Classification of Staining Patterns on HEp-2 Specimen Images

A THESIS

submitted by

KRATI GUPTA

for the award of the degree

of

DOCTOR OF PHILOSOPHY

SCHOOL OF COMPUTING AND ELECTRICAL ENGINEERING

INDIAN INSTITUTE OF TECHNOLOGY MANDI

November 24, 2020

Through selfless service, you will always be fruitful and find the fulfillment of your desires!

Bhagavat Gita

To My Grandmother

Smt. Kamlesh Gupta

And To My Family

Declaration

I hereby declare that the entire work embodied in this thesis is the result of investigations carried out by me in the **School of Computing and Electrical Engineering, Indian Institute of Technology Mandi**, under the supervision of **Dr. Anil Kumar Sao** and **Dr. Arnav Bhavsar**, and that it has not been submitted elsewhere for any degree or diploma. In keeping with the general practice, due acknowledgments have been made wherever the work described is based on finding of other investigators.

Mandi, 175005

Date:

Krati Gupta

THESIS CERTIFICATE

This is to certify that the thesis titled **CLASSIFICATION OF STAINING PATTERNS ON HEP-2 SPECIMEN IMAGES**, submitted by **Krati Gupta**, to the Indian Institute of Technology, Mandi, for the award of the degree of **Doctor of Philosophy**, is a bonafide record of the research work done by her under our supervision. The contents of this thesis, in full or in parts, have not been submitted to any other institute or university for the award of any degree or diploma.

Mandi, 175005
Date:

Dr. Anil Kumar Sao
(Ph.D Supervisor)

Dr. Arnav Bhavsar
(Ph.D Supervisor)

Acknowledgements

First and foremost, I would like to thank my respected advisors, Dr. Anil K. Sao & Dr. Arnav Bhavsar for their inspiration, guidance and incredible support, throughout my PhD years. Their timely interactions, insightful discussions, scientific advices and expertise were incredible and made my journey very enthusiastic, lively and energetic. Also, I would like to thank my doctoral committee members, Dr. Renu M. Rameshan, Dr. Bhakti Joshi, Dr. Ajay Soni, and Dr. B. S. Rajpurohit. They have inspired me greatly though their own research. Their useful suggestions and timely feedback have evolved me greatly over the years.

I want to thank Dr. Dileep A.D. and Dr. Aditya Nigam for their guidance at several times, during my PhD thesis work. Our interactions gave me an opportunity to deepen my understanding of the fundamentals of pattern analysis and deep learning. Also I am thankful to Prof. Hema Murthy and Dr. C. Chandra Shekhar for important courses taught, during my course work, which enhances my knowledge to pursue my research effectively. I thank Dr. Timothy A. Gonsalves, Director, IIT Mandi, for academic support and amenities provided to us, to pursue our research effectively. Also, I want to thank all the faculty members of MANAS lab group. Dr. Padmanabhan Rajan, and Dr. Shubhajit Roy Chowdhary have always been very supportive, and ready to solve the queries, regarding research problems.

I thank Dr. Vani RaviKumar, from R.V. Metropolis Diagnostic & Healthcare Centre Pvt. Ltd., Bangalore, for her immense explanation on medical context of the problem statement and for her valuable time, spent on validation of some part of the thesis work. I want to thank another special person Dr. Purnima Bajwe, Counseler, IIT Mandi, for her support and timely suggestions for my professional and personal life, during this time.

In my daily working hours, I have been blessed with a friendly and cheerful group of fellow scholars at MANAS lab, and those are Shikha, Anshul, Srishti, Arshdeep, Ranjeet,

Jyoti, Prabhjot, Priyanka, Preethi, Gaurav, Rahul, Snehal, Mohana, and many more. They provided me a friendly and cooperative atmosphere at work and also provided useful feedbacks and insightful comments on my work. Moreover, I am forever thankful to my friend and colleague Daksh Thapar for his frequent help. I want to mention the name of a very special friend Naina, who has helped me a lot like a sister. She frequently cleared my concepts related to medical context and helped me step out of her comfort zone.

I greatly acknowledge the research funding received towards my PhD from the HTRA fellowship, provided by Ministry of Human Resource Development, India. Additionally, I would like to thank Kuldeep, Deshraj, Rakhi, Nalini, Monu, and Sushma for providing assistance with administrative tasks and network related issues.

Words cannot express how grateful I am to my parents Mr. Arvind Kumar Gupta & Mrs. Neelam Gupta and my brother Madhav, for their unconditional love and support throughout my life and especially during my PhD journey. They believed in me and allowed me to pursue my dreams. Their prayer for me was what sustained me thus far. I am grateful to some of my relatives, Mr. Prashant & Mrs. Shalini Mittal, and Ms. Deeksha who have supported me along the way and provided me mental strength during my tough time of this journey. I am also grateful to some of my campus friends Vijender, Deepika, Dr. Avinash, and Mamta, who took good care of me, during my busy days of PhD. Most importantly, I express my heartfelt gratitude to another couple, Mr. Manvender & Mrs. Seema Kapoor, who provided me affection, full space, and liberty to complete my work and to fulfill my dreams. There is one another person, Manav, who deserves a special mention here, as he made me confident, strong, robust, and potent, and always pushed me to fulfill my dreams. He has made this life-changing opportunity possible for me and this would not have been possible without his constant encouragement and support.

Finally, I would like to thank those who directly or indirectly, helped me to finish my thesis work. Last but not the least, I want to thank the almighty God for providing me a blessed life, health, and environment, that pushed me towards my dreams and aspirations.

Krati Gupta

Abstract

Indirect immunofluorescence (IIF) imaging of human epithelium type-2 (HEp-2) cell substrate has been considered as the 'gold standard' test for diagnosis of autoimmune disorders. However, manual diagnosis assessment involves tedious and labor-intensive workflow, and can also result in with high intra-personnel and intra-laboratory variations. In this thesis, an image-processing based algorithmic framework is presented for identification of different staining patterns manifested on HEp-2 cell substrate, which can contribute to the computer-assisted diagnosis of autoimmune disorders.

The visual staining patterns can be broadly divided into mitotic patterns and interphase patterns, based on their manifestation during different phases of the cell-cycle. Owing to the fact that mitosis is a transition phase, the appearance of patterns associated with the mitotic phase is quite rare, which makes the identification of mitotic patterns more challenging and important. Another challenge considered in this work includes designing the framework using only primary channel images, without the use of DAPI pattern images. The DAPI is a secondary chemical dye, which is required for the acquisition of segmentation masks for cells, but not recommended in practice. In addition, less inter-class and high intra-class variations among samples make the classification problem more difficult to solve.

Considering above mentioned challenges, the contributions of the thesis include novel cell-based, region-based, and specimen-based approaches for identification of patterns on specimens. The cell-based approaches are designed to identify the rare or minority based patterns, as the traditional classification paradigm gets biased towards majority class samples in specimen-based approaches. Hence, minority patterns can be identified via cell-level processing. These approaches use segmentation mask images for cell segmentation and cell extraction from specimen images. In cell-based approaches, the first proposed framework is

the identification of rare mitotic phase cells in specimen images. Importantly, this considers the data imbalance between mitotic patterns and non-mitotic/interphase patterns in classification paradigm, and we use different data-skew balancing strategies. The next framework focuses on mitigating some drawbacks of data-skew balancing strategies, and considers mitotic pattern cells as anomaly, in the specimen images, that consist of largely interphase pattern cells and few mitotic pattern cells. This task is done by one class classifier, used as an anomaly detection framework. The next approach involves a similarity-learning based framework, for identification of the mitotic patterns in specimen images. In this approach, a distance metric computation is integrated with deep convolutional neural networks (D-CNN), aiming to learn useful embeddings of the data, using triplet-loss based function. All the cell-based strategies also involve a novel and separate decision-making criterion for mitotic specimen, that is based on the count of a minimum number of mitotic samples, present in the specimens. This criterion is termed as 'threshold-based decision-making criterion'.

Following the proposed cell-based approaches, the next task of this thesis work is to address the identification of mitotic/minority patterns in specimen images, via image-based approaches, in order to avoid the use of the provided segmentation masks, which require the DAPI dye. This problem statement is also addressed in three different ways, where the first idea is to perform classification via a segmentation approach. Though, this pipeline is also based on a cell-based approach, the segmentation masks are computer automatically. Here, a U-Net neural network-based segmentation framework is used, followed by a 2-class classification framework. The second approach divides specimen images in local uniform regions, where these regions are considered as input images to a CNN-based classifier. The third and final method considers mitotic patterns as distinct objects in specimen images, due to their rare appearance, and a good object-detection framework is applied for detection of such patterns. Hence, these three approaches address the identification of minority patterns in complete specimen images.

Following the various strategies for identification of mitotic cells, as a final task of the thesis, a framework is designed for the identification and classification of different interphase type patterns, which can be discriminated based on their morphology and appearance. Hence, considering the characteristic of each class, some class-specific feature-

representations are designed to capture such types of discriminative characteristics of each pattern. Similar to the mitotic class, here also, D-CNN based framework is used for the identification of different interphase patterns. Finally, another novel attempt is made, in order to generate synthetic samples for rare classes, so that the synthetic samples can contribute to the training of the classifier, where the data across classes was imbalanced in the original scenario. For this task, a generative adversarial network-based framework is used for mitotic patterns, which is validated experimentally and subjectively by medical experts.

In experimental results and analysis, it is demonstrated that the proposed frameworks perform quite well for the identification of mitotic patterns, as well as for the classification of different interphase patterns. Overall, the best performance (in terms of Matthews correlation coefficient) for mitotic class (for single cell images) is 0.99. For specimen images, the best achieved performance for mitotic class, and interphase classes are 0.96 and 0.98, respectively. The study also shows a comparative analysis amongst different proposed frameworks and with existing works. Thus, the proposed methods are validated, with good and notable performance, for identification of different staining patterns, on an important problem definition.

Contents

Acknowledgement **i**

Abstract **iv**

List of Tables **xiv**

List of Figures **xv**

Abbreviations **xix**

1 Introduction **1**

1.1 Diagnostic test for autoimmune disorders 3

1.2 Problem statement & motivation of the thesis work 7

1.3 Research challenges of the problem statement 8

1.4 A brief literature review 10

1.5 Objectives and scope of the thesis 16

1.6 Contributions of the thesis 18

1.7 Outline of the thesis 20

2 Preliminaries **23**

2.1 Feature representation used in the thesis 23

2.1.1 For mitotic v/s interphase staining patterns classification 23

2.1.1.1 Morphology-based features 24

2.1.1.2 Texture-based features 24

2.1.1.3 Convolutional neural network (CNN)-based features 25

2.1.1.4 Bag-of-words (BoW) representation 25

2.1.2 For classification among different interphase type patterns 26

2.2 Classifiers used in the thesis . . . 27
2.2.1 Support vector machines . . . 27
2.2.2 Convolutional neural network (CNN)-based classifiers . . . 29
2.3 Evaluation metrics . . . 30
2.4 Data dividing protocol . . . 31
2.4.1 Random cross-validation protocol . . . 31
2.4.2 Leave-one-out (LOO) protocol . . . 32
2.5 Datasets . . . 33
2.5.1 UQ-SNP Task-2 dataset . . . 33
2.5.2 UQ-SNP Task-3 dataset . . . 36
2.6 Summary . . . 37

3 Detection of Mitotic Patterns: Hand-Crafted v/s CNN features, and Data Skew Balancing 39

3.1 Literature review . . . 41
3.2 Proposed framework . . . 42
3.2.1 Data skew balancing strategies . . . 42
3.2.1.1 Balancing number of samples in classes . . . 43
3.2.1.2 Weight assignment to classes in optimization function . . . 45
3.2.2 Feature representation scheme . . . 47
3.2.2.1 Pre-processing of images . . . 47
3.2.2.2 Feature representation: UQ-SNP Task-3 single cell dataset . . . 47
3.2.2.3 Feature representation: UQ-SNP Task-2 specimen dataset . . . 49
3.2.3 Classification . . . 51
3.2.4 Decision-making criterion for specimens: Threshold-based scheme . . . 52
3.3 Experiments and results: UQ-SNP Task-3 dataset . . . 52
3.3.1 Analysis of features and data skew-balancing strategies . . . 54
3.3.1.1 Morphology-based features . . . 56
3.3.1.2 LM filter bank . . . 56
3.3.1.3 Gabor filter bank . . . 59
3.3.1.4 CNN-based features . . . 62
3.3.1.5 Fine-tuned CNN features . . . 65

3.3.2 Comparative analysis 66
3.3.2.1 Comparative analysis of traditional v/s CNN features 67
3.3.2.2 Comparative analysis of skewed datasets evaluation schemes . . 68
3.3.2.3 Comparative analysis with other CNN frameworks 68
3.4 Experiments and results: UQ-SNP Task-2 specimen dataset 69
3.4.1 Analysis of data-skew balancing strategy 70
3.4.2 Analysis of feature representation 72
3.5 Summary 75

4 Anomaly based Identification of Mitotic Patterns in HEp-2 Images 77
4.1 Literature review 79
4.2 Proposed framework 80
4.2.1 Feature representation scheme 80
4.2.2 One class classifier (OCC): One class support vector machines (OC-SVM) 82
4.3 Experiments and Results: UQ-SNP Task-3 dataset 83
4.3.1 Analysis of feature representation 84
4.3.1.1 Morphology, LM & Gabor filter bank features 85
4.3.1.2 CNN-based learned features 86
4.3.1.3 Fine-tuning of network 88
4.3.2 Comparative analysis 89
4.3.2.1 Comparison with BC-SVM 89
4.3.2.2 Comparison with CNN baseline classifier 90
4.4 Experiments and results: UQ-SNP Task-2 dataset 92
4.4.1 Analysis of feature representation 93
4.4.1.1 Random cross-validation (RCV) protocol 93
4.4.1.2 Leave-one-out (LOO) protocol 93
4.4.2 Comparative analysis 95
4.4.2.1 Comparison with BC-SVM (without data augmentation) 95
4.4.2.2 Comparison with BC-SVM (with data augmentation) 96
4.5 Summary 96

5 Identification of Mitotic Patterns: a Deep Metric Learning Approach 99
5.1 Literature review 101

5.2 Proposed framework . . . 102
5.2.1 Triplet Loss function . . . 102
5.2.2 Triplet Selection: Hard Sample Mining . . . 103
5.2.3 CNN-based embeddings . . . 104
5.2.4 Classification . . . 104
5.3 Experiments and results: UQ-SNP Task-3 dataset . . . 105
5.3.1 Comparative analysis . . . 107
5.3.1.1 Comparison with cross-entropy loss . . . 108
5.3.1.2 Comparison with other frameworks . . . 109
5.4 Experiments and Results: UQ-SNP Task-2 dataset . . . 110
5.4.1 Results with leave-one-out (LOO) protocol . . . 110
5.4.2 Results with random cross-validation protocol . . . 111
5.4.3 Comparison with previous frameworks . . . 113
5.5 Summary . . . 113

6 Specimen-level Strategies for Identification of Mitotic Patterns 115

6.1 Literature review . . . 116
6.2 Classification via DNN-based segmentation framework . . . 118
6.2.1 Classification framework: Cell-based approach . . . 120
6.2.1.1 Feature representation: Texture-based features . . . 120
6.2.1.2 Classifiers: Identification of individual cells . . . 121
6.2.1.3 Decision-making criteria for specimen images . . . 121
6.3 Classification framework: Region-based approach . . . 121
6.3.1 Feature extraction . . . 122
6.3.2 Analysis of probability maps . . . 122
6.3.3 CNN architecture and fine-tuning . . . 123
6.3.4 Decision-making criterion: Threshold on MS regions . . . 124
6.4 Classification framework: Image-based approach . . . 124
6.5 Experiments and results . . . 127
6.5.1 Dataset and evaluation protocol . . . 127
6.5.2 Results for segmentation based classification framework . . . 130
6.5.2.1 Classification performance on complete specimen images . . . 131

6.5.2.2 Classification performance with segmentation 131
6.5.3 Results for region-based approach 135
6.5.4 Results for image-based approach 136
6.5.5 Comparison of segmentation task with prior approaches 139
6.5.6 Comparison with existing approaches 140
6.6 Summary 143

7 Classification of Different Interphase Type Staining Patterns 145
7.1 Literature review 147
7.2 Proposed framework 148
7.2.1 Class-specific characteristics 149
7.2.2 Decision-tree based classification framework 151
7.2.3 CNN-based framework 153
7.2.4 Decision-making criterion for interphase specimens 154
7.3 Experiments and results 155
7.3.1 Experimental results for decision-tree based framework 155
7.3.2 Experimental results for CNN-based approach 158
7.3.3 Comparison between class-specific and CNN-based framework 160
7.3.4 Comparison with existing approaches 161
7.4 Summary 163

8 GAN-based Generation of Synthetic Minority Class Samples 165
8.1 Literature review 167
8.2 Proposed framework 169
8.2.1 Generative Adversarial Networks (GAN) 169
8.2.2 Modified DC-GAN framework 171
8.3 Experiments and results 173
8.3.1 Experimental Validation: classification (training) with original mitotic and generated samples 174
8.3.2 Experimental Validation: classification (training) without including original mitotic samples 176
8.3.3 GAN-based sample generation for cytoplasmic patterns 177
8.3.4 Subjective (clinical) validation by medical experts: The visual Turing test . 178

8.3.4.1 Details of visual Turing test 179
8.3.4.2 Results & analysis of visual test 181
8.3.5 Comparative analysis with oversampling approach 181
8.4 Summary 183

9 Conclusion & Future Research Directions **185**
9.1 Limitations of the framework 188
9.2 Future research directions 188

References **191**

List of Publications **204**