mitosis, since daughter cells are always smaller at the beginning than their mother cell. If the tracking found a new cell, they checked if it is in contact with another cell (the other possible daughter cell), and calculated the size of the possible mother and daughter cells. To be a valid candidate, the radius of the mother cell had to be at least 10% bigger than the average radius of the daughter cells.

In the last step they validated the candidates using four Hidden Conditional Random Fields (HCRFs) modelling cell size changes of non-mitotic cells, mother and daughter cells.

The framework of their mitosis detection can be seen on Figure 2.2.

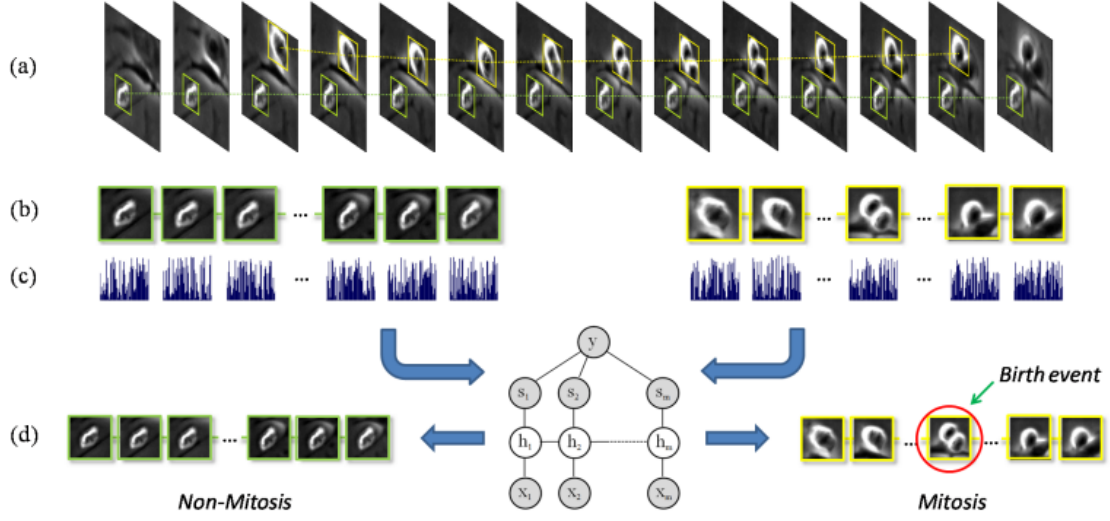


Figure 2.2: Framework of the mitosis detection algorithm by Huh et al. [8]

After step 3 they achieved 97.4% precision, 96.6% recall and 97% F1 score. Their detailed results can be seen in Table 2.2. They compared the found mitosis candidates to the ground truth annotation based on 2 criteria: matching of spatial and temporal position. They used a threshold of 5 pixels and 10 frames respectively, which worked well for their dataset. It can depend on the size of the cells on the images and the speed of the mitosis events on the frames, which can be effected by the cell type, image modality, and frame frequency.

	annotation	detection	TP	FN	FP	precision	recall	F1
After step 2	117	234	114	3	120	0,49	0,97	0,65
After step 3	117	116	113	4	3	0,974	0,966	0,97

Table 2.2: Results of the automatic mitosis detection of Huh et al. [8]

2.1.3 Deep-learning based methods

Among the deep-learning based methods one of the most promising is the unsupervised CNN-LSTM network [11], which groups various spatio-temporal patterns, and clusters various cell events, including mitosis. Some of the possible deep learning methods for object detection and classification are Deep convolutional neural networks (DCNN) [12], which can be improved with hierarchical DCNN [13]. 3D CNNs [14] can extract temporal features from image sequences and thus perform better than handcrafted features. Mao et al. [15], [3] proposed an approach for mitosis detection and stage localization, where as the first step they used a Low-Rank Matrix Recovery model to extract mitosis candidates, and then a Hierarchical Convolutional Neural Network (HCNN) to classify these events based on the temporal and motion features. The framework of their HCNN can be seen on Figure 2.3 and 2.4

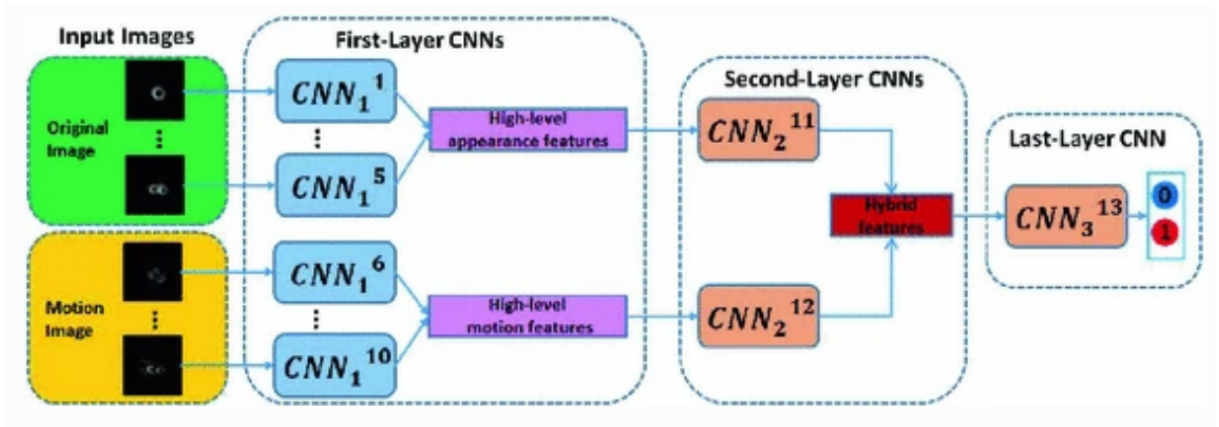
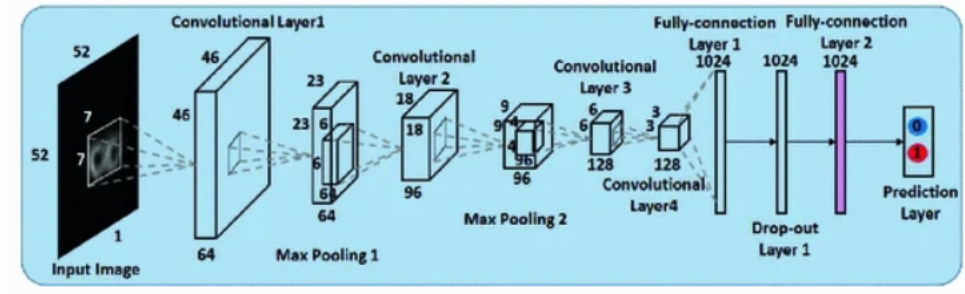
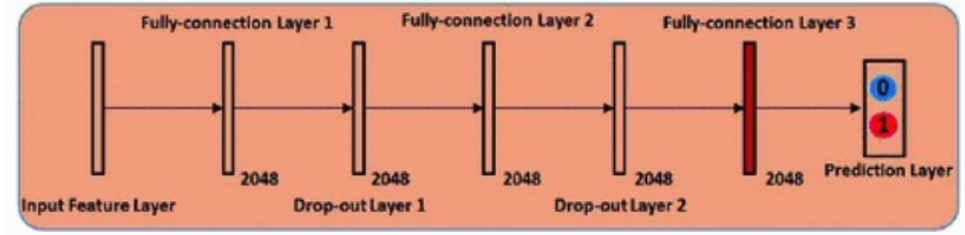


Figure 2.3: Framework of the Hierarchical Convolutional Neural Network (HCNN) used by Mao et al. [15]

They achieved a 99.1% precision and 97.2% recall, which shows the potential advantage of deep-learning based methods compared to the previously described techniques. However, a disadvantage of deep learning methods is that they are computationally expensive, the training requires a lot of time and in most cases it needs annotated training data.



(a)



(b)

Figure 2.4: The architecture of CNNs in the HCNN used by Mao et al. [15] a) first layer b) second and last layer

2.1.4 Hybrid methods

Many researches combine the advantages of the tracking-free and tracking-based methods. Our approach is a hybrid technique as well, using a combination of tracking and visual feature extraction. The goal of the research was to develop an algorithm that is capable of automatic mitosis detection, and can assist the creation of an annotated training database through a desktop application, which in turn can be used for supervised neural network training. The visually detectable features were extracted based on the advice of the biologist supervisor of the team and the temporal information was collected using an improved pretrained segmentation model.

2.2. Existing methods for cell segmentation

Independently from mitosis detection, cell segmentation is a crucial step in image-based cellular research. Since this will be the first step of our system as well, we checked the previous solutions of the field. In this section we present two methods that we have considered.

2.2.1 Usiigaci

Usiigaci is an all-in-one semi-automated pipeline that aims to segment, track, and visualize cell movement and morphological changes. It has a workflow consisting of three modules: a

segmentation module, a tracking module, and a data processing module (Figure 2.5). The approach was introduced in the paper [16] on stain-free phase contrast microscopy (PCM) images. The stain-free, instance-aware segmentation is accomplished using a mask regional convolutional neural network (Mask R-CNN). The results were compared with other techniques: in fluorescence (FL) frames automatic thresholding, in PCM images the PHANTAST [17], Fogbank [18] and Deepcell [19] models. The segmentation similarity achieved by Fogbank and PHANTAST is high, but single-cell tracking with high cell density is not effective, because individual cells cannot be distinguished [16]. The Deepcell model uses a pixel-level classification technique which leads to poor segmentation results if the cells do not have clear boundaries [16]. Although the paper provides accurate results in cell movement and morphological information for quantitative cell migration analysis, the pre-trained model was overfitted to the dataset, which were used in the training and it was not robust to adapt to the slight changes in other sequence characteristics.

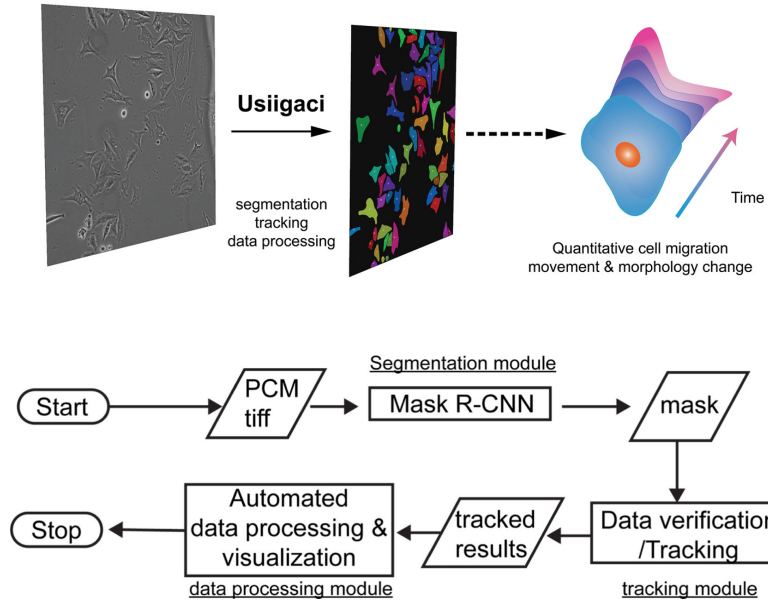


Figure 2.5: Usiigaci workflow [16]

2.2.2 Cellpose

The goal of the research was to develop a generalist, deep-learning-based segmentation algorithm which is able to segment cell images on various types of data without retraining the model or adjusting its parameters. The architecture accomplishes instance segmentation so it not only detects if a pixel belongs to a cell or to the background but identifies each object. Cellpose [20] is a U-Net-based architecture which has two main parts: the upsampling and the downsampling. There are skip connections between layers that have the same size. Additionally, at the lowest resolution, the image styles are computed and linked to all consecutive computations with global

skip connections. A representation of the image style is computed by global average pooling on the smallest layer. Instead of predicting the segmentation masks directly, it estimates a flow-based representation which is based on a heat-diffusion simulation. The network predicts the horizontal and vertical flow of the input. Furthermore, a semantic segmentation mask is also computed which tells if a pixel belongs to a cell or not. These three masks are combined into a spatial flow field. Based on the acquired information of the objects, it can determine which pixels converge to the same fixed point and assign these pixels to the same mask. Figure 2.6 shows how the model works.

The training data was composed of various types of images such as fluorescent cells and brightfield microscopy. It contained images of various types of cells (neurons, muscles, plants) and even some pictures that did not have any cells, but consisted of many repeated objects (fruits, rocks). The presumption was that with these many diverse data the network will be able to generalize better. The training set was composed of 547 images, while the test set had 69. These contained more than 70000 objects. This algorithm outperformed other state-of-the-art segmentation methods like Stardist [21], [22] and Mask-RCNN.

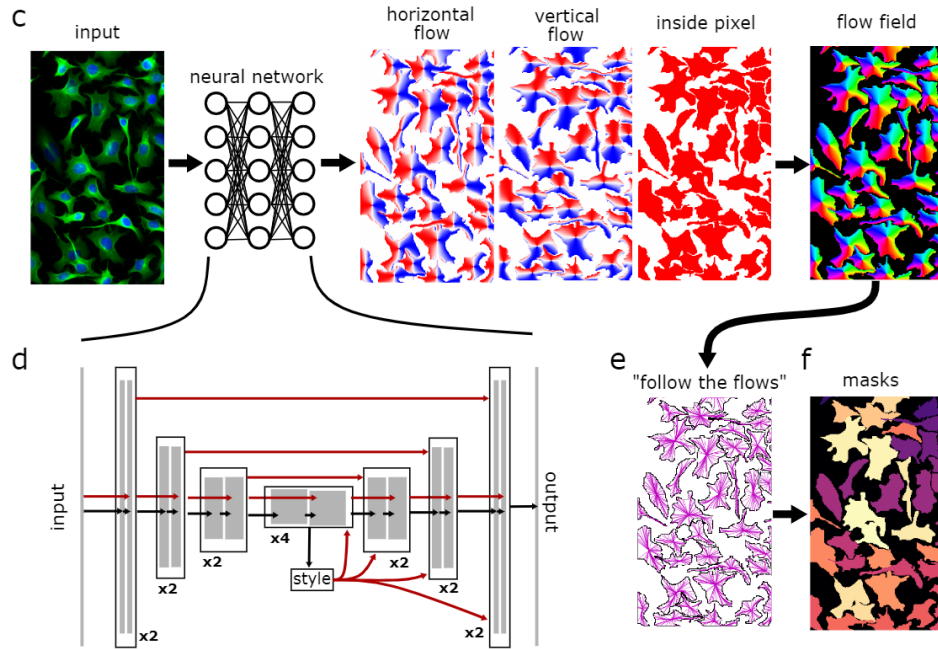


Figure 2.6: Cellpose model [20]. c, The output of the network is a horizontal and vertical flow and semantic segmentation mask, these are combined into the flow field. d, the structure of the neural network, U-net architecture with skip connections. e, Result of the network, from the flow field the masks are calculated based on the convergence of the pixels.

2.3. Existing methods for cell tracking

There are many existing solutions for cell tracking for different purposes, mitosis detection being one of them. For example, in 2008 CellTrack was implemented by Sacan et al. [23], Piccinini et al. [24] introduced CellTracker in 2015, Ghannoum et al. [25] developed CellMAPtracer in 2021. A not complete list can be seen in Table 2.3.

Name	Year	Authors
CellTrack	2008	Sacan et al. [23]
CellProfiler 2.0	2011	Kamentsky et al. [26]
CellTracker	2016	Piccinini et al. [24]
Lineage Mapper	2016	Chalfoun et al. [27]
TrackMate	2017	Tinevez et al. [28]
MMHelper	2019	Smith et al. [29]
Usiigaci	2019	Tsai et al. [16]
LIM Tracker	2022	Aragaki et al. [30]
CellMAPtracer	2021	Ghannoum et al. [25]

Table 2.3: Cell tracking softwares

Lineage Mapper [27] is an open-source overlap-based cell tracking system. It can be used for time-lapse images of biological cells, colonies, and particles. Their tracking algorithmic consists of 5 steps:

1. cost computation between cells from consecutive frames
2. cell collision detection
3. mitosis detection
4. track assignment between cells
5. tracking output creation

The framework of their system can be seen on Figure 2.7.

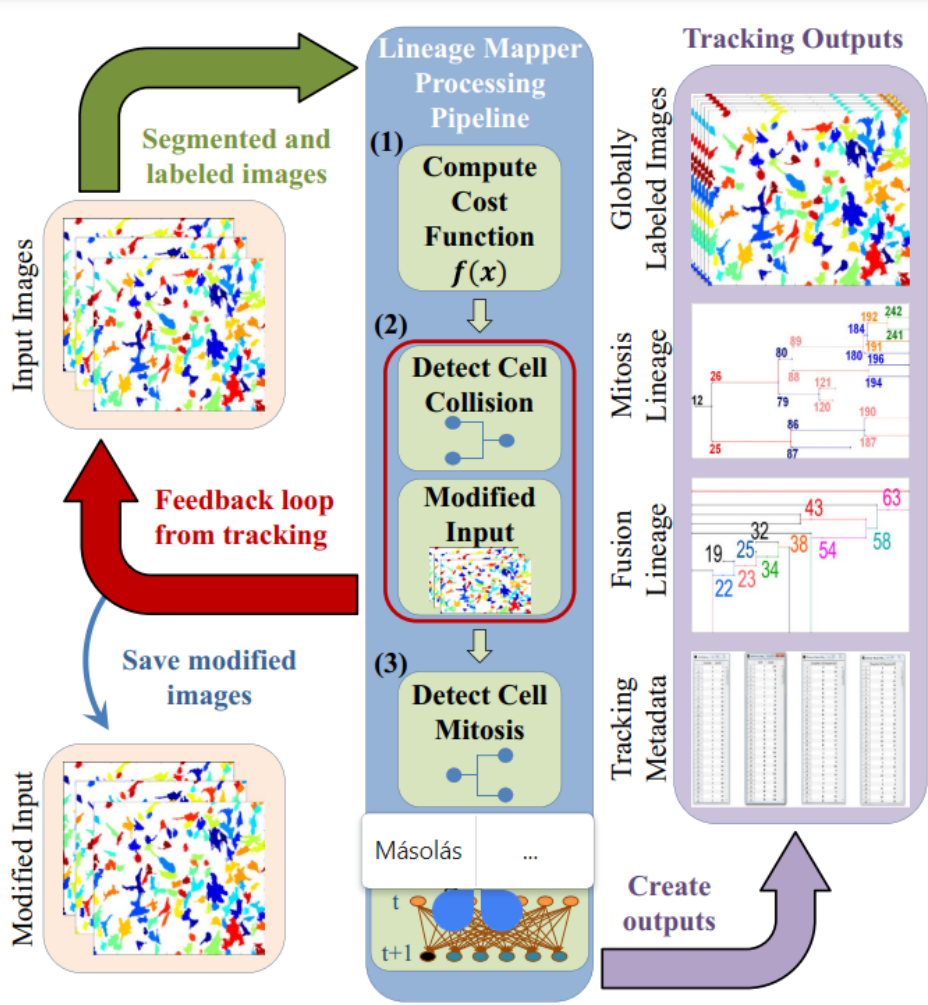


Figure 2.7: Framework of the Lineage Mapper tracking software [27]

Despite of all the previously mentioned softwares, cell tracking still faces some challenges. Gwatimba et al. described some of the possible problems of cell tracking in their publication in 2022 [31]. Since tracking is based on the aforementioned segmentation of the images, which can be difficult in case of high density cell cultures, the accuracy of the segmentation will influence the result of the tracking heavily. Some tracking algorithms are not fully automatic, and require some input from the user, which can be time consuming in the case of a large dataset. Low resolution of the images and low frame-rate can also cause problems. If the time interval between the frames is too long, and it is a dense cell culture, some cells can change their position more than the distance between them and the neighboring cells, which makes the tracking harder and less reliable. In the case of low resolution, the detection of the cells is much more difficult.

Chapter 3

Methods

3.1. Dataset

3.1.1 Phase contrast microscopy imaging

Phase contrast microscopy is a technique that uses transmitted illumination to observe colorless, transparent, and unstained specimens that don't absorb light. The most important advantage is that living cells and tissues can be examined in a natural state, without staining, and it can provide well-contrasted, high-quality images. However, mitosis detection on phase-contrast microscopy images has numerous challenges. The visual appearance of different types of cells can be really varied, and it also changes for the same cell during the phases of the cell cycle as described above (Figure 1.1). In the case of dense cell cultures it can be also difficult to distinguish the cells from each other as well, since they can be very close. For this reason we also have to follow the temporal changes to be able to tell the difference between mitotic events and really close or even overlapping neighboring cells (Figure 3.3).

3.1.2 Data analysis

The dataset was provided by the Pázmány Péter Catholic University (PPCU) and composed of a total of 1367 video sequences. For this research, 580 video sequences were manually selected by a biologist expert from the PPCU, which were blur-free, had proper focus settings, and were captured at the right time for these measurements. The selected video sequences were captured by a phase contrast microscope every 5 or 10 minutes with a duration of multiple (3-6) days. The sequences contain corresponding frames with an average size of $576 \pm 2 \times 768 \pm 4$, so there are only small variations regarding the size of the images. The total number of frames was 1,184,516, and none of them contained any annotation. Figure 3.1 shows multiple different frames of various image sequences of the available data. The red bounding boxes denote multipolar mitosis events.

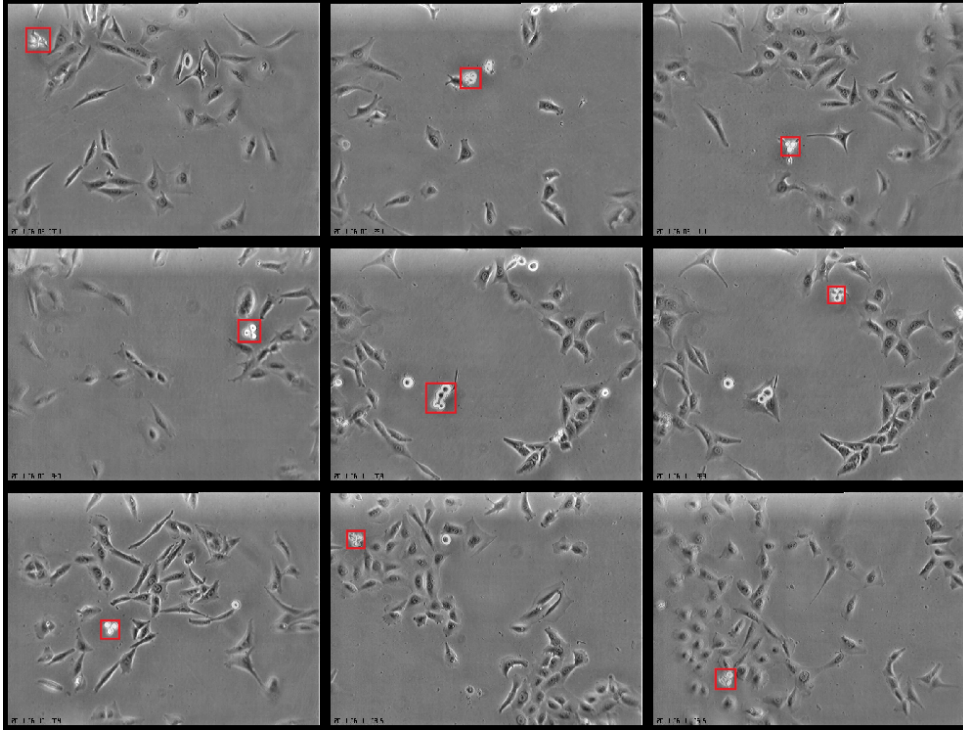


Figure 3.1: Examples of the data, multipolar mitoses highlighted

3.1.3 Data annotation

Since the given dataset did not contain any labels, we needed to annotate some of the image sequences in order to evaluate the performance of our algorithms. First we used an online tool, called makesense.ai [32], and later our own sequence analyser and annotating software, that we will describe in Sections 3.5 and 4.1. The annotation process had two parts:

- **Single cell annotation:** We annotated the cells of 24 sequences to measure the performance of the cell detection and cell estimation algorithms. Considering the long time of the annotation process, we annotated only the 10 starting frames of each sequence, which seemed to be a representative subset for the rest of the sequences. On the other hand, we chose point annotation precisely at the center of the cells, which was completed to bounding boxes with a default box size. The point annotations took approximately 3 minutes per frame. The selected points were stored in *.csv* files containing the annotation ID, the x & y coordinates, the image name, and the width & height of the image. Figure 3.2 shows an annotated frame with different bounding box sizes.
- **Mitosis annotation:** The mitotic events were also marked by our team on ten sequences (957 frames each) using point annotation. It's important to mention that human annotation of mitosis events is not free from errors either. Finding all mitosis events on all frames,

and differentiating between normal and multipolar mitosis is a difficult task even for the experts. To measure this uncertainty, 2 different persons annotated the mitosis events on the sequences, and we compared their annotations as part of the evaluation process.

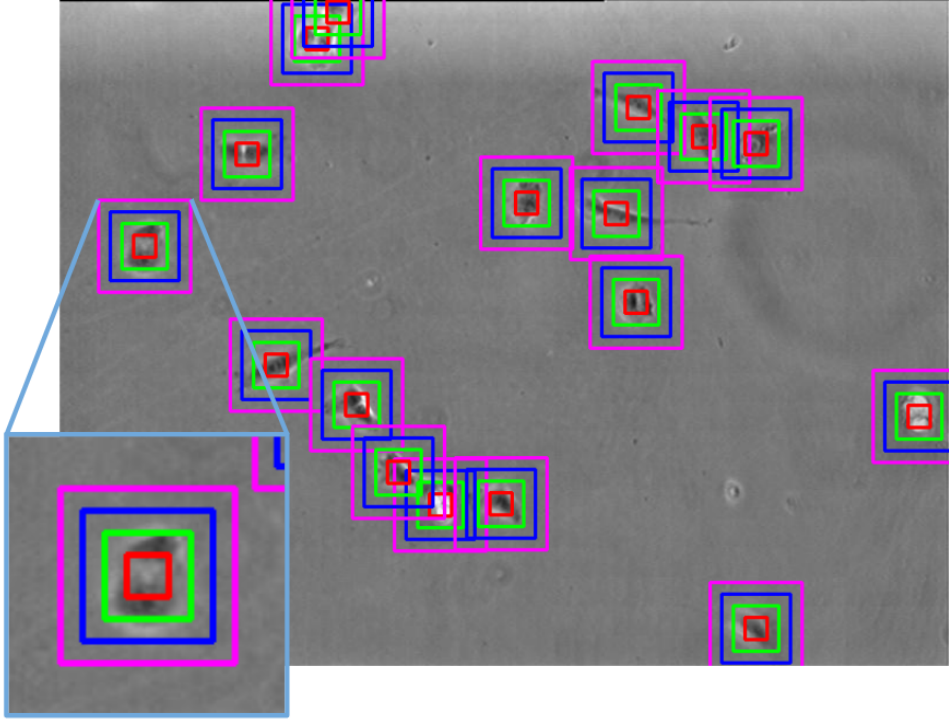
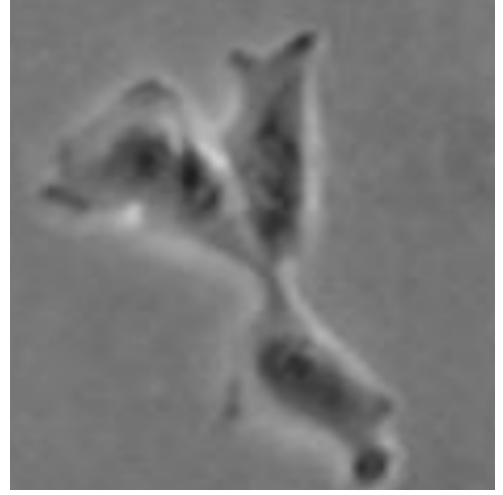


Figure 3.2: Result of point annotation of a frame with different size bounding boxes. Red: 20x20, Green: 40x40, Blue: 60x60, Purple: 80x80 pixels.

Understanding and analyzing the database there are several ways to solve this problem. The naive solution is a detection frame-by-frame, which could lead to inaccurate results. As you can observe in Figure 3.3, in absence of temporal information there is less chance to make accurate decisions about detection. In order to get precise results about the ratio of mitotic events the most important aspect is to analyze the temporal characteristics of the cell events, which will be introduced in our approach in Section 3.4.



(a)



(b)

Figure 3.3: Difference between mitotic event and neighbor cells a) Triple mitosis b) 3 neighbor cells

3.2. Cell segmentation

We tested several pre-trained Usiigaci models for our input data with different configurations, like resolution alignment, contrast limited adaptive histogram equalization, brightness, illumination, and contrast adjustment the results were still inconsiderable. As you can see in Figure 3.4, the model gives accurate results on the dataset which was used in the publication, however, it performed on our data unacceptable segmentation.

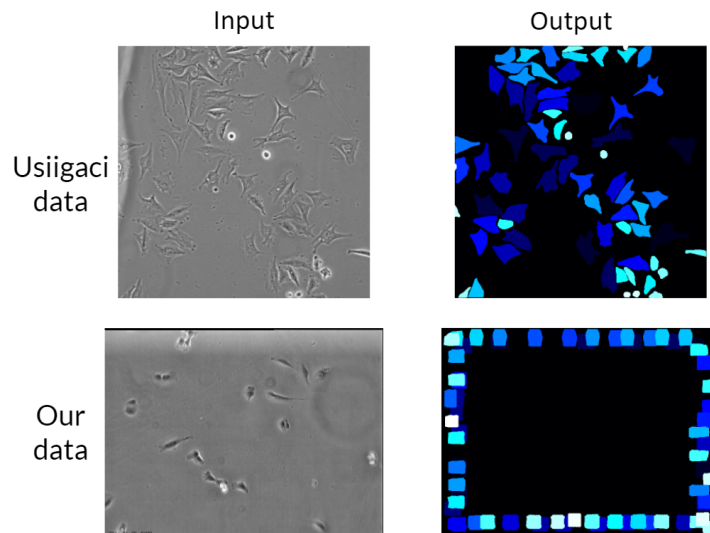


Figure 3.4: The input and output of the Usiigaci network for our data and on the data the network was originally trained.

Then we decided to use the Cellpose network (described in Section 2) instead, that worked well for our data. Figure 3.5 shows the result of the Cellpose network for some images, plotted on the input image.

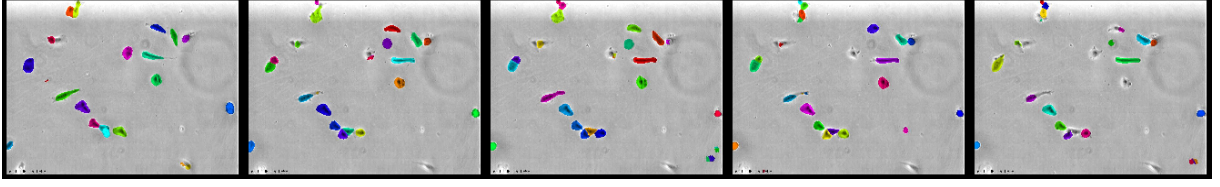


Figure 3.5: Result of Cellpose on 5 successive frames: there is no tracking, only segmentation.

3.3. Cell tracking

We improved the results of the Cellpose segmentation by connecting the segmentation results across frames. The fact that pre-trained parameters are available for the Cellpose network and the main idea behind it is the generalization, that makes this model ideal for our problem. The disadvantage of the method was that it is a segmentation network developed to work on images, not on videos or image sequences. This means that every image input is evaluated individually, and there is no connection between the subsequent frames.

To overcome this problem a fundamental tracking algorithm was implemented on the output of the Cellpose. The segmentation provided a mask for every input with the same size as the original image. Zeros defined the background while the different cells were determined by different numbers. With this information about the objects, a bounding box was assigned for all of the predicted cells.

Cells below a certain size were removed as it is most probably noise. We calculated an adaptive threshold for this so it can be as robust for the various image sequences as possible. To track cells across frames we searched for possible matches on consecutive frames. The cells with the highest IoU (Intersection over Union) value were assigned the same ID. Cells without a match were added later with a new ID. Figure 3.6 shows the result of the tracking algorithm on some frames.

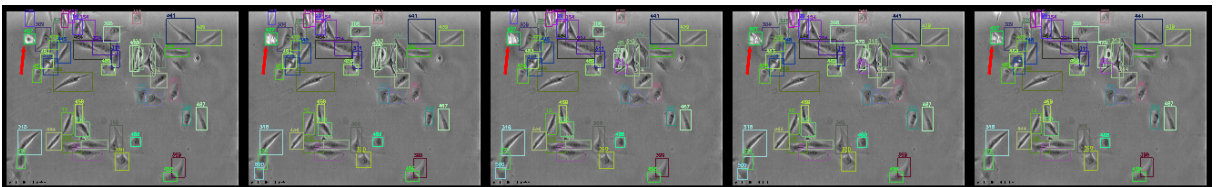


Figure 3.6: Result of Tracking on 5 successive frames. The red arrow points to multipolar mitosis.

If the segmentation could not find a cell in one frame but it could in previous and following images then the tracking also failed and the cell was assigned a new ID. This was not a rare occurrence as the segmentation was not perfect. To overcome this obstacle, as the next step a time parameter was implemented. Hereby it is possible to set that on how many consecutive frames we tolerate missing detections. Also, if a cell was detected for fewer subsequent frames than this time parameter, it was removed as noise. Figure 3.7 shows a comparison of the segmentation and the instance aware detection for a part of three consequent frames.



Figure 3.7: Difference between a) segmentation and b) instance aware tracking with missing detection correction

We can calculate much biologically relevant information from the tracking, such as the number of cells in a sequence, their average migration, the distance they traveled, and the average speed of the cells. These can be used by biologists as a baseline for the comparison of cell cultures. Long-term cell tracking is also a useful and powerful tool for examining a cell culture.

3.4. Mitosis detection

The mitotic activity index is a key prognostic measure in tumor grading [33]. In the case of normal mitosis, the cell divides into two, and in the case of multipolar mitosis events into three or more cells. Calculating the ratio of the mitotic events in the video sequences helps biologists to evaluate cancer treatments. The problem of mitosis detection is classified into 3 levels [34]: Firstly the classification of mitotic images, which aims to mark the frames in a sequence, where the mitotic event occurs. The second level is a spatial and temporal localization of the events in the microscopy images. The third level is the segmentation of the events into temporal stages, which is irrelevant in our study, considering the low resolution of the images, without any inside information about the cells. However, the cell cycle could be analyzed, as the cell grows in the interphase, executing the mitosis by the prophase, metaphase, and anaphase, which terminates with the cell division at the telophase, when the daughter cells form and start to separate. The cell cycle can be seen in Figure 1.1 described in Section 1.

Our approach is focusing on mitosis detection based on handcrafted features. The extrac-

tions were executed along the biological characteristics of the mitotic event. Each instance was examined and filtered out due to the followings properties:

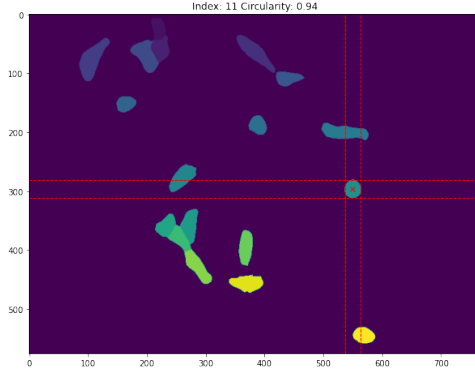
1. Filter out **noise**: The algorithm aims to filter out capturing noise but keeping the cells that are small enough, considering that the size of the cell decreases at the start of mitosis. The parameter which determines the threshold value is the minimum segmentation size in pixels.
2. Filtering out **non-circular cells**: After the cells start the mitosis it becomes more circular. The circularity was measured on the Cellpose segmentation result and filtered out according to the minimum circularity parameter on each instance. The cells which are not rounded enough were removed from the candidates, in order to find the cells with the optimal characteristics. The circularity was calculated as you can see in Equation 3.1:

$$C = \frac{4 \cdot \pi \cdot A}{P^2} \quad (3.1)$$

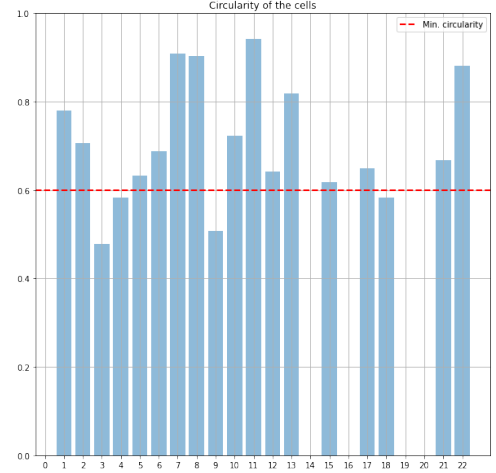
where A is the area, and P is the perimeter of the blob.

3. Filter out **non-bright cells**: As mitosis starts, the brightness of the cells increases. The mean brightness value was calculated for the cells for each frame from the intensity values of the original image covered by a segmentation mask. The minimum brightness parameter filter out the darker cells.
4. **Connecting mitoses** instances: Since the cells are using their mitotic spindles for the division which is also used for migration, hence they can not move at the same time. In order to connect the mitoses detection candidates on the frames, belonging to the same instance, an IoU threshold was set to check the region overlap ratio and a search range to check overlapping in time.
5. Filtering out the **moving cells**: The algorithm examines the same region looking for daughter cells, where the mitosis occurs along a patience parameter (the number of frames after mitosis) and an IoU threshold between the region and the daughter cells.
6. Filtering along the **number of daughter cells**: Since mitosis is a cell division, if the cell is separating into at least 2 daughters, we consider it as mitosis.

Our approach is taking into consideration on one hand the appearance of the cells at the beginning of the mitosis (Properties 1, 2, 3) on the other hand the temporal information came from the tracking results (Properties 5, 6) after the formation of the daughter cells. Since these parameter groups are independent, the best parameters were calculated by a genetic algorithm,



(a) Circularity of Cell₁₁ = 0.94



(b) Circularity histogram for one sample frame

Figure 3.8: Minimum circularity filtering

using population size = 50, mutation probability = 0.1, elit ratio = 0.0, crossover probability = 0.5, parents portion = 0.3, crossover type: uniform. The algorithm stops the training after 14 iterations (due to early stopping), providing the following optimal values: minimum segmentation size = 500 pixels, minimum circularity = 0.6, and minimum brightness value = 0.6. The optimal value for the search range is 80 due to the presence of the mitosis takes usually many frames, where the IoU threshold was set to be higher than 0 allowing only small spatial drifts. You can observe the result of the minimum circularity filtering in Figure 3.8, and an example image (Figure 3.9) about the final filtering output displaying the radius around the center of gravity points of the mitosis candidates.

The optimal parameters for the properties 5, 6 were set after a grid search using the best values of the validation resulting in the following optimal parameters: maximum patience value = 100 frames, IoU threshold > 0.

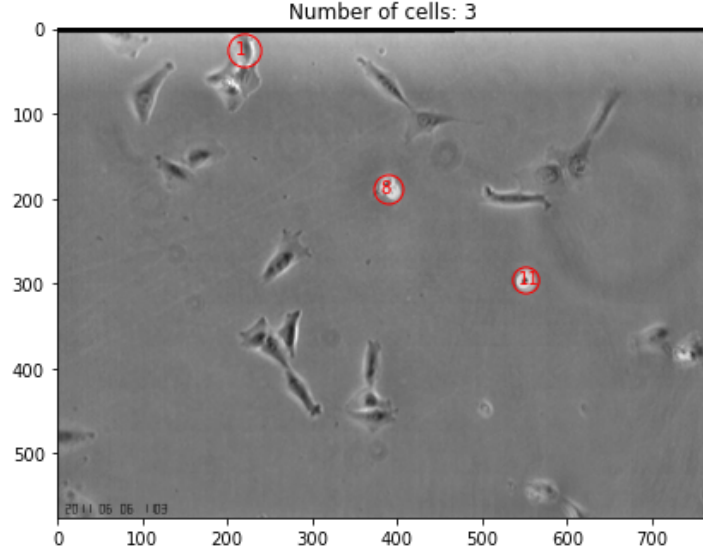
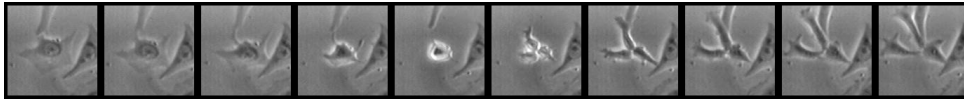


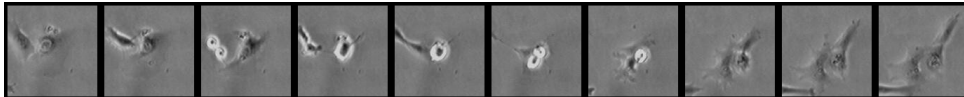
Figure 3.9: Brightness filtering. TP: Cell₈, Cell₁₁, FP: Cell₁

3.5. Desktop application

In order to make the algorithms easy to use and to help the researchers in manual annotation and visualization of the results, or comparing different mitosis lists (between two manual annotation, or an annotation and the mitosis detection algorithm), we created a semi-automated sequence analyser desktop application in Python. For the graphical user interface, we chose PyQt, which is the most popular Python library for using the C++-based, platform-independent Qt framework. Apart from the benefits in diagnosis, the software stores the detection result (corrected by the user if needed) in a frame sequence as well, which is demonstrated in Figure 3.10. This way the application can help to create an annotated database that could be used for applying supervised algorithms on the data. It can also be used to visually check the results of the algorithms.



(a) Multipolar mitosis



(b) Normal mitosis

Figure 3.10: Samples of a 60 frames long sequence from the future training data

3.6. Metrics

3.6.1 Region overlap

The region overlap (or intersection over union) metric was used for multiple tasks such as for implementing the tracking (Section 3.3) and evaluating the accuracy of the segmentation (Section 4.2). The region overlap [35] is often used as a metric in object tracking. It is computed as the fraction of the intersection and the overlap of the estimation and the ground truth (Equation 3.2).

$$\Phi(\Lambda^G, \Lambda^T) = \{\phi_t\}_{t=1}^N, \phi_t = \frac{R_t^G \cap R_t^T}{R_t^G \cup R_t^T} \quad (3.2)$$

The value is between 0 and 1 with the former meaning that there is no overlap and the latter occurring when the two objects are identical (Figure 3.11).

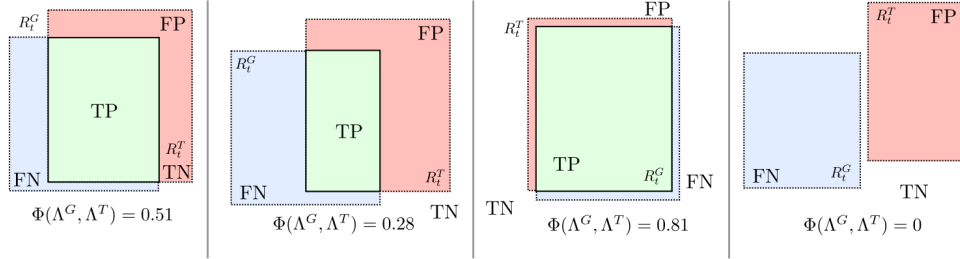


Figure 3.11: Examples of the region overlap for bounding boxes.

3.6.2 Recall, Precision, F1 score

To evaluate the detection algorithm's performance, we used a commonly used metric, the confusion matrix. We calculated the true positive, false positive and false negative mitosis events. The number of true positives means the mitotic candidates that are correctly identified as positive (mitotic). False positives represent the nonmitotic candidates that are incorrectly classified as positive (mitotic) and false negatives show the mitotic candidates that are incorrectly classified as negative (nonmitotic) [34]. As can be seen on Figure 3.12, the confusion matrix has a fourth element, false negative. However in our case, all the background could be considered true negative, which is hard to quantify. For this reason, we didn't calculate the accuracy of the results, only recall, precision and F1-score.

		Does The Effect Exist?	
		Effect Exists	Effect Doesn't Exist
Was The Effect Observed?	Effect Observed	Hit True Positive	False Alarm False Positive Type I Error
	Effect Not Observed	Miss False Negative Type II Error	Correct Rejection True Negative

Figure 3.12: Confusion matrix [36]

Recall [37] measures how well we can find all the ground truth elements (Equation 3.3).

$$R = \frac{TP}{TP + FN} \quad (3.3)$$

Precision [37] measures how accurate are the detections (Equation 3.4), as:

$$P = \frac{TP}{TP + FP} \quad (3.4)$$

F1 score [37] is defined as the harmonic mean between precision and recall (Equation 3.5), as:

$$F1 = 2 \cdot \frac{\text{precision} \cdot \text{recall}}{\text{precision} + \text{recall}} \quad (3.5)$$

Chapter 4

Results

4.1. Semi-automated sequence analyser tool

The main window is divided into three parts, as can be seen in Figure 4.1. The center region contains the images as a video sequence with the corresponding control buttons after selecting the folder to be analyzed. The left side is about displaying some information about the images and the analysis, and also has some display settings that can be changed dynamically during run-time. Finally the right side shows the history of the found mitosis events, where they can be accepted as either normal or multipolar mitosis, or be declined, and at the bottom of the panel has a button to generate the report after the analysis is done.

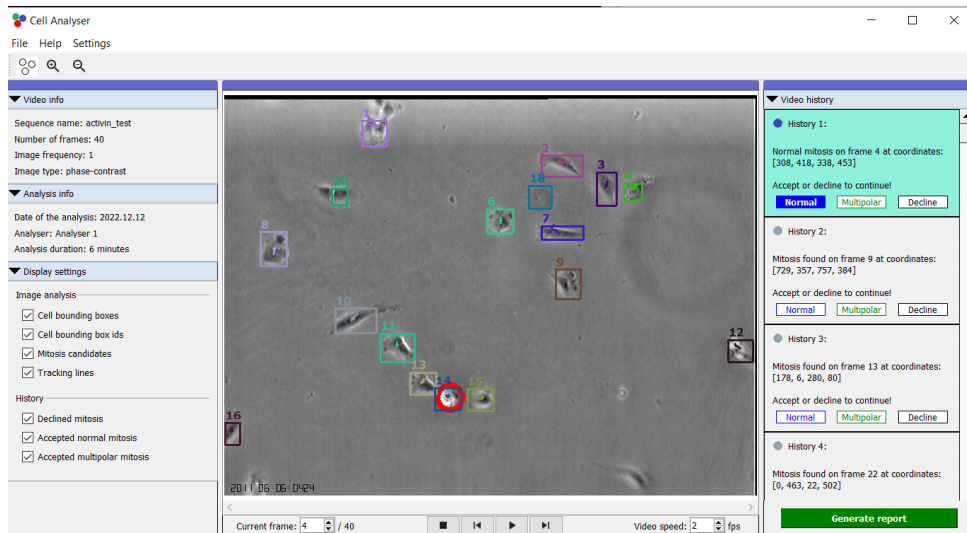


Figure 4.1: Semi-automated sequence analyser tool implemented in PyQt

We can load the image sequence to the application with the File menu / Open action. In the resulting file dialog (Figure 4.2) we can set some parameters like the image frequency, image type, and the cell size threshold for the segmentation. This can also be set to use an automatic

threshold. When the folder is selected and the parameters are set, the application will load the first image to the center region, fill in the information in the left panel, and start the segmentation, tracking and mitosis detection algorithm in the background. After the algorithm is done with the calculations, the mitosis history will be loaded to the right panel.

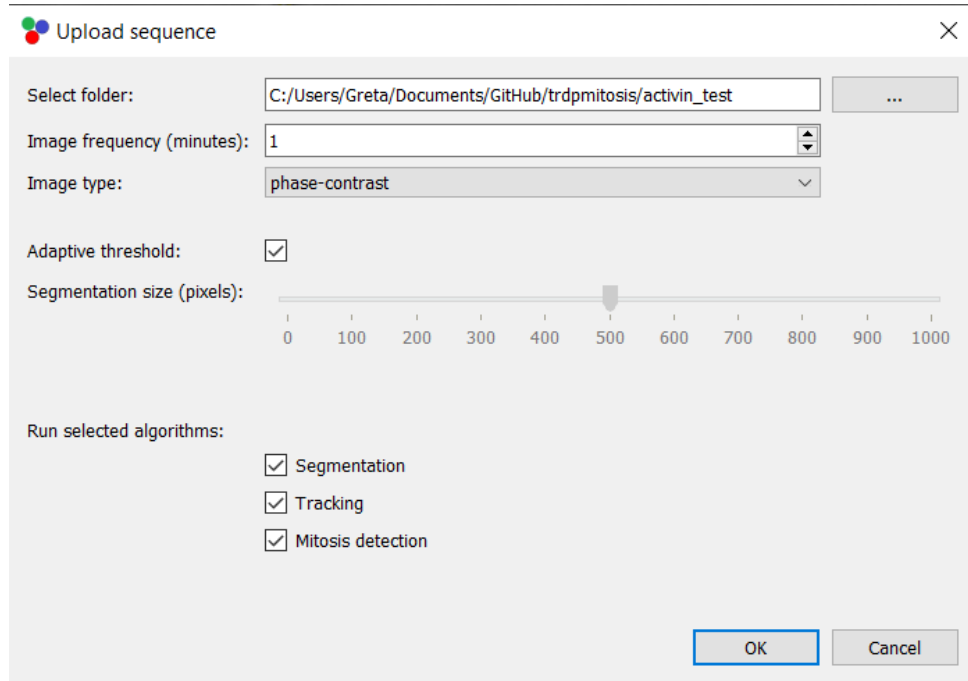


Figure 4.2: Selection of the video sequence and parameter settings

The user can categorize the mitosis events, and add new ones in case they were missed by the algorithm, using the first button of the toolbar. It's also possible to zoom in and out of the images, or change whether the bounding boxes and ids of the cell segmentation, the mitosis candidates, or the tracking lines of the cells are shown on the images or not, making it easier to concentrate on the most important information. When checking the results of the mitosis detection, sometimes it is useful to be able to visually verify the position of the mitosis on the image, this can be helped by showing a grid on the image. We show the mitosis events by circles to visually separate them from the bounding boxes if both are shown at the same time. At first we highlighted the mitosis events only on the frame where based on our calculations the mitosis is the most detectable. However, mitosis is a longer process, often lasting for at least 10-20 frames from the formation of the bright, round cell form to the appearance of the resulting daughter cells. To help the user follow the process more, a frame window can be set around the detected frames where a bigger circle shows the area of interest. In case of comparing two lists of mitosis events, for example the manual annotation (ground truth) and the algorithm results, the two sets can be visualized with a different color as shown on Figure 4.3.

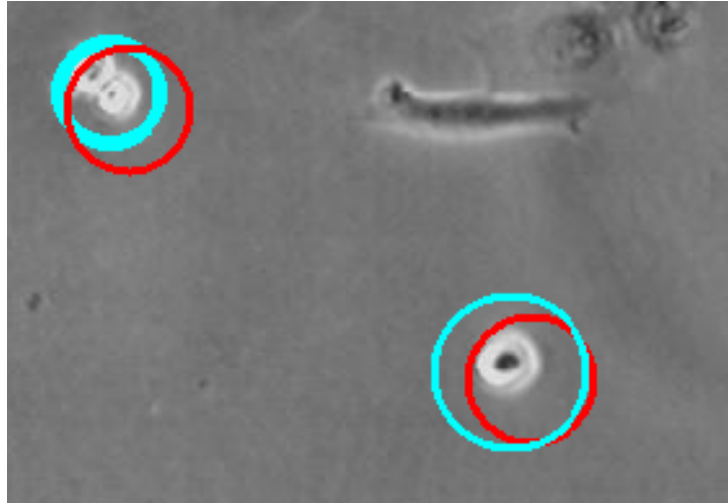


Figure 4.3: Comparing manual annotation (blue) with a mitosis detection result (red) in the analyser tool

The display settings of the mitosis events, and also the grid can be changed in the settings (Figure 4.4). We are planning to implement more changeable options based on the user feedback later.

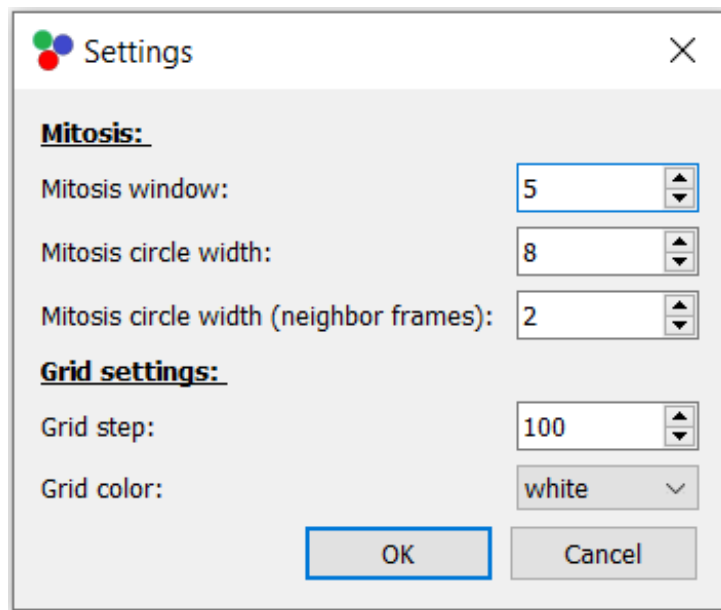


Figure 4.4: Display settings of the sequence analyser tool

In addition to several csv files saved during the segmentation, tracking and detection that can be analyzed separately, a Pdf report can be generated as well. The generated report includes all information about the image sequence, either given by the user at the opening of the sequence (like the image type) or calculated by the program (for example the number of frames, or the duration of the analysis). It also contains the main results of the segmentation and the tracking:

the number of cells in the sequence, the average cell speed, and the average migration of the cells. After that, it describes the mitotic events by the number of normal, multipolar and declined mitosis, and finally includes the mitosis history with the frame index and coordinates of the mitosis events.

The software can also calculate the precision, recall and F1-score of a mitosis detection result (or two annotations) with a built-in mitosis comparison tool. To match the mitosis events between the two lists, we used the same method as the work of Huh et al. [8], described in Section 2.1. A detected mitosis event is matched with a ground-truth mitosis if they are close enough both spatially and in time. The user can set the pixel and frame thresholds in the application (Figure 4.5).

Mitosis comparison results	
Annotated:	64
Automatic:	79
True positive:	52
False negative:	12
False positive:	27
Precision:	0.6582278481012658
Recall:	0.8125
F1 score:	0.7272727272727273

Figure 4.5: Mitosis comparison tool

4.2. Cell tracking results

Our research goal for the first phase was to achieve instance-aware cell tracking results. There are multiple techniques to solve this computer vision problem as it was introduced with PHANTAST [17], Fogbank [18] and Deepcell [19] in Section 2, but these methods all use annotated data. The best performance results were provided by the Usiigaci [16] paper, which was the only available pre-trained model for our research. As we tried this model after several preprocessing methods, our conclusion was that the model is overfitted to the training dataset, and it was not robust to our data. Our approach is based on the Cellpose [20] segmentation pre-trained model improved by tracking (Section 3.3) to reach instance-aware cell segmentation. We used this method for

evaluation with the following parameters:

- IoU threshold to matching cells between frames: 0.2
- IoU threshold to avoid multiple detections: 0.7
- time patience for tracking: 3 frames
- minimum area of bounding box: adaptive method

The results were evaluated on a subset of 24 sequences annotated by our team with point annotation as it was described in Section 3.1.2 and we used the detailed metrics in Section 3.6. We evaluated the sequences with 40x40 bounding box size around the ground truth point annotation, and minimum 0.2 IoU for matching the ground truth with the detection results. With a lower threshold, the results were better but a significant overlap is necessary. The results were calculated for each frame in the sequences and gives averaged results for the sequences for recall, precision, and F1 score (displayed in Figure 4.6). We calculated these metrics for the tracking and the detection, so for the bounding boxes around the segmentation results without connecting them in time.

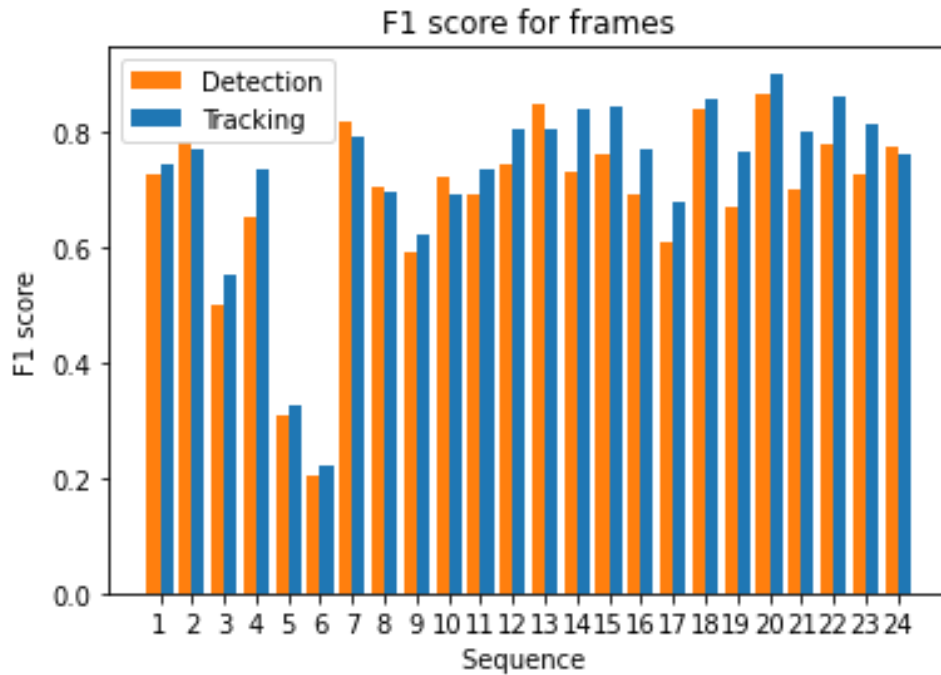


Figure 4.6: Tracking and detection F1 score results

The results can be found in Table 4.1. As the numeric results show the tracking shows a big improvement compared to the detection for all three of the analyzed metrics. With the tracking algorithm, we could successfully connect the cells in time, and follow them for multiple frames.

It solves multiple shortcomings of the detection, such as if a cell was not detected on a frame, but it was detected on the previous and the later ones, it will be added on the middle frames. Furthermore, as it was mentioned short detections were deleted. Another filtering was done by removing cells below a certain size in an adaptive manner, so the threshold for noise filtering depends on the sequence.

Detection features	<i>Detection</i>	<i>Tracking</i>
Precision	0.6089	0.7387
Recall	0.7066	0.7198
F1 score	0.6502	0.7247

Table 4.1: Tracking and Detection evaluation

4.3. Mitosis detection results

The results of the automatic mitosis detection are shown in Table 4.2. For the mitosis event matching described earlier, we have set a threshold of 30 pixel, 10 backward and 50 forward frames. The reason for the rather high forward frame threshold is that we have seen that sometimes mitosis could last around 40-50 frames, and our algorithm detects the start of the mitosis, while the annotation usually marks the "8" form. Regarding the pixel threshold most of the mitosis events were also found even with lower threshold, however, after manually checking some false positives we noticed that the detection was correct, but the positioning of either the annotation or the detection was not precise enough for the matching with lower threshold. We are planning to improve that and evaluate our results again with lower thresholds. With these parameters we found that our algorithm have an average recall of 0.75, which means that it can correctly detect 75% of the ground-truth mitosis events. False negative events can be an effect of the mitosis happening too close to the border of the image, when one of the daughter cells can even leave the field of view in the subsequent frames, or when the mitosis process is faster than usual, and the cell doesn't take up the real circular form completely. The precision was lower since there were some false positive events. We were able to filter out some of them by adding more conditions to the mitosis detection, for example we removed too long mitosis events (more than 450 frames), because these are not biologically probable. Most of them were caused by illumination differences, the top region of the frames is brighter than the rest, and we have seen a lot of false detections in that area. The false positives that we were not able to remove were either caused by background noises, or they were selected correctly based on our definition

of mitosis (the round, bright cell phase), but in some cases this cell form was not followed by cell division, the cell either died, changed back to its moving form, or the sequence ended before the mitosis might have happened. As we mentioned before, in manual annotation the labeling happens on the frame where the cell has already divided and reached the "8" shaped form, and the annotating expert can say that the mitosis has clearly happened. Due to this the automatic detection usually labels the same mitosis events on earlier frames. In most cases, this means only a couple of frames, since cell division usually lasts for about an hour (12 frames), and our matching algorithm can handle this, so we still consider the mitosis candidate as true positive. However in some cases of slow mitosis, the 2 phases can happen from 40-50 frames from each other (3-4 hours), which can lead to false positives and false negatives as well. We would like to emphasize that although our algorithm has to be improved to give better results and be able to support fully automated mitosis detection, it could find some mitosis events missed by the annotators (and validated later as real mitosis events when checking the false positives), who were also not be able to annotate the sequences with the same results compared to each other, which means that the automatic detection can still be a very helpful tool in assisting manual annotation.

	annotation	program	TP	FN	FP	precision	recall	F1
aktivin 13	64	79	52	12	27	0.66	0.81	0.73
aktivin 14	64	70	51	13	19	0.73	0.8	0.76
aktivin 15	79	91	56	23	35	0.62	0.71	0.66
aktivin 16	72	76	55	17	21	0.82	0.58	0.68
aktivin 17	74	83	49	25	34	0.59	0.66	0.62
aktivin 18	62	64	45	17	19	0.7	0.73	0.71
aktivin 19	72	79	53	19	26	0.67	0.74	0.70
aktivin 20	84	102	65	19	37	0.64	0.77	0.70
aktivin 21	70	95	60	10	35	0.63	0.86	0.73
aktivin 22	79	86	54	25	32	0.63	0.68	0.65
Average						0.66	0.75	0.70

Table 4.2: Evaluation results of the mitosis detection for 10 different image sequences and their average

4.4. Conclusions

We would like to dedicate this section to emphasize the limitations and difficulties of this research, but also highlight the achievements. We faced the biggest obstacle at the very beginning of this project, because we did not have annotated data. Although the amount and the quality of the images were sufficient, annotating mitotic events without an efficient tool that can help in the process would take a lot of time, so our team could not annotate enough data for a sufficient training set, only for the evaluation of the developed algorithms. During the research, we tried to select the most promising methods: since the only pre-trained model we found (Usiigaci phase-contrast microscopic cell tracking model) was overfitted to the training data, we chose a semantic segmentation (Cellpose) and improved it with a tracking implementation.

Despite our team has the expertise to annotate cells on the image sequences, as the comparison between two annotators showed we do not have the ability to perfectly annotate every normal and multipolar mitotic event, so we can expect some uncertainty even for the ground-truth.

The current semi-automated method has some false positive and false negative mitotic events, however, it localizes accurately in space and time around 75% of the ground-truth mitosis candidates using tracking information and visual features. Improving the segmentation and tracking results could aid the semi-automated application by providing confident suggestions for the type of mitotic events as well. The developed application proved to be a useful tool in annotating sequences and evaluating detection results both qualitatively and quantitatively.

4.5. Future plans

This research aims to detect atypical cell events in order to measure the effectiveness of different chemotherapy treatments. Detecting multipolar mitoses manually is a hard and time-consuming task, not even mentioning the possibility, that the manual analysis of these sequences leads to less accurate results due to human mistakes and the interpolation or estimation from a subset to the whole sequence. The long-term goal of this research is to create a tool, which takes over the monotonous analysis processes from the biologists and provides an evaluation of the given treatments. As discussed in Section 4.4 using the presented algorithms a unique training dataset can be created for mitosis detection, composed of cell events across frames, which could be used for training a 3D CNN model proposed by our supervisor. This model would be designed for detecting cell events, such as multipolar mitosis and normal mitosis in phase-contrast microscopy image sequences, speeding up the chemotherapy treatments.

Chapter 5

Summary

Since cancer is one of the leading causes of death across the world, chemotherapy treatments are in the focus of many researches. The stage of the cancer and the effectiveness of the treatment can both be evaluated by examining the cellular processes of tumors, especially normal and multipolar mitosis events. Mitosis is the most important part of the cell cycle for these evaluations, since cancer cells have accelerated growth and division rate. Multipolar mitosis is an atypical form of cell division when the mother cell is divided into three or more daughter cells instead of two, which can lead to genome instability and tumor-specific apoptosis. One of the main goals of target-specific chemotherapy is to induce these apoptotic events in cancer cells, which made the calculation of the ratio of the atypical and normal mitosis events a powerful tool to measure the efficiency of cancer treatments. This calculation can be done manually by checking image sequences of cell cultures before, during, and after the treatment, however, manual mitosis detection requires a lot of time.

In the first phase of our work, we detected the cells on the provided image sequences with the help of a segmentation algorithm, and then implemented a tracking algorithm to track the spatial and temporal movement and changes of the cells. In the second phase, we developed a desktop application in Python using PyQt, which takes the selected series of images and performs the segmentation and tracking algorithms on them, and also contains a third algorithm suitable for mitosis detection. Its automatic detection can be corrected with manual annotations, for which the program offers an easy-to-use interface for the users. Our future plan is to create a training database that can be suitable in the third phase of our research to be used for training neural networks and improve the results of our automatic mitosis detection.

Bibliography

- [1] mhg_{cancer}, *Global Cancer Facts and Figures 2021*, <https://miskawaanhealth.com/cancer/global-cancer-statistics/>, [Online; accessed 2023-06-07], Jul. 2021.
- [2] *Global Cancer Observatory*, <https://gco.iarc.fr/>, [Online; accessed 2023-06-07].
- [3] Y. Mao, L. Han, and Z. Yin, “Cell mitosis event analysis in phase contrast microscopy images using deep learning,” *Medical Image Analysis*, vol. 57, pp. 32–43, 2019, ISSN: 1361-8415. DOI: <https://doi.org/10.1016/j.media.2019.06.011>. [Online]. Available: <https://www.sciencedirect.com/science/article/pii/S1361841518304730>.
- [4] H. Takahashi, M. Oshi, M. Asaoka, L. Yan, I. Endo, and K. Takabe, “Molecular Biological Features of Nottingham Histological Grade 3 Breast Cancers,” *Annals of Surgical Oncology*, vol. 27, no. 11, pp. 4475–4485, May 2020, [Online; accessed 2023-06-09].
- [5] Y.-T. Su, Y. Lu, J. Liu, M. Chen, and A.-A. Liu, “Spatio-temporal mitosis detection in time-lapse phase-contrast microscopy image sequences: A benchmark,” *IEEE Transactions on Medical Imaging*, vol. 40, no. 5, pp. 1319–1328, 2021. DOI: [10.1109/TMI.2021.3052854](https://doi.org/10.1109/TMI.2021.3052854).
- [6] H. Irshad, “Automated mitosis detection in histopathology using morphological and multi-channel statistics features,” *Journal of Pathology Informatics*, vol. 4, no. 1, p. 10, Jan. 2013, [Online; accessed 2023-06-09].
- [7] H. Chang, L. A. Loss, and B. Parvin, “Nuclear segmentation in h&e sections via multi-reference graph cut (mrgc),” in *International symposium biomedical imaging*, 2012.
- [8] S. Huh, S. Eom, D. F. E. Ker, L. Weiss, and T. Kanade, “Mitosis detection of hematopoietic stem cell populations in time-lapse phase-contrast microscopy images,” in *2012 9th IEEE International Symposium on Biomedical Imaging (ISBI)*, [Online; accessed 2023-06-07], IEEE, May 2012.

- [9] S. Eom, R. Bise, and T. Kanade, “Detection of hematopoietic stem cells in microscopy images using a bank of ring filters,” in *2010 IEEE International Symposium on Biomedical Imaging: From Nano to Macro*, [Online; accessed 2023-06-07], IEEE, 2010.
- [10] F. Bourgeois and J.-C. Lassalle, “An extension of the Munkres algorithm for the assignment problem to rectangular matrices,” *Communications of the ACM*, vol. 14, no. 12, pp. 802–804, Dec. 1971, [Online; accessed 2023-06-07].
- [11] H. T. H. Phan, A. Kumar, D. Feng, M. Fulham, and J. Kim, “Unsupervised two-path neural network for cell event detection and classification using spatiotemporal patterns,” *IEEE Transactions on Medical Imaging*, vol. 38, no. 6, pp. 1477–1487, 2019. DOI: [10.1109/TMI.2018.2885572](https://doi.org/10.1109/TMI.2018.2885572).
- [12] D. C. Cireşan, A. Giusti, L. M. Gambardella, and J. Schmidhuber, “Mitosis detection in breast cancer histology images with deep neural networks,” in *Medical Image Computing and Computer-Assisted Intervention – MICCAI 2013*, K. Mori, I. Sakuma, Y. Sato, C. Barillot, and N. Navab, Eds., Berlin, Heidelberg: Springer Berlin Heidelberg, 2013, pp. 411–418, ISBN: 978-3-642-40763-5.
- [13] Z. Yan, H. Zhang, R. Piramuthu, *et al.*, “Hd-cnn: Hierarchical deep convolutional neural networks for large scale visual recognition,” in *2015 IEEE International Conference on Computer Vision (ICCV)*, 2015, pp. 2740–2748. DOI: [10.1109/ICCV.2015.314](https://doi.org/10.1109/ICCV.2015.314).
- [14] S. Ji, W. Xu, M. Yang, and K. Yu, “3d convolutional neural networks for human action recognition,” *IEEE Transactions on Pattern Analysis and Machine Intelligence*, vol. 35, no. 1, pp. 221–231, 2013. DOI: [10.1109/TPAMI.2012.59](https://doi.org/10.1109/TPAMI.2012.59).
- [15] Y. Mao and Z. Yin, “A Hierarchical Convolutional Neural Network for Mitosis Detection in Phase-Contrast Microscopy Images,” in *Medical Image Computing and Computer-Assisted Intervention – MICCAI 2016*. Cham: Springer International Publishing, 2016, pp. 685–692, [Online; accessed 2023-06-09].
- [16] H.-F. Tsai, J. Gajda, T. F. Sloan, A. Rares, and A. Q. Shen, “Usiigaci: Instance-aware cell tracking in stain-free phase contrast microscopy enabled by machine learning,” *SoftwareX*, vol. 9, pp. 230–237, 2019, ISSN: 2352-7110. DOI: <https://doi.org/10.1016/j.softx.2019.02.007>. [Online]. Available: <https://www.sciencedirect.com/science/article/pii/S2352711018301882>.
- [17] N. Jaccard, L. D. Griffin, A. Keser, *et al.*, “Automated method for the rapid and precise estimation of adherent cell culture characteristics from phase contrast microscopy images,” *Biotechnology and Bioengineering*, vol. 111, no. 3, pp. 504–517, 2014. DOI: <https://doi.org/10.1002/bt.22882>.

- i.org/10.1002/bit.25115. eprint: <https://onlinelibrary.wiley.com/doi/pdf/10.1002/bit.25115>. [Online]. Available: <https://onlinelibrary.wiley.com/doi/abs/10.1002/bit.25115>.
- [18] J. Chalfoun, M. Majurski, A. Dima, C. Stuelten, A. Peskin, and M. Brady, “Fogbank: A single cell segmentation across multiple cell lines and image modalities,” *Bmc Bioinformatics*, vol. 15, no. 1, pp. 1–12, 2014.
 - [19] D. A. Van Valen, T. Kudo, K. M. Lane, *et al.*, “Deep learning automates the quantitative analysis of individual cells in live-cell imaging experiments,” *PLoS computational biology*, vol. 12, no. 11, e1005177, 2016.
 - [20] C. Stringer, M. Michaelos, and M. Pachitariu, “Cellpose: A generalist algorithm for cellular segmentation,” *bioRxiv*, 2020. DOI: 10.1101/2020.02.02.931238. eprint: <https://www.biorxiv.org/content/early/2020/02/03/2020.02.02.931238.full.pdf>. [Online]. Available: <https://www.biorxiv.org/content/early/2020/02/03/2020.02.02.931238>.
 - [21] U. Schmidt, M. Weigert, C. Broaddus, and G. Myers, “Cell detection with star-convex polygons,” in *Medical Image Computing and Computer Assisted Intervention - MICCAI 2018 - 21st International Conference, Granada, Spain, September 16-20, 2018, Proceedings, Part II*, 2018, pp. 265–273. DOI: 10.1007/978-3-030-00934-2_30.
 - [22] M. Weigert, U. Schmidt, R. Haase, K. Sugawara, and G. Myers, “Star-convex polyhedra for 3d object detection and segmentation in microscopy,” in *The IEEE Winter Conference on Applications of Computer Vision (WACV)*, Mar. 2020. DOI: 10.1109/WACV45572.2020.9093435.
 - [23] A. Sacan, H. Ferhatosmanoglu, and H. Coskun, “Celltrack: An open-source software for cell tracking and motility analysis,” *Bioinformatics*, vol. 24, no. 14, pp. 1647–1649, May 2008, [Online; accessed 2023-06-07].
 - [24] F. Piccinini, A. Kiss, and P. Horvath, “Celltracker (not only) for dummies,” *Bioinformatics*, vol. 32, no. 6, pp. 955–957, Nov. 2015, [Online; accessed 2023-06-07].
 - [25] S. Ghannoum, K. Antos, W. Leoncio Netto, C. Gomes, A. Köhn-Luque, and H. Farhan, “Cellmaptracer: A User-Friendly Tracking Tool for Long-Term Migratory and Proliferating Cells Associated with FUCCI Systems,” *Cells*, vol. 10, no. 2, p. 469, Feb. 2021, [Online; accessed 2023-06-07].

- [26] L. Kamentsky, T. R. Jones, A. Fraser, *et al.*, “Improved structure, function and compatibility for CellProfiler: Modular high-throughput image analysis software,” *Bioinformatics*, vol. 27, no. 8, pp. 1179–1180, Feb. 2011, [Online; accessed 2023-06-07].
- [27] J. Chalfoun, M. Majurski, A. Dima, M. Halter, K. Bhadriraju, and M. Brady, “Lineage mapper: A versatile cell and particle tracker,” *Scientific Reports*, vol. 6, no. 1, Nov. 2016, [Online; accessed 2023-06-07].
- [28] J.-Y. Tinevez, N. Perry, J. Schindelin, *et al.*, “Trackmate: An open and extensible platform for single-particle tracking,” *Methods*, vol. 115, pp. 80–90, Feb. 2017, [Online; accessed 2023-06-07].
- [29] A. Smith, J. Metz, and S. Pagliara, “Mmhelper: An automated framework for the analysis of microscopy images acquired with the mother machine,” *Scientific Reports*, vol. 9, no. 1, pp. 1–12, Jul. 2019.
- [30] H. Aragaki, K. Ogoh, Y. Kondo, and K. Aoki, “Lim Tracker: A software package for cell tracking and analysis with advanced interactivity,” *Scientific Reports*, vol. 12, no. 1, Feb. 2022, [Online; accessed 2023-06-07].
- [31] A. Gwatimba, T. Rosenow, S. M. Stick, A. Kicic, T. Iosifidis, and Y. V. Karpievitch, “Ai-Driven Cell Tracking to Enable High-Throughput Drug Screening Targeting Airway Epithelial Repair for Children with Asthma,” *Journal of Personalized Medicine*, vol. 12, no. 5, p. 809, May 2022, [Online; accessed 2023-06-09].
- [32] *Make sense*, <https://www.makesense.ai/>, (Accessed on 05/22/2022).
- [33] A. Sohail, A. Khan, N. Wahab, A. Zameer, and S. Khan, “A multi-phase deep cnn based mitosis detection framework for breast cancer histopathological images,” *Scientific Reports*, vol. 11, no. 1, pp. 1–18, 2021.
- [34] Y. Lu, A.-A. Liu, and Y.-T. Su, “Chapter 6 - mitosis detection in biomedical images,” in *Computer Vision for Microscopy Image Analysis*, ser. Computer Vision and Pattern Recognition, M. Chen, Ed., Academic Press, 2021, pp. 131–157, ISBN: 978-0-12-814972-0. DOI: <https://doi.org/10.1016/B978-0-12-814972-0.00006-0>. [Online]. Available: <https://www.sciencedirect.com/science/article/pii/B9780128149720000060>.
- [35] L. Čehovin, A. Leonardis, and M. Kristan, “Visual object tracking performance measures revisited,” *IEEE Transactions on Image Processing*, vol. 25, no. 3, pp. 1261–1274, 2016. DOI: [10.1109/TIP.2016.2520370](https://doi.org/10.1109/TIP.2016.2520370).

- [36] *Confused by The Confusion Matrix: What's the difference between Hit Rate, True Positive Rate, Sensitivity, Recall and Statistical Power?* <https://learncuriously.wordpress.com/2018/10/21/confused-by-the-confusion-matrix/>, [Online; accessed 2023-06-09], Oct. 2018.
- [37] M. Hossin and S. M.N, “A review on evaluation metrics for data classification evaluations,” *International Journal of Data Mining & Knowledge Management Process*, vol. 5, pp. 01–11, Mar. 2015. DOI: [10.5121/ijdkp.2015.5201](https://doi.org/10.5121/ijdkp.2015.5201).