Automated Classification of Haematopoietic Compartments in the Human Bone Marrow using Reservoir Computing

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1 INTRODUCTION

Digital pathology is an emerging field in medicine (Cross et al., 2002) and – among others – includes sub-disciplines like telepathology, virtual microscopy and digital image processing. As digitalization devices became more precise over the past years and whole slide imaging has been well accepted as an alternative to the conventional slides, lots of new means in the slide analysis process were revealed (Riber-Hansen et al., 2012; Hamilton et al., 2012). Digital slides may be used in a variety of applications like education, digital diagnostics, research, or digital archiving (Al-Janabi et al., 2011; Riber-Hansen et al., 2012; Hamilton et al., 2012). Every day, hundreds of glass slides are processed manually (Huang et al., 2011), which is a tedious and error-prone activity.

Background, Relevance and Motivation. The histological examination of bone marrow is considered in the diagnostic process of a wide range of diseases including leukemia, anemia or lymphoma. In the current routine diagnosis process the quantification of the cellularity in the human bone marrow can be determined using histological sections. Though, the technology of digitized glass slides has been known for several years, using digital images in the daily diagnosis process in pathology is currently being established (Kayser et al., 2012).

The cellularity of a bone marrow specimen is expressed as percentage of the different haematopoietic compartments. A multi-potent stem cell is the origin of all types of bone marrow cells: erythrocytes, granulocytes, monocytes, megakaryocytes and its corresponding precursors as well as macrophages and mast cells. The different bone marrow cell types can be discriminated by morphology and spatial distribution within the bone marrow (Bain et al., 2000). Currently, histomorphometry of erythrocytopoiesis, granulocytopoiesis, and megakaryocytopoiesis is visually performed by the pathologist. Both the overall quantity and an increased or decreased quantity of haematopoietic precursors is assessed. This method heavily relies on the experience of the pathologist. Generally, six development stages of erythrocytes, six of granulocytes and three of megakaryocytes are known. A correct discrimination of the development stages is inherently quite difficult, since the evolution is continuous. The subjective emphasis of morphological criteria may lead to an under- or overestimation of the cellularity of the different components of haematopoiesis (Bain et al., 2000). Consequently, both intra- and inter-observer variability is in certain cases significantly high, as reported from several other research fields in digital pathology (Foss et al., 2012; Trocchi et al., 2012; Riber-Hansen et al., 2012; Cooper et al., 2012; Revell, 1983).

An automated detection of cell types and, as a consequence thereof, the quantification of the percentage of haematopoietic compartments would be of greatest benefit for medical diagnostics based on histological images. A method like this would improve accuracy, sensitivity, and specificity and support the diagnose process quantitatively and qualitatively. Although there has been a lot of progress in research, no single quantitative method has been proposed as a gold standard in digital pathology so far.

1.1 Computational Intelligence and Machine Learning

Computational Intelligence (CI) refers to a subbranch of Artificial Intelligence (AI). Evolutionary algorithms, fuzzy logic, Artificial Neural Networks (ANN) (Haykin, 1999), Reservoir Computing paradigms (Schrauwen et al., 2007) like Liquid State Machines (Maass, 2010) and Echo State Networks

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(Jaeger, 2001), and swarm intelligence are some CI paradigms used to facilitate "intelligent" behaviour. Whilst these paradigms solely have been successfully applied to real-world-problems, trends towards combinations of these algorithms can be observed (Verstraeten et al., 2007; Engelbrecht, 2007; Hassanien et al., 2008; Yao, 1999).

CI methods can be applied to various problems including classification, (non-linear) regression and clustering (Bishop, 2006). Regarding the classification the learning system tempts to develop classification rules in order to determine a specific class out of an input pattern. In case of regression problems input data of a set A are tried to be mapped on target values of a set B by fitting the parameters of a mathematical model. By doing this, future attribute values can be predicted. Clustering is based on the idea of letting the learning system suggest suitable classifications out of available patterns without predefining the target class. Commonly known clustering algorithms are (fuzzy) k-Means clustering, or Estimation Maximization (Bishop, 2006; Gonzalez and Woods, 2008).

In addition to intelligent algorithms statistical methods, e.g. variance and correlation analyses, or entropy are used supplementary. Those are often used for the purpose of pre-processing raw data for certain learning algorithms. The representation of input data contributes significantly to a proper performance of intelligent algorithms. Though, poorly processed data, e.g. inexact measurements, or noisy data, may cause a bad learning behaviour, CI algorithms are somehow error-resistant (Engelbrecht, 2007), because the "natural" variance of data can also be learned.

The methods of CI are applied more and more to biomedical and biochemical problem domains (Fogel, 2008; Mitra and Pal, 2005). Medical imaging is a data intensive research area (Cooper et al., 2012; Al-Janabi et al., 2011; Mori et al., 2008) and thus, another advantage of these methods is to be able to process more data in less time when compared to a human. They are suitable for automated generation, or derivation, respectively, of new knowledge out of large data sets and take up, where human cognition is limited in time and complexity. For instance, CI methods are used to discover coherences in high-dimensional data spaces (associations) or for pattern recognition in digital images and videos (classification). Combinations of different methods may yield better results in developing a learning model or in pre-processing the raw data (Dullin et al., 2007; Aizenberg et al., 2001). Other CI methods for image processing have also been proposed, like convolutional neural networks (Chua, 1998), pulse-coupled neural networks (Wang et al., 2010; Kuntimad and Ranganath, 1999; Ranganath and Kuntimad, 1999) or probabilistic neural networks (Specht, 1990), and have been successfully applied to image segmentation and object recognition problems.

1.2 Reservoir Computing

Reservoir computing (RC) (Schrauwen et al., 2007) refers to a quite novel paradigm in CI dealing with separate training of recurrent neural networks (the "reservoir") and its readouts (Verstraeten et al., 2007; Lukoševičius et al., 2012). RC and its underlying theory – computational neuroscience – recently became an emerging field in information processing (Abbott, 2008; Lukoševičius and Jaeger, 2009).

Liquid State Machines (LSMs) (Maass et al., 2002a) are one possible implementation of the RC paradigm and have been developed at the Graz University of Technology. A basic LSM architecture (Maass et al., 2002b) consists of an input layer of input neurons, a recurrent neural network, the neural microcircuit (NMC), of biologically realistic spiking neurons ("liquid", or "reservoir") and a readout layer, which can be constructed of another ANN, or other types of neurons (Maass et al., 2002a). The neurons within the NMC are randomly connected, though, their initial connectivity (dynamic spiking synapses (Maass and Markram, 2004)) is constrained by certain probabilities, depending on whether two neurons are of inhibitory or excitatory kind and their spatial distance, see (Burgsteiner, 2006) for an example model. The connections within the NMC are not altered during the learning procedure, but just the weights between readout layer and microcircuit are learned within a supervised training procedure (Goodman and Ventura, 2005). The basic principle behind LSMs is that the input layer continuously feeds the input to the reservoir which maps the input space non-linearly to a very high-dimensional feature space, where – according to Cover's theorem (Cover, 1965) – linear separability is more likely. The reservoir's state is recorded over time and the readout layer is trained on the feature space in order to accomplish a given task with a minimal error and greatest possible generalization capability (Bishop, 1995).

Concurrently to the LSM, Echo State Networks (ESN) have been proposed as a similar approach, but due to our hypothesis this project will eventually try to implement a LSM because of a biologically more realistic approach than other RC paradigms (ESN (Jaeger, 2001; Jaeger et al., 2007), Backpropagation-Decorrelation (Steil, 2004), and Temporal Recurrent Networks (Dominey and Ramus, 2000), reviewed in

(Lukoševičius and Jaeger, 2009)). LSMs facilitate real-time parallel computation by exploiting the non-linear computational power of just one "liquid" and are very well suited for temporal classification tasks (Verstraeten et al., 2006). Since the LSM's first occurrence in (Maass et al., 2002a), improvements of the LSM architecture and training have been proposed in (Norton and Ventura, 2006; Norton and Ventura, 2010; Hazan and Manevitz, 2010).

2 STATE OF THE ART

There has been a lot of research in AI, digital pathology, and digital histological images (Kayser et al., 2008a; Kayser et al., 2008b; Kayser et al., 2009; Kayser, 2011; Molnar et al., 2003). Applications of CI methods to images of histological specimen yielded promising results (Sjöström et al., 1999). ANN were used in combination with standard methods of image analysis for cell counting in histological images (100× magnification, digital microscopy, single band 8 bit grey value images). The developed machine learning system was up to six times faster than an experienced human, and in contrast to the human, the system produced fewer and more constant errors. The software used for cell counting was not capable of dealing with noisy images, hence just semi-automatic counting could be performed. Additionally, the authors found out, that the background of an image can substantially compromise the performance of an automated learning system. They chose ANN for pattern recognition, because setting a simple histogram threshold and the search for grey value peaks in an image of Hematoxylin-Eosin (HE) stained specimen was not expedient. Some cells in the digitalized microscopy had multiple grey value peaks and the threshold algorithm separated the single cell into two distinct ones. The authors additionally tried to train separate ANNs to determine the cell type by its morphology, but the data was not included in this publication. Similar work has been done by (Schaberg et al., 1992), because ANNs are able to tolerate negligible image noise to a certain extent (Lin et al., 1998). In another work (Zheng et al., 2004) directly fed cropped image regions of 32×32 pixels into ANNs of different architectures and concluded that the classification using a two-layer Feed-Forward-ANN (FF-ANN) with shared weights yielded remarkable results ($98 - 99\%$ correct classification rates). Others have already experience with bone marrow material and proposed an automated segmentation and classification method based on features of the nuclei of white blood cells (Shivhare and Shrivastava, 2012). Despite their efforts and acceptable classification results using FF-ANN they were not able to achieve automated cell segmentation.

A huge obstacle in automated image analysis is the pre-processing of image data for classifiers. A common approach for reducing the dimension of a problem domain is the representation of complex information as features. The Principal Component Analysis (PCA) can be used for feature extraction (Bishop, 2006) and is applied even in other fields of image processing for feature ranking (Zhang and Wu, 2011). Decision trees can be used for main feature extraction of more abstract data like features of digital images, too (Lu and Yang, 2009). There is no golden rule, whether raw pixel data or more compressed information in features works best for a given problem; both approaches yielded good results.

In histological images one can distinguish between the object space, which (probably) contains objects (e.g. cells) and the background, containing (probably) no objects. Frequently it is sufficient to determine appropriate threshold values in the gray value spectrum of an image in order to separate the background from the object space (Kayser et al., 2009). One problem still remains: some objects may seem to be connected, although they are solitary, e.g. if they overlap in the histological section. Additional color channels (e.g. in RGB color space) or color space conversions are possible solutions to the problem of searching for an appropriate threshold. Common standard image processing methods like edge detection algorithms, morphological operations, texture based filters (Gonzalez and Woods, 2008) or pixel-size filters may serve as valuable links in the pre-processing chain for CI algorithms.

In their review about histopathological image analysis (Gurcan et al., 2009) point out that there is a clear need for quantitative methods in disease grading and that computer-aided diagnosis processes are substantial to modern processes in pathology departments. Machine learning algorithms are powerful tools to support the daily life in digital pathology, if properly implemented in process-oriented software. CI has the vast potential to overcome existing barriers and eradicate weaknesses of current standard methods. As a result, the application of its methods promotes the progress in medical research.

3 RESEARCH PROBLEM

The research question addressed in this PhD project can be separated in two domains: (i) the cell segmentation and (ii) the object recognition and classifica-

tion. Classical quantitative image analysis deals with pattern recognition based on grey level intensities in pixels and neighbourhoods. Texture is a feature used to partition images into regions of interest and to categorize those regions into object classes (Tuceryan and Jain, 1998). It provides information on the spatial arrangement of pixel colours, or intensities, respectively, in an image. Both texture classification and segmentation are common methods for pattern recognition of different kinds of tissues. Texture classification aims at matching given image regions of interest with existing texture classes. Applying texture classification solely is problematic due to the natural variety of cell morphology. Texture segmentation follows an automated processing approach to determine distinct texture regions within an image. Statistical measures like the Grey Level Co-Occurrence Matrix (GLCM), run-length statistics, contrast, entropy, variance, or energy (Gonzalez and Woods, 2008) are useful, if micro-textures are the observation element of interest. A commonly known problem when processing histological images is the explicit determination of an object (e.g. a cell) using parameters of the standard image processing, i.e. simple grey level intensities without information on their spatial arrangement (Sjöström et al., 1999). Nevertheless, a final classification of a (segmented) sample or image must always be performed by the pathologists themselves.

Complementary to the aforementioned image processing methods, approaches using machine learning systems are increasingly becoming popular in image analysis and computer vision. Several methods for both the classical and the neural image processing approach have been proposed and applied to specific problems in image processing domains. The automated segmentation and classification of objects in the human bone marrow has not yet been solved satisfactorily and digital pathology is demanding new solutions for reliable decision support based on objective, robust, and reproducible results. Attempts have been undertaken using these kind of methods in medical image processing and there is progress in similar research areas, but there is currently no solution to our problem, or a proposed standard method, respectively.

However, there does not exist *the* one method solving all problems, because each single problem requires a critical view on the method to be applied due to its characteristics (Fogel, 2008).

4 OUTLINE OF OBJECTIVES

Hypotheses. The following hypotheses are to be evaluated in the scope of this PhD project.

1. Computational Intelligence algorithms can be used to solve the problem of automated relative quantification of erythropoiesis, granulopoiesis and megakaryopoiesis in histo(patho)logical images of stained human bone marrow specimen.
2. An appropriate biologically realistic neural network can be implemented in order to classify and quantify automatically three distinct cell types in digital images of bone marrow.
3. New automated diagnosis methods using Computational Intelligence will keep the intra- and inter-observer variability of histological and histopathological images at a lower and more constant level when compared to humans.

Goals. The goals of the proposed PhD thesis are as follows.

1. *Evaluation of the best standard staining techniques for bone marrow diagnosis and digitalization of the histological slides.*
   At the beginning of the project we will focus on chemical standard staining techniques of healthy human bone marrow specimen. In further research we also regard the examination of pathological specimen. High image quality and resolution is critical to succeeding image processing and classification methods, especially in our case, since we strive for classifying up to 13 cell classes out of a single image.
2. *Extension of the Liquid State Machine (LSM, recurrent neural network) paradigm to the haematopoiesis classification problem in the bone marrow.*
   As described in section 1.1, the LSM has mainly been designed for dealing with time-variant input- and output-data. Within this project we face static, incoherent 2D whole slide images, where experienced pathologists are going to label (classify) the cells for training. We will have to extend the LSM paradigm in terms of finding a way to generate temporal-like input from static data and presenting it to the LSM's input layer. This stream generation process is a crucial advancement in research and has not been proposed yet. The human visual perception and its biological signal processing mechanism in the visual cortex is complex and holds tremendous computational power. Therefore, the information processing chain from the retina to the brain is taken as inspiring example for our neural network architecture.
3. *Application, testing and evaluation of the adapted Liquid State Machine on cells of the bone marrow.*
   We plan on applying the visual capability of the

LSM to single images of the stained human bone marrow and imitate the human visual perception. The performance of the system will be evaluated comparing the trained classifier's quantification to the quantification of several experienced pathologists on the same image. Additionally, we will run benchmark tests, comparing our classifier to other, commonly used neural computation methods in image processing like Hopfield networks, multilayer perceptrons, support vector machines or radial basis function networks. Another goal is, to evaluate the proposed approach to classic object recognition methods like template matching.

5 METHODOLOGY

Major tasks of this dissertation project are:

1. the generation of the data sets for training classification algorithms, comprising
   (a) digitalization of histological sections,
   (b) the segmentation of the cells in the virtual slides,
   (c) data pre-processing for the algorithms, and
2. the design of the machine learning system capable of classifying the haematopoietic compartments within an histological image of the bone marrow.

5.1 Material and Data Acquisition

Bone marrow material is harvested from the human iliac crest during examinations in the clinical practice at Graz University Hospital. The material is embedded in acrylate and stained at the Institute of Pathology of the University Hospital. As a first step, and in order to train a classifier on cell image data of the bone marrow, we will use images of healthy bone marrow since the intra-class cell morphology is rather stable than in neoplastic tissue. In further experiments we consider pathological tissue grading, too. Experiments will determine suitable staining techniques. The sections will be stained using at least the following standard staining techniques: Hematoxylin-Eosin (HE), May-Grünwald Giemsa (MGG), Toluidin Blue (TB), Gomori's Silvering (GOM), and Periodic Acid Schiff (PAS), where the first two are diagnostically most conclusive for pathologists. Figure 1 shows a cropped image of HE and MGG stained healthy human bone marrow as an example. Additional custom staining techniques will be taken into account, if the standard techniques are insufficient for the automated image analysis. The glass slides are digitized at 40× magnification using an Aperio ScanScope scanner,

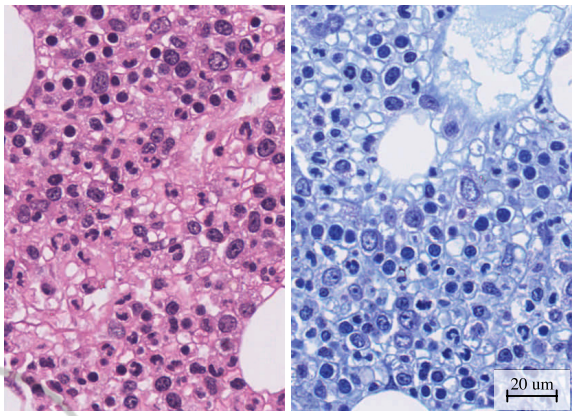

Figure 1: This figure shows a scanned image of healthy human bone marrow stained with Hematoxylin-Eosin (HE, left), and May-Grünwald Giemsa (MGG, right), respectively, at 40× magnification.

available at the Center for Medical Research ZMF at the Medical University of Graz. Specimen sizes varied between 5×10 mm and 7×14 mm, resulting in 24 bit, JPEG-compressed RGB images of about $25\,000 \times 50\,000$ pixels and about 200 MB per file.

5.2 Data Set Generation

Since we plan on experimenting with different supervised and unsupervised methods, training, validation and test data set are required. Supervised methods need target outputs (classes) representing the different development stages of the haematopoietic compartments. The inputs of RC approaches like ESN and LSM are usually several temporal signals and therefore a couple of concurrent 1D signals. In order to generate temporal-like 1D signals from static 2D images, we pursue the following approach.

5.2.1 Image Pre-processing and Pathway Generation

Image pre-processing is an important step in the preparation of raw images for intelligent methods and is partly performed in ImageJ[1], IQM[2] and MATLAB[3], where a large library of classical image processing and enhancement methods is already available. As a first step, a window containing the most significant information on the classes to be detected is chosen from the source image and the virtual slide

[1] Available from http://rsb.info.nih.gov/ij/.

[2] Available from https://sf.net/projects/iqm/. This software is developed by our research group *Quantitative Morphology and non-linear Methods* at the Institute of Biophysics, Medical University of Graz.

[3] Available from http://www.mathworks.com/.

is cropped to the window's dimensions. The cells C_i, with $i = 1,\ldots,K$, of interest are segmented by standard image processing methods. Hereinafter, the cell nuclei's centers are determined and marked (depicted as white dots in Figure 2). These centers delineate a plane of nodes N_i, where the minimum Euclidean distance $\min(\{D(a,b_j)\})$ between a node a and its adjacent nodes b_j determines the path to the next node. We are going to apply certain restrictions in order to determine a practical neighbourhood $\{b_j\} \subseteq \{N_i\}$ for node a. This successively constructs a directed graph for subsequent extraction tasks. If it turns out that this naive approach is insufficient, we consider using a more sophisticated algorithm for the pathway construction. Since the number of input neurons is immutable after learning a special task, we have to be flexible in dealing with the varying shape and size of C_i and cannot use a fixed patch size for the extraction. Thus, the minimum circumscribing circle O_i with radius r_i of the corresponding C_i is determined. Sub-images S_i (patches, regions of interest ROIs) of size $2(r_i+\xi_i) \times 2(r_i+\xi_i)$ pixels are extracted, where ξ_i denotes an additional object border tolerance. Each S_i contains only a single cell nucleus at its center. S_i centers and O_i centers are congruent. With three distinct cell types and its development stages we will get up to 13 classes in total: C_i is either a sub-class of erythropoiesis $E_{c\in\{1,\ldots,6\}}$, granulopoiesis $G_{c\in\{1,\ldots,6\}}$, or megakaryopoiesis $M_{c\in\{1\}}$, with c as sub-class index for each cell type.

5.2.2 Class Labeling and Input Stream Generation

The cropped virtual slides are viewed in ImageJ image processing software, where experienced pathologists manually label the ROIs with the correct target class. The labeled patches are rotated centrically by some angle α (e.g. $\alpha = 0,\ldots,359°$). This rotation transforms the anisotropy of shape and staining in a temporal-like input for the classifier. Due to both varying intra- and inter-cell class shape and size, and consequently the varying magnitude of r_i, the patches have to be scaled ($S_i \rightarrow S_i'$) in order to fit the artificial retina's visual field[4] before rotation. Proportions of the objects are preserved. All normalized and rotated patches $S_i'(\alpha)$ are concatenated and form one input stream Θ_i of one cell. The grey value variations of all pixels $P_{x,y}$ within the stream serve as the basis for the required input signals for the classifier. Finally, we get a set of input streams $\{\Theta_i\}$ from a single image. This process is repeated for each available cropped virtual slide. The entire input stream generation process for one cell class is depicted in Figure 2. The complex models used in CI approaches require a huge set of different training examples to be able to learn a given task. Additionally, some of the examples have to be reserved for testing and validation. The proposed data set generation approach facilitates the creation of an extensive data set and enables us to cope with intra-class variability and develop a more robust rotation- and translation-invariant classifier. The data set we will get out of all available virtual slides will be divided into training, validation and test data set (e.g. 75%, 10% and 15%).

[4]The concept and architecture of the artificial retina is explained in section 5.3.

5.3 System Architecture and Training Procedure

Each cropped image contains lots of training examples (labelled cells) of different classes. Learning one class from a single patch comprises (a) initializing (resetting) the reservoir, (b) feeding one encoded stream into the input layer, (c) recording the reservoir's activity in state vectors and (d) learning the synaptic weights in the readout layer according to the target class. Hence, we propose the following architecture.

5.3.1 Architecture

Based on the biological model of the retina, a 2D array of n input neurons arranged in a circular field of vision V_{ret} with diameter d_{ret} holds responsible for receiving the input. We will refer hereto as the artificial retina (AR). One can choose out of several neuron models and input encodings in order to construct the input layer of a LSM, e.g. spiking neurons or linear neurons, and spike train generators like Poisson code generator (Burgsteiner et al., 2007) or Bens Spiker Algorithm (Schrauwen and Van Campenhout, 2003), respectively. ESN on the other hand use artificial neurons and do not require spike trains. The input layer is connected to the reservoir by feed-forward synapses. An additional input parameter, the scaling factor, is considered to be included as a priori knowledge into the architecture, since the patches have to be scaled in order to fit V_{ret}. The optimal reservoir architecture and connectivity of its computational units will be determined in our experiments. The output layer is composed of three to 13 readout neurons, depending on the given task. However, it is not yet specified, which kind of neuron is suited best for our retina model or the output layer.

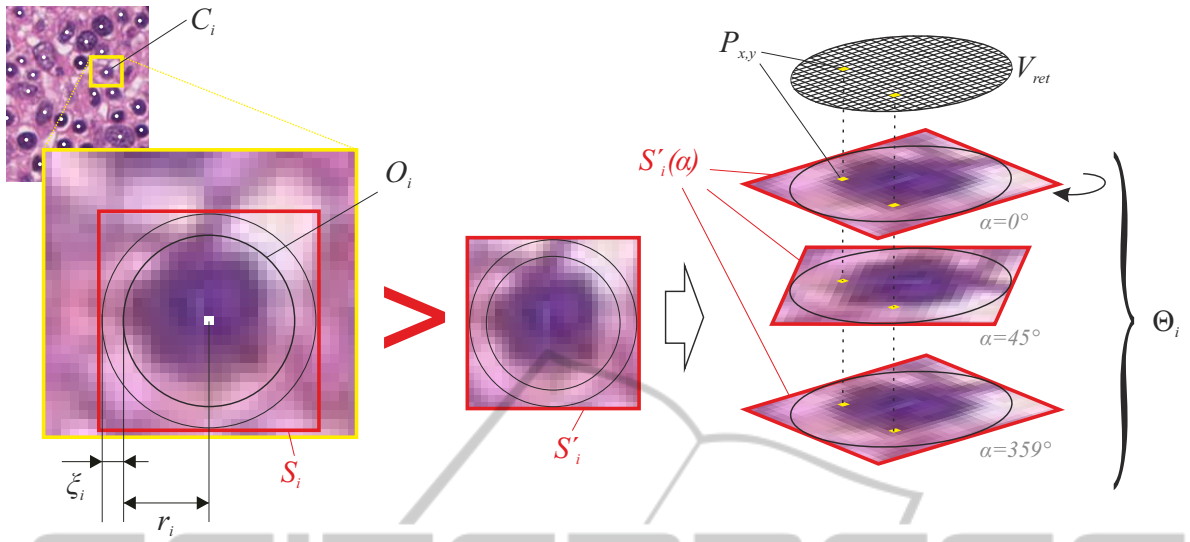

Figure 2: This figure illustrates the generation of the image input stream Θ_i for one cell class within a single image. This process is repeated for each detected cell C_i within an image.

5.3.2 Training

The initial classification task is the detection and recognition of one of the three main cell types. A further task is to train the classifier on all meaningful sub-classes of each cell type.

As stated above, the cropped image is scanned through along the pathway, continually feeding a sub-image stream into the AR and learning the target's (super)class. After rotation, each image in the stream is of different size, but since the retina is of circular shape, all pixels outside V_{ret} may be omitted. The AR receives the input vectors $u(t)$ (e.g. spike trains) and forwards them to the reservoir. This causes activity of the computational units, which is mapped to a state vector $x(t)$ at each sampling time-step t. From $x(t)$, the readout layer computes the output $y(t)$. The actual training is done by adjusting the synaptic weights between the reservoir and the readout neurons using a suitable algorithm. It is not yet determined, which training algorithm we will use eventually, but linear regression training has already been applied successively by others (Burgsteiner et al., 2007; Hourdakis and Trahanias, 2011) and is our first candidate. Another approach for multi-class discrimination is the winner-take-all-principle, where the neuron with the highest output activity solely determines the output (Verstraeten et al., 2005).

5.3.3 Extensions

The cell types may express some characteristic neighbourhood properties during their development, e.g. erythroblastic islands (Bain et al., 2000). So far we just regarded pixel grey values for training, but we definitely consider incorporating such a priori knowledge into our method. Another approach is the generation of significant image features, which will vastly decrease the input dimensionality. Neighbourhood conditions can be taken into account and influence – at best enhance – the next classification output, e.g. if both the correct classification and a distance measure to the previously correct classified cell are fed back into the network. Using the committee paradigm (Bishop, 1995), the training of additional classifiers on specific cell classes will increase the accuracy of the overall classification. With this system, we are also able to train the algorithm not only on these three cell types and its stages of development, but on fat and other tissue, too. Other interesting research questions are expected to rise as the project progresses.

5.4 Application and Evaluation

Similar to the training procedure, the starting point for the application is a cropped histological image of bone marrow, where the classifier is guided through the pathway, generating classification outputs from the observed cells. These outputs are recorded. Thereafter, we calculate the relative amount of each haematopoietic compartment within the image. Other approaches like training another, subsequent (CI) algorithm on the output patterns in order to decrease the learning effort are considered as well.

The performance of the system is evaluated using several experiments and is analysed statistically. Some experienced pathologists will independently

classify samples from the test data set and produce the output (relative quantity of the haematopoietic compartments) via their current standard method. Interesting benchmarks will be performed, namely the comparison between the trained system and the experienced humans, in terms of overall error, intra- and inter-observer variability, reliability, and robustness. Furthermore, we will compare our classifier to proven standard image processing methods (e.g. template matching) as well as to other neural computing approaches in image processing (Hopfield networks, FF-ANN, support vector machines, radial basis functions).

5.5 Software Implementation of a Prototype Learning System

Since the image analysis software IQM, developed by our group is written in Java, we plan to stick to this language for our prototype system. However, MATLAB is a powerful tool for signal and image processing. The Neural Network Toolbox and the Reservoir Computing Toolbox for instance are libraries providing implementations of different neural network architectures ready to be used in some of our experiments. In conjunction with other MATLAB toolboxes we are be able to implement – at least parts of – the prototype machine learning system. OpenCV[5] is a large open-source library for computer vision and image processing tasks. It has been extended recently and now provides a Java binding for the C/C++ library. This advancement enables us to optimize our pre-processing Java routines in terms of computational expenses. Furthermore, Java-ML[6] is another machine learning library providing algorithms e.g. for feature selection, clustering, classification, and data filtering out-of-the-box. Java-ML has been used successfully in several studies (Abeel et al., 2009) and is taken into account as a candidate for particular tasks of the prototype development. Depending on the classifier, e.g. LSM or ESN, we use other toolboxes and simulation environments like PCSIM[7] or Oger[8], too.

6 EXPECTED OUTCOME

In this project we are going to develop an automated quantitative approach for the diagnosis of the cellularity of the haematopoietic compartments and its precursors in the bone marrow.

The results of our research will substantially contribute to the evolution of digital pathology, namely by providing an innovative and robust machine learning system in order to stabilize and accelerate diagnosis processes using state of the art information technology. Having a controlled error behaviour will decrease inter- and intra-observer variability and avoid individual errors. It has to be noted that the developed machine learning system will not be restricted to haematopoiesis classification; it can be trained on any arbitrary task where training and test data is available. Moreover, new insights regarding biological aspects and knowledge on the visual perception and computation of visual stimuli may be revealed by this research.

7 STAGE OF THE RESEARCH

Data Acquisition. Essential tasks like the acquisition and digitalization of histological sections of healthy human bone marrow have successfully been accomplished. Our initial approach uses HE and MGG stainings, but we are currently working on evaluating other staining techniques for the sections in order to ease the segmentation and recognition problem. Substantial time has been spent on software development for image pre-processing. The open-source plugin *Cell Counter*[9] for ImageJ has been extended to our requirements. It is capable of recording the pathologists' classification of the cells. So far, we have labeled 400 patches containing different cell types from these images, where 55% are granulopoietic, 44% are erythropoietic and $\approx 1\%$ are megakaryopoietic compartments. More labeled patches are generated continuously and our data set is constantly growing.

System Design. First experiments are currently planned using the ESN approach, where we manually crop the cells from the image and omit the preceding automated segmentation process. In order to simplify the object recognition and discrimination, we intend to use only two highly distinguishable classes from our data set (early and late granulopoietic classes) in the first place. A problem we are currently working on is finding suitable target functions for the classes. Approximating different constant functions are considered for the binary classification tasks. If more and more classes are added to be learned, the functions must be more discriminative. Another approach we consider is pooled decision making in the readout

[5] Available from http://opencv.org/.

[6] Available from http://java-ml.sourceforge.net/.

[7] Available from https://sf.net/projects/pcsim.

[8] Available from http://organic.elis.ugent.be/oger.

[9] Available from http://rsbweb.nih.gov/ij/plugins/cell-counter.html.

layer, such that a combination of output units decides about the categorization of the input signal.

If the results of these experiments are promising, we plan on adapting the design to more complex problems in terms of (i) learning more than two classes concurrently, (ii) using spiking neural networks as reservoirs, and (iii) pursue a fully automated approach for both segmentation and classification.

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