ISTANBUL TECHNICAL UNIVERSITY ★ INFORMATICS INSTITUTE

AN INNOVATIVE AND ACCURATE DEEP LEARNING BASED HER2 SCORING METHOD HER2-UNET

M.Sc. THESIS

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Informatics Institute

Computer Science Program

DECEMBER 2018

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İSTANBUL TEKNİK ÜNİVERSİTESİ ★ BİLİŞİM ENSTİTÜSÜ

HER2 TÜMÖR HÜCRELERİNİN SEGMENTASYON İÇİN DERİN ÖĞRENME TABANLI YENİ BİR YAKLAŞIM

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Fariba DAMBAND KHAMENEH, a M.Sc. student of ITU Informatics Institute 704161007 successfully defended the thesis entitled "AN INNOVATIVE AND ACCURATE DEEP LEARNING BASED HER2 SCORING METHOD HER2-UNET", which he/she prepared after fulfilling the requirements specified in the associated legislations, before the jury whose signatures are below.

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To my spouse and my family

FOREWORD

This thesis is written as a completion to the M.Sc. program, at Istanbul Technical University. The subject of this thesis, *An Innovative and Accurate Deep Learning Based Her2 Scoring Method Her2-UNet*, falls in the field of deep learning in digital pathology. Accomplishing the work presented in this thesis was challenging but so joyful and full of learning in a professional and friendly environment. Firstly, I would like to express my sincere gratitude to my advisor Mustafa Ersel Kamaşak for his patience, motivation and immense knowledge. His insights on how to perform and convey research had a huge influence on me. He has always been so supportive and the manner of his leadership made his students so confident in themselves and decision makers in the course of research, which I am not an exception to that. I would also like to thanks my team leader, Salar Razavi for being an excellent mentor during my work. Also, I am so grateful from Virasoft Company to provide the data.

Finally, I would like to thank my family for their love and support. They always encouraged me to pursue my dreams, which I'm so grateful to them.

November 2018

Fariba DAMBAND KHAMENEH
(Computer Vision expert)

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ABBREVIATIONS

HER2	**:** Human Epidermal Growth Factor Receptor 2
ISH	**:** In Situ Hybridization
DAB	**:** Diaminobenzidin
IHC	**:** Immunohistochemistry
WSI	**:** Whole Slide Image
ML	**:** Machine Learning
SVM	**:** Support Vector Machine
CNN	**:** Convolutional Neural Network
SLIC	**:** Simple Linear Iterative Clustering
LSTM	**:** Long Short Term Memory

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AN INNOVATIVE AND ACCURATE DEEP LEARNING BASED HER2 SCORING METHOD HER2-UNET

SUMMARY

Breast cancer is the second most common form of cancer among women in the US that leads to death. The uncontrollable growth of cells in the breast tissue causes breast cancer. Identification of biomarkers in tissues carry significant biological information. Evaluating the expression level in some biomarkers play an essential role in cancer diagnosis. Digital pathology proposes an appreciable way to prevail the non-objectivity by analysing the biological images. Immunohistochemistry (IHC) analysis is a method for demonstrating the presence and location of proteins in tissue sections which introduce new demands on the reproducibility, accuracy, and specificity of the extracted information. The automated analysis in Whole Slide Image (WSI) has recently achieved considerable attention because of the accessibility of digital slide scanners and the increasing importance of tissue-based biomarkers of stratified medicine. Several biomarkers have been identified for breast cancer. Normally, Human Epidermal Growth Factor Receptor 2 (HER2) proteins are responsible for division and growth of healthy breast cells. HER2 status is currently assessed using IHC as well as In Situ Hybridization (ISH) in equivocal cases. Manual HER2 evaluation of IHC stained microscopic images involves error-prone, tedious, inter-observer variable, and time-consuming routine lab work due to diverse staining, overlapped regions, and non-homogeneous remarkable large slides. To address these issues, digital pathology offers reproducible, automatic, and objective analysis and interpretation of WSI. Since HER2 is associated with tumors of an epithelial region and most of the breast tumors originate in epithelial tissue, it is crucial to develop an approach to segment different tissue structures. The proposed technique has comprised of three steps. In the first step, a superpixel based Support Vector Machine (SVM) feature learning classifier is proposed to classify epithelial and stromal regions from WSI. In the second stage, on epithelial regions, a Convolutional Neural Network (CNN) based segmentation method is applied to segment membrane regions. Finally, divided tiles are merged and the overall score of each slide is evaluated. Experimental results for 50 slides are presented and compared with state-of-the-art handcraft and deep learning approaches. The experiments demonstrate that the proposed method achieved promising performance on IHC stained data. Our automated algorithm was shown to outperform other approaches in terms of superpixel based classifying of epithelial regions and segmentation of membrane staining using CNN.

HER2 TÜMÖR HÜCRELERİNİN SEGMENTASYON İÇİN DERİN ÖĞRENME TABANLI YENİ BİR YAKLAŞIM

ÖZET

Günümüzde, meme kanseri, yaygınlaşan bir hastalık olarak incelenip, bilimsel çalış malara sıklıkla konu olmaktadır. Meme dokularında, mutasyona uğramış HER2 tümörlü hücreler, hücre çeperinde bulunan HER2 proteininin artışıyla tespit edilmektedir. Protein artışının görünür kılınıp patologlar tarafından değerlendirilebilmesi için, FISH (Fluorescent in Situ Hybridization) ve İmmuno Histo Kimya (İHK) tabanlı olmak üzere iki farklı boyama tekniği kullanılmaktadır. Bu tekniklerden, zaman ve maliyet açısından daha uygun olan ve ASCO / CAP 2013 önerilerine göre dört dereceli bir skor ölçeği içeren İHK yaklaşımı, meme kanseri tedavi sürecinin belirlenmesinde sıklıkla kullanılmaktadır.

Aşırı ekspresyonun analizi İHK'sal olarak yapılmaktadır. İHK 3+ olarak değerlendirilen hastalarda, aşırı ekspresyonu vardır şeklinde bildirilir ve bu hastalara istisnai durumlar haricinde Herceptin tedavisi uygulanır. Bu tedavinin uygulanabilmesi için hastanın İHK'sal skoru 3+ ya da 2+ olmalıdır. Diğer skorlar (1+ ve 0) negatif olarak değerlendirilir ve hastanın HER2 ekspresyonu normaldir şeklinde değerlendirilir. 3+ hastalarda Herceptin kullanılanbilir ancak, 2+ hastalar için kuşkulu yaklaşım sergilenir ve İHK'sal teste ek olarak, FISH tekniği ile yeni bir analiz yapılarak, kuşkulu skor desteklenir ya da negatif olarak değerlendirilir.
Çevresel membran hücre boyamasının tamamlanmamış olduğu durumlarda ve boyama zayıf (soluk) olduğu durumlarda, tümör hücrelerinin %10'un üzerinde olduğu saptanırsa, İHK 2+ olarak değerlendirilir. İHK 2+ değerlendirmeye alınan bir diğer durum ise, çevresel membran boyanmasının yoğun ve tamamlanmış olduğu ancak, tümör hücrelerinin %10'un altında yoğunluk gösterdiği durumdur. Eğer çevresel membran boyanması tamamlanmamış veya boyamanın az çok fark edilebilir nitelikte olduğu tümör hücrelerinin yoğunluğu %10'un üzerinde ise İHK 1+ olarak değerlendirilir. Çevresel membran boyanmasının tamamlanmamış olduğu ya da boyanmanın olmadıgı ve tümör hücrelerinin yoğunluğunun %10'un altında olduğu durumlarda ise, İHK 0 olarak de¨gerlendirilir. İHK 1+ ve İHK 0 sonuçları negatif, İHK2+ kuşkulu ve İHK 3+ pozitif olarak sınıflandırılır . Önerdigimiz yöntem ile İHK'sal olarak HER2 analizini otomatik gerçekleştirecek ve İHK'sal skorunu bildirecek bir çalışma anlatılmaktadır.

İHK ile boyanmış mikroskopik görüntülerin manuel HER2 değerlendirmesi boyanma çeşitliliği, üst üste binmiş bölgeler ve homojen olmayan çok büyük slaytın görülebilmesi sebebiyle hata yapmaya açık, zahmetli, gözlemciler arası değişken ve zaman alıcı rutin laboratuvar işleri işerir. Bu sorunları gidermek için dijital patoloji, tüm slayt görüntüsünün analiz edilmesinde ve yorumlanmasında, tekrarlanabilir, otomatik ve objektif bir değerlendirme sunar. HER2 epitel doku tümörleri ile ilişkili olduğundan ve meme tümörlerinin çoğu epitel dokudan kaynaklandığından, farklı doku yapılarını ayırmak için bir yaklaşım geliştirmek çok önemlidir. Bu tez

çalışmasında, meme kanseri görüntülerinde HER2'nin İHK skorunu otomatik olarak değerlerlendirme yapmak için bilgisayar destekli tüm slayt bazlı derin öğrenme metodunu tanıttık. Histopatoloji görüntülerinin yorumlanmasındaki subjektiflik ve patologlar arası uyuşmazlıklar nedeniyle, üzerinde anlaşılmış ve yinelenebilen methodların belirlenmesi gerekmektedir. HER2 değerlendirmesindeki bir önceki methodun aksine, bu araştırmada tüm slayt görüntülerinde, hücre zarının derin öğrenme temelli segmentasyonu kullanılarak HER2'nin sınıflandırılması gerçekleştirilir. Analiz sonuçları ağin membrane hücre boyanması ve sitoplazmik veya hatalı boyanma arasındaki farkı ayırt etmeyi başardığını gösterir. Test veri seti eğitim için kullanılmadığından, dikkate değer yüksek doğruluk, modelin hücre zarlarını doğru şekilde bölümlere ayırmayı iyi bir şekilde öğrendiğini gösterir. Patch'ler temel alarak slaytları skorlayan ve sınıflandıran diğer derin öğrenme metodlarına karşın, tüm slayt görüntülerini patologlar tarafından kabul edilen değerlendirme kılavuzlarını dikkate alarak değerlendirdik. Önerilen teknik üç adımdan oluşmuştur.

Birinci aşama olarak, bütün slayt görüntüleri üzerindeki epitel ve stroma bölgelerini sınıflandırabilmek için süperpiksel tabanlı öznitelik öğrenme sınıflandırıcısı olan Destek Vektör Makinası (SVM) kullanılmıştır. Bu tezin ilk bölümünde, epitel ve epitel olmayan (stroma) bölgelerini sınıflandırılmasında geleneksel makine öğrenmesi algoritması geliştirilmiştir. Sınıflandırmanın söz konusu olduvu problemin çözümüne yönelik algoritmalarda, SVM sıkça kullanılan bir makine öğrenmesi tekniğidir. Bütün slayt görüntülerindeki bölgelerin, ikili örüntü (LBP) ve renk histogramı gibi bazı doku ve renk öznitelikleri ekstrakt edilerek Destek Vektör Makinasına verilir. İlk olarak, görüntü analizinde temel ön işlem protokollerinin başında gelen normalizasyon işlemi uygulanır. Normalizasyon işleminin uygulanmasındaki amaç, kullanılacak olan her öznitelik türü arasındaki aralık farklılıklarının yarattığı yanlılıkları (bias) ortadan kaldırmaktır. Görüntü elde edilirken kullanılan farklı tarayıcılar ve farklı boyama tekniklerinden kaynaklanan problemlerin önüne geçebilmek için piksel yoğunluğu dağılımlarını standartlaştıran normalizasyon tekniği uygulanması gerekmektedir.

Normalizasyon tekniği uygularken kullanılan histogram dağılım değerini, belirlemiş olduğumuz spesifik bir referans görüntünün (Diaminobenzidin uygulaması) mavi ve kahverengi kanallarının histogram değerlerine bakarak elde ettik. Denetlenen sınıflandırıcıda, büyük slayt görüntülerindeki ilgili alanların (ROI'lerin) belirlenip, özelliklerine göre sınıflandırılıp etiketlendirilebilmesi için el yapıcı öznitelikler kullanılmıştır.

Bu aşamadan sonraki işlemler, büyük slayt görüntülerindeki tüm pikseller yerine, artık belirlenmiş süperpiksellere uygulanmıştır. Anlamlı ve benzer bölgelerin kümeselleştirildiği (gereksiz kısımların arıtıldığı) süperpikseller üzerinden öznitelik çıkarmak performansı arttırmakla kalmamış, sonraki sınıflandırma aşaması için değişken sayısını azaltmıştır. SVM, sınıflandırıcısını oluşturmak için gereken etiketlenmiş ve anote edilmiş veriler, konusunda uzman patalog tarafından temin edilmesi gerekmektedir. Patalog bu anotasyon işlemini gerçekleştirirken, İmmünohistokimyasal (İHK) bütün slayt görüntülerindeki karmaşıklığın önüne geçebilmesi için etiketleme işlemini küçük, detaylı ve tanımlayıcı epitel ve stroma bölgeleri üzerinde yapması gerekmektedir.

İkinci aşamada, epitel bölgeler üzerinde, Konvolüsyenel Sinir Ağları (KSA) tabanlı segmentasyon yöntemi membranöz bölgeleri bölütlemek için kullanılmıştır. KSA genellikle sınıflandırma işlerinde kullanılır, fakat UNet mimarisi karmaşık yapıları

sınıflandırmak ve bölümlere ayırmak için insan görsel algılama sistemi tarafından tanınabilir yerelleştirilmiş öznitelikler ekstrakt eder. Bu öznitelikler, UNet'in her piksele etiket atamayı mümkün kılan alt örnekleme özelliği kullanılarak sağlanır. Biyomedikal uygulamalarda, karmaşıklık ve yüksek veri toplama maliyeti gibi sebeplerden dolayı, dijital analiz ortamda insan gözüne yakın seviyede sonuçlar elde edilmeye çalışılırken problemler yaşanmaktadır. Makine öğrenmesi metotlarıyla, çok az sayıda eğitim örneği ile iyi sonuçlar elde edilerek bu zorlukların üstesinden gelinebilir.

Anote edilmiş eğitim görüntülerini eğitebilmek için Keras kütüphanesini kullandık. UNet mimarisi çok az sayıda eğitim görüntüsünde çalışabilir hâle gelebilmek için değiştirilen ve genişletilen her evrişimin geçerli bölümünü kullanır. Mevcut ağı desteklemek ve başarılı bir sonuç almak için, pooling öperatörü yöntemi yerine üstörnekleme yöntemi kullandı. Üstörnekleme yöntemi her bir girdinin çözünürlüğünü arttırmış oldu. Daha yerel ve kusursuz sonuç elde edebilmek için yüksek çözünürlüklü öznitelikler ekstrakt edildi. UNet mimarisi her bir kıvrımın sadece geçerli parçasını kullanır. Tüm parça bölütleme haritasının piksellerini barındıran girdi resminde mevcuttur. UNet aşağı örnekleme (solda) ve üst örnekleme olarak bilinen geniş bir patika yolundan oluşur. İki adet 3×3 konvolüsyon devamlı olacak şekilde ve her birinin ardından Rectified Linear Unit (ReLU) uygulanılacak şekilde kullanıldı. Ayrıca mimari, 2 birim kaydırmalı ve 2×2 maksimum pooling operatörü içermektedir. Her alt örnekleme adımında, kanallardaki öznitelik sayısıiki katına çıkar. Bunun sonucu olarak, her geniş haritadaki özellik haritasının bir örneklemesi 2×2'lik üst evriçim içermektedir. İstenilen sayıda sınıfı bulabilmek için 1×1'lik tabakanın her 64 bileşen özniteliği vektörünü son tabakada işlemesi gerekmektedir. Mimari toplamda 23 konvolüsyenel tabakaya sahiptir. Sonuç olarak, bölünmüş fayanslar birleştirilir ve her slaytın toplam puanı değerlendirilir. 50 slayt için elde edilen sonuçlar, el yapımı öznitelikler ve derin öğrenme öğretileriyle karşılaştırılır. Her bir fayansın ayrı olarak işlenmesinden sonra, tüm fayans sonuçları birliştirilip, genel bir skor elde edilir.

Bölme işlemi esnasında, bazı hücre çekirdekleri kusurlu olan ve normal olan hücre çekirdeği diye ayıklanır. Bu ayıklanma sürecinde bazı zorluklarla karşılaşılmıştır. Hücre çekirdekleri iki fayans bölgesinin arasında kaldığında problemlem oluşabilmektedir. Olabilecek bir yanlışlıktan kaçınmak için, küçük fayans bölgeleri köşe tarafları gözetilerek ekstrakt edilmiştir. Yataysal birleştirilme yapılırken, sağdaki görüntüyü içeren fayans bölgesinin sol kısmının diğer fayansa yakın olan parçasıile, soldaki görüntüyü içeren fayans bölgesinin sağ kısmının sağdaki fayansa yakın olan bölgesi kombine edilmiştir. Buna benzer olarak, dikey birleştirme aşamasında da, üst görüntüyü içeren fayans bölgesinin alt kısmı ile, alt görüntüyü içeren fayans görüntüsünün üst kısmı birleştirilmiştir.

Elde edilen deneyler, boyalı veriler içeren İHK görüntüleri üzerinde, önerilen algoritmamızın ümit verici performans elde ettiğini göstermektedir. Geliştirmiş olduğumuz yazılımımızın, diğer literatürde bulunan epitel bölgelerideki süperpiksel bazlı sınıflandırma yöntemlerini içeren ve KSA kullanılarak yapılmış membranöz hücre boyamaların bölütlenmesinde kullanılmış diğer algoritmaları içeren yaklaşımlardan daha iyi performans gösterdiği gözlemlenmiştir.

1. INTRODUCTION

Cancer has been a major health problem worldwide, for a long time, and yet the incidence of this group of related diseases is increasing. There were an estimated 3.9 million new cases of cancer and 1.9 million deaths from cancer in Europe in 2018. The most common cancer sites were cancers of the female breast, followed by colorectal, lung and prostate cancer [1]. Breast cancer is the most prevalent form of cancers among women. Currently, based on American cancer society (ASCO), there is a 1 in 8 American women would develop breast cancer in their life. Due to the extensive research, new treatment methods and drugs have been developed, and diverse molecular mechanisms affecting the onset and development of breast cancer have been discovered in the past decades. Several prognostic and predictive markers have been established. The most important and widely researched molecular factors is Human epidermal growth factor receptor 2 (HER2), estrogen receptor HER2 could play an important role in the development of breast cancer. HER2 proteins are responsible for how cells grow and divide. Therefore, HER2 therapy in combination with chemotherapy or/and endocrine therapy could be the most efficient treatment. Furthermore, in breast cancer treatment, trastuzumab and lapatinib therapies are implied to be effective in HER2 amplified cases. The traditional system of HER2 breast cancer assessment deficits from accuracy on detection of correct patients overexpressing HER2.

1.1 Purpose Of Thesis

The prevalence of HER2 overexpression is associated with invasive breast cancer in about 20 percent of breast cancers. Admittedly, precise and fast HER2 assessment is crucial to consider the appropriate action for patients. The HER2 biomarker is over-expressed, amplified, or both, in 15%-20% of high-grade invasive breast cancers and has been associated with fast tumor growth, increased risk of recurrence after surgery, and poor response to shortened survival [2]. Quantitative image analysis

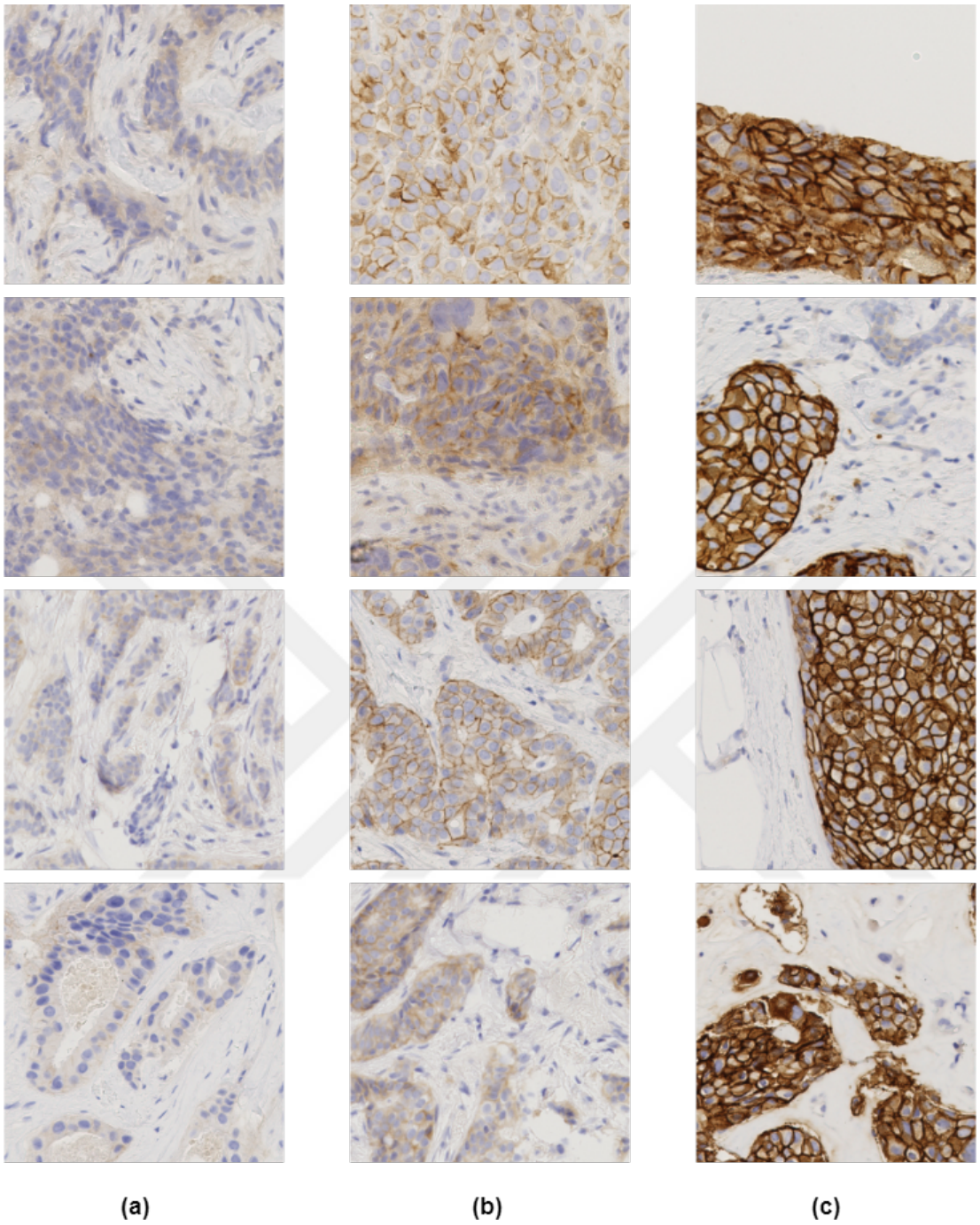

Figure 1.1 : Sample breast tissue images with (a) Score 0/1+, (b) Score 2,and (c) Score 3.

of digitalized slides decreases human error, increases the accuracy of diagnosis, reduces the workload of pathologists, and standardizes scoring systems. In HER2 assessment of IHC slides, to address ambiguities and subjectivities of manual scoring, computer-aided solutions are provided to simplify the overall progress. With the advent of image analysis in digital pathology, a huge interest has focused on digital slide scanners to process and evaluate typical pathology lab workload in a digital, fast,

Table 1.1 : Evaluation criteria for HER2 (ERBB2) protein expression by IHC assay of the invasive component of a breast cancer specimen.

Specimen Staining Pattern	Score	Classification
Incomplete membrane staining that is faint or barely perceptible and within $\leq$ 10% of the invasive tumor cells or no staining observed	0	Negative
Incomplete membrane staining that is faint/barely perceptible in $\geq$10% of tumor cells	1+	Negative
Weak to moderate complete membrane staining observed in $\geq$10% of tumor cells	2+	Equivocal
Circumferential membrane staining that is complete, intense and in $\geq$10% of tumor cells	3+	Positive

accurate, and efficient way. According to ASCO/CAP guideline [3] shown in Table 1.1, in IHC slides if more than 10% of the whole tissue comprises strong tumor cells the case displays 3+ which is accepted as positive and are allowed for therapies. If the ratio for moderate tumor cells is more than 10% the case is considered as equivocal 2+ and reflexed to ISH test to assess HER2 status. In no staining or weak conditions, the case is HER2 negative. Some tissue samples have been shown in the Figure 1.1. Recently, machine learning methods have considerably enhanced the ability of computers to automatically diagnose various components in biomedical images. Investigating and processing microscopic images is an extremely tedious and time consuming procedure. Computer based machine learning tools assists pathologists to work on stained tissues to segment, recognize, classify, recognize, and reveal important information about the samples. In HER2 membrane scoring, it is important to count the number of closed and open membranes that are in epithelial area. In this thesis, we combined both local color histogram and LBP features together to build an automatic classification solution to reduce inter-observer variability and increase the accuracy of the procedure. The main reason to do this is to remove tiles that contain white pixels and reduce the computational complexity. In a normal slide about 30 percent of tiles with pixel size 512$\times$512 are redundant and empty. Hence we can increase the speed of the deep learning task by skipping aside redundant tissue blocks. In the patients with breast cancer, HER2 state is critical as the variations would specify

the type of therapies. IHC is a special staining method to discover HER2 protein in the cancer cells that works based on detecting particular antigens in tissues. The IHC stained slides are comprised of brown channel (diaminobenzidine DAB signal) and counterstain blue-violet channel (hematoxylin signal). This color based reaction produces different structures like nucleus, membrane or cytoplasm. In IHC HER2, the membrane and nuclei detection methodologies are performed on the brown and blue channels, respectively. Therefore, extracting ROI depends on color information of the superpixels. We use local color histogram and distribution of superpixels in clustered uniform patterns. The color bin of each histogram is computed based on areas containing pixels in superpixels. The LBP algorithm is a robust and powerful descriptor that thresholds the neighboring pixels based on a center pixel. Here, we would consider each superpixel separately and evaluate the histogram of each pixel. This uniform LBP achieves rotation invariant descriptor of the pattern. The histogram of the pixels inside a superpixel is concatenated and for each superpixel a unique feature and label is assigned.

1.2 Literature Review

The recent studies report a wide range of automated methods for HER2 scoring algorithms. The ImmunoMembrane application [4] is an open source software for digital image analysis of HER2 IHC. ImmunoMembrane analysis the completeness and intensity of the cell membrane staining reaction, based on the IHC interpretation criteria of the ASCO/CAP guidelines [5] [6].

IHC analysis is a method for demonstrating the presence and location of proteins in tissue sections which is placing new demands on the reproducibility, accuracy, and specificity of the extracted information [7]. The automated analysis in Whole Slide Image (WSI) has recently achieved considerable attention because of the accessibility of digital slide scanners and the increasing importance of tissue-based biomarkers of stratified medicine [8]. Fernandez et al. [9] proposed a method to deal with the color variation and distortion problem by describing a density tool that has been implemented to measure the positive IHC stain areas in WSI. Among pixel-based clustering methods, Simple Linear Iterative Clustering (SLIC) [10] is studied in histopathological segmentation tasks. SLIC is a clustering method that agglomerates

similar and nearby pixels and is accepted as a superior method in terms of accuracy and efficiency. Jiří Borovec [11] employed SLIC as a preliminary step in histopathological images to increase the efficiency of Graph-cut method. In [12], epithelium and stroma regions in histopathological images is segmented using a hierarchal fuzzy c-means method. Babak *et al.* [13], detected regions of interest in whole slide images using a multi-scale superpixel classification approach that classifies at different scales based on the acquired details of the region of interest (ROI). Authors in [14] also used texture features to classify malignant and benign breast histopathology images. Most of the developed approaches are related to automate classifying of Hematoxylin and Eosin (H&E) tissue images [15]. In automated HER2 assessment from IHC slides, several classical and handcrafted approaches are presented [16] [17] [18] . Most of these methods are about threshold based approaches as in [19] by using an optimal threshold value the percentage of the stained area and the score is evaluated. In a work by Morteza *et al* [20], a WSI based classifier using robust local binary pattern LBP and characteristic features is provided. The extracted characteristic is scored through a naive rule-based classifier. The rotation invariant LBP are used to classify selected ROI. A membrane connectivity based algorithm that automatically specifies the HER2 status in preselected sections of the tissues is presented in [21]. The method segments brown pixels and each slide is scored using the skeletonized connected membrane. In [22], basolateral membranous activity and neoplastic cell count are evaluated using segmentation and thresholding methods and the results of the computer-aided analysis are compared with manual evaluation. In another study [23], manual outlined ROIs are transformed into HIS color space and various features are extracted to train a super vector machine classifier. In the test step, the classified image was classified through a voting system.

In IHC membrane staining, one of a most challenging issue is the reconstruction of the membranes that are not revealed. A method based on nuclear membranes and approximating cellular membranes to automatically detect bounding membrane of each cell is available in [24]. In Immunumembrane, a set of thresholding, morphology, segmentation, and a point-based membrane evaluation is presented. Recently, state-of-the-art approaches like deep learning have intensely attracted the attention of researchers. Due to rapid growth in large medical datasets, new interesting machine learning challenges rises which are supposed to give promising results under

uncertain conditions.

During the recent few years, deep learning has gained a central position toward the automation of our daily life and delivered considerable improvements as compared to classical machine learning algorithms. Based on their improvement in performance, most researchers believe that within next 15 years, deep learning based applications will take over human and most of the daily activities with be performed by autonomous machines. However, penetration of deep learning in healthcare especially in medical image analysis is quite slow compared to the other real world problems.

Deep learning is a computational model that resembled from human cognition system that can be used efficiently in different applications [25]. In practice, an artificial neural network that has more than one hidden layer could be considered as a deep learning architecture. Currently, medical imaging and digital pathology community is showing increasing interest in deep learning as demonstrated by various studies.

CNNs that are one form of deep learning, have been well suited to medical data and have been incorporated successfully in different segmentation [10, 26–28], classification [27, 29–31], and detection [29, 32, 33] tasks.

Among deep learning models, CNN is the most commonly studied method in medical image understanding tasks. One of the implementations of CNN in HER2 assessment was presented in [34], where detected cells from IHC stained tissues are classified after some morphological operations.

The proposed method was based on whole slide cell classification using CNN and the results were considerably better than classical machine classification methods. In [35], 128×128 blocks of four labels at a low resolution are considered as training data. For each slide, the ratio of blocks with each label to total blocks is considered to determine the HER2 score for a WSI.

The proposed methods in deep learning for HER2 assessment are all about cell [34] or tile based classification [36] . The best of knowledge, for HER2 scoring, none work has been done based on segmentation of cell membranes. Ideally, deeper architectures in CNN represent better results. In [36], long short term memory (LSTM) architecture is proposed to detect cell membrane and nucleus in small patches. However, in HER2 assessment usually WSI is considered to evaluate the overall result.

1.3 Hypothesis

In this thesis, we propose a novel architecture for HER2 assessment of IHC biomarker. Our architecture exploits a simple linear iterative clustering (SLIC) clustering, SVM classifier, and CNN segmentation. We would investigate different variables which take into account segmentation of epithelial area as well as classifying WSI as positive, equivocal, or negative. The main goal of this study is (1) to segment and classify epithelium areas from stromal parts of slides correctly and (2) to apply a precise membrane segmentation using a convolutional auto-encoder that unlike sliding-window convolutional networks, relies on a strong data augmentation that efficiently trains with very few annotated samples and leads precise segmentation.

2. METHODS AND DATA

In Figure 2.1 the overview of the proposed methodology is shown. The proposed method for HER2 scoring consists of preprocessing procedures, patch-based preprocessing, feature detection, and techniques to score the WSI.

2.1 Proposed Method

One of the important steps in automatically analyzing the WSI in immunohistochemical images is discriminating between epithelial and stromal tissues. In the first part of this thesis, the traditional machine learning algorithm is developed for classifying the epithelial and non-epithelial areas. The SVM classification is a widely algorithms in classifying techniques. It is applied by employing some texture and color features such as LBP and color histogram. An illustration of tissue segmentation part is presented in Figure 2.2. In addition to the reasons mentioned above, considering other factors

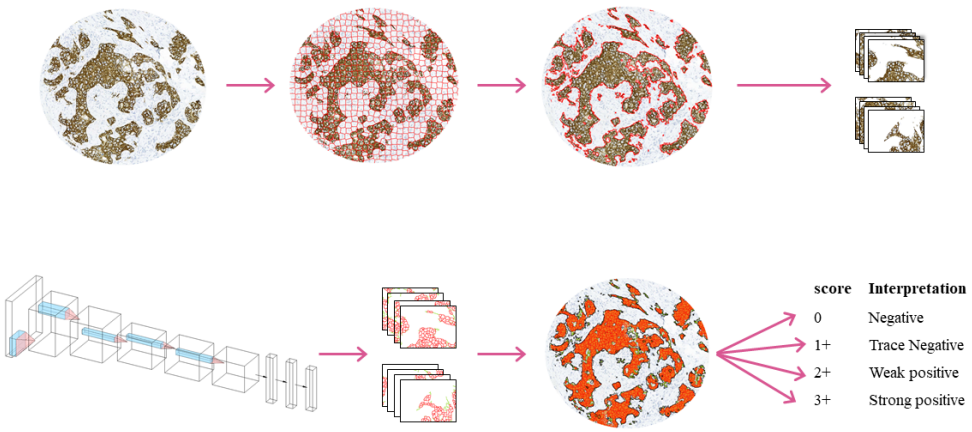

Figure 2.1 : The diagram of the applied method to segment cell membrane in HER2 stained IHC specimen which is the combination of deep learning and traditional machine learning algorithms. First, superpixel breaks the image to manageable parts. Hand crafted features are extracted to classify each superpixel to epithelial or stromal. Deep learning part in is an end-to-end method that takes images as input and learns a U-net model to produce segmentation result. The WSI merged from all tiles is obtained to get overall score of the specimen.

of the efficiency beside consistent magnification level could also substantiate. To delineate, CNN based solution reaches to higher accuracy compared to traditional image processing methods. To be impartial, working with various datasets would result in a rich trained model which would segment membranes at various zoom level and size.

2.1.1 Normalization

Normalization is an essential preprocessing step in image analysis. Normalization is applied in order to eliminate biases created by the range differences between each feature type. As the image acquisition is performed by different scanners with different staining, normalization should apply in order to standardized the range of pixel intensity. We have used histogram of one specific image in three channels of diaminobenzidine (DAB) staining. We normalize the images based on the histogram of the blue and brown channels of that specific reference image.

2.1.2 Tissue segmentation

Regions of cancer are typically obtained from the epithelium parts so separating epithelium and stroma leads to better statics through analysis. The supervised classifier uses handcrafted features to determine and specify regions of interest for each WSI. In [37] they have presented a machine learning based approach for detecting metastatic tissue regions that accomplishes in blockwise detection of breast cancer metastases from lymph node tissue sections. It was applied for hemotexolyin and eosin WSIs. They have divided each image to the small blocks and they have used Random forest method for classifying the blocks into metastases from lymph nodes. In our proposed method the entire processes such as feature extraction, and training and predicting are applied in the superpixels instead of working on all pixels. Extracting features from superpixels on meaningful and similar regions not only to increase the performance but also decreased the number of variables for the subsequent classification step.

2.1.2.1 Superpixel

Simple Linear Iterative Clustering (SLIC) [10] is a clustering method that clusters pixels according to their color and distance space to create similar areas named as superpixels. Rich quality segmentation, consistent size, and low computational cost

are the most prominent advantages in comparing of other feature extraction methods. This approach performs based on 5-dimensional feature space defined by [*labxy*] space in which *l*, *a*, *b* are the values of CIELAB color space and *x*, *y* stand for coordination of the point. This method is a special form of k-means clustering adapted to local uniform group of pixels without redundant distance calculations. We have used SLIC for generating superpixels due to its strong performance and better adherence to the boundaries. And also using superpixel map is computationally efficient as it reduces the complexity of examining thousands of pixels to only a few hundred superpixels. It is also perceptually meaningful because each superpixel has uniformity. Also, each superpixel considers as a uniform unit, especially in their color and texture.

2.1.2.2 Manual sampling or using previous models

Training superpixels were selected by a pathologist as normal and tumor tissues. These negative and positive samples were given as a training dataset to the classification model. Sufficient amount of samples should be selected from different kinds of regions for each category in order to train a robust model. Consequently, the trained models can be used for other samples without the repeat of manual sampling.

2.1.2.3 Feature extraction

Each image has a special texture and color features. Texture and color are considered as the main features of any image. The texture of each tissue was further described using local binary patterns (LBP) and Gray level co-occurrence matrix (GLCM). In addition to the texture features, mean color and color histogram features are extracted from each image by considering each channel in RGB and HSV color spaces.

2.1.2.4 Classifier

To assess the performance of epithelial and stromal area classification using the local color histogram and LBP superpixel-level features a support vector machine (SVM) classifier is applied to predict the label of superpixels. For implementing SVM, LIBSVM library is required. In order to apply SVM on the superpixels, a ground truth needed and this ground truth obtained by the pathologists that manually sampled each superpixel to stromal and tumoral (foreground and background). To build the SVM classifier, labeled training data provided by an expert is desired. Due to the complexity

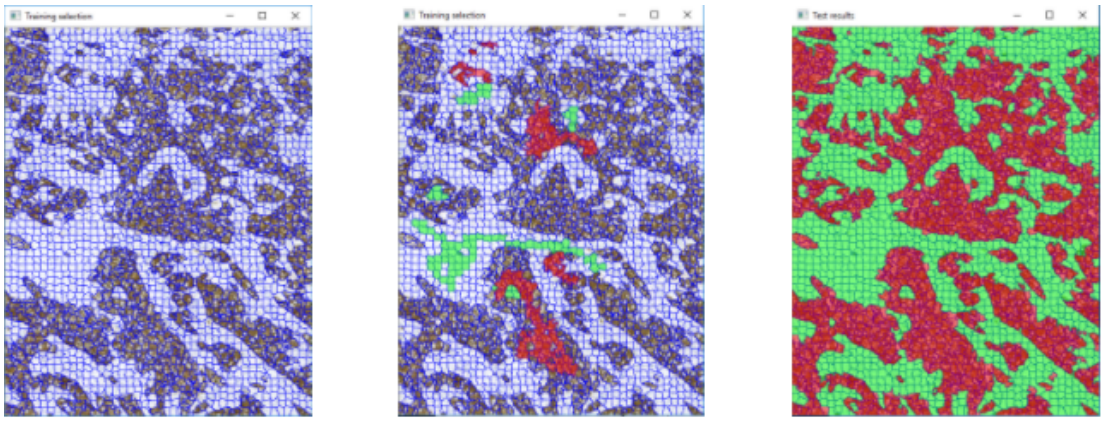

Figure 2.2 : Example of tissue segmentation by SVM classifier on the selected area. (A) Image generated by SLIC algorithm. (B) Labelling superpixels as tumor and normal tissues. (C) Result of the SVM classification.

of the whole IHC image, a pathologist should label small and descriptive epithelial and stromal patterns to segment the whole slide. This process is performed once as we would use this trained model for further samples.

2.1.3 Tile extraction

Loading excessively large images into the memory is one of the fundamental challenges in the digital pathology. For instance, a typical image can have 200000 $\times$ 100000 pixels. An obvious approach of manipulating the WSI is dividing the image into smaller tiles. The method should apply in the region of interest (ROI) that reaches from the tissue segmentation. Since the ROIs have an extremely high

Figure 2.3 : (a) A sample of WSI. (b) Result of tissue segmentation which is extracted as a mask. (c) Extract tiles into the none overlapping 512$\times$512 images.

resolution, loading such images are extremely difficult for an application. To overcome

the memory usage problem in WSI, the best solution is to keep the images as small as possible and process images in parallel. None overlapping tiles has been extracted as 512 $\times$ 512 images in 0.23 μ / pixels (25$\times$ magnification) to be examined separately. Images from different microscopes with diverse formats have various zoom levels so working on μ / pixels gives us reliable and constant proximity to slides. In general, each slide is divided into about 7k tiles as shown in Figure 2.3.

2.1.4 UNet model

UNet uses the valid part of each convolution which modify and extend for working on very few training images. Upsampling have used instead of pooling operator to supplement a usual and successive contracting network. Using Upsampling cause to increase the resolution of the input in each layer. High resolution features are extracted and combined to localize more precise output. UNet only uses the valid part of each convolution. The full context is available in the input image which contains the pixels of the segmentation map.

UNet consists of contarcting path which is downsampling (left) and an expansive path which is known as upsampling. contracting side works like usual architecture of a convolutional network. Continual application of two 3$\times$3 convolutions is utilized and each of them followed by Rectified Linear Unit (ReLU). It is also has a 2$\times$2 max pooling operation with stride 2. The number of feature channels is doubled in each downsampling step. Consequently, an upsampling of the feature map in each expansive map contain 2$\times$2 up-convolution. At final layer a 1$\times$1 layer is required to map each 64 component feature vector to find the desirable number of classes. The network has 23 convolutional layers entirely. CNNs are typically used for classification tasks, however the UNet architecture extracts localized features recognizable by human visual system to classify and segment complex structures. This is provided by the downsampling feature of the UNet that makes it possible to assign a label to each pixel. Furthermore, the success of convolutional networks are usually dependent on the size of training dataset. In biomedical applications, due to complexity and high cost of data collection, machine learning methods would work elegantly with very few training samples. In UNet by acquiring upsampling layers instead of pooling layers the resolution of the input image is increased which enables the successive convolution layer to learn

more accurate result. In this type of network architecture, low-level feature maps are combined with higher-level ones to precisely locate. We considered training images with corresponding annotations to train our network with implementation of Keras. To use GPU memory efficiently, we favor large input tiles over a large batch size and discard white empty tiles eliminated from classification part. The segmentation problem for HER2 is assigning one and zero pixels to each pixel in each tile. The network architecture is illustrated in Figure 2.4. It is consisted of two encoding (left) and decoding (right) sides. The encoding path is comprised of repeated convolutions, followed by rectified linear unit ReLU and max pooling layers. In right side after upsampling features maps, a cropped feature map from encoding part is concatenated which is followed by convolution and ReLU layers. Finally, a convolution layer with 1×1 size is used to map 64 component feature map to the number of output class.

2.1.5 Merging tiles

After processing in each tile separately, all tile's result should be merged to get the total result. During the dividing step, some of the cells have been divided into the defective cells. To avoid this fallacy, the small tiles (127×127) are extracted by considering edge parts of each tile. During a horizontal merge, the left-most part of the right image is combined with the right-most part of the left image. Similarly, during a vertical merge, the bottom-most part of the top image is combined with the top-most part of the bottom image. Extracted parts are wide enough to encompass the largest possible cell size so that no cell will be cut in half. The algorithm is run in this newly created region, but only the particles (complete cells and negative nucleus) that intersect with the center line are considered for calculations and overlays. The center line in the combined image corresponds to the merging edges of the original tiles. While processing each tile, particles that are found to intersect the edges are excluded from the calculations, thus making sure that no particle will be considered twice. Each will be considered only once, either in the original tiles or in one of the generated merging tiles. Figure 2.5 shows the merging results that consider the edge parts of right and bottom tiles.

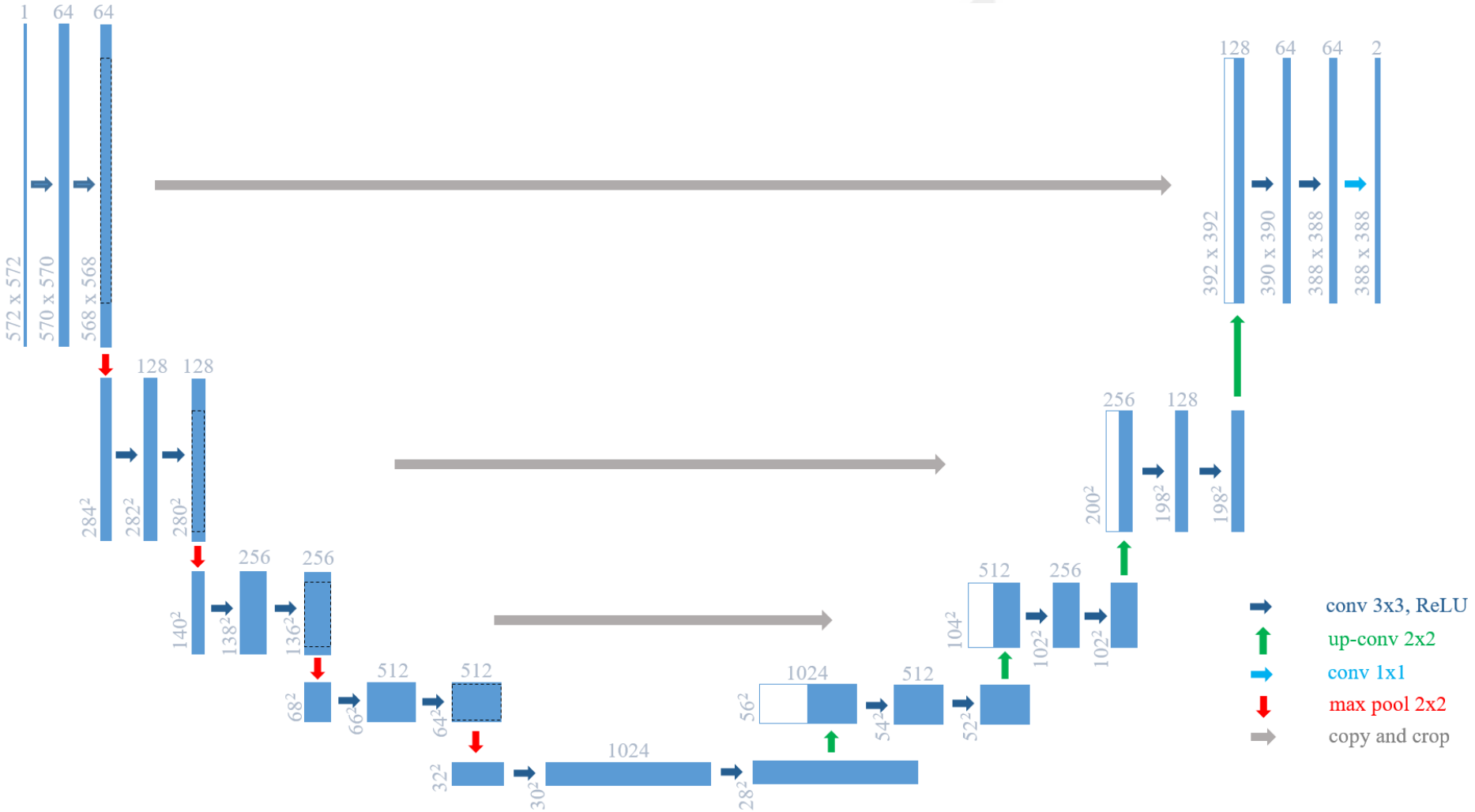

Figure 2.4 : HER2/neu image fragments and corresponding segmentation results of deep learning output compared to ground truth.

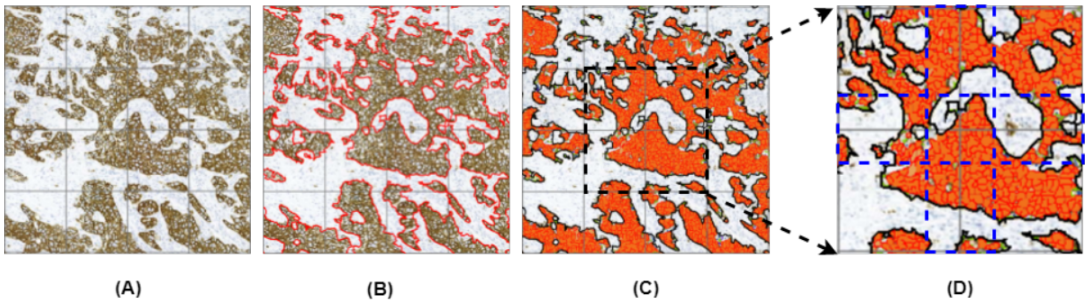

Figure 2.5 : Each tile considers its right and below edge tiles. (A) The original image. (B) Result after applying Tissue segmentation. (C) Applying merging method by considering neiboring tiles. (D) Four tiles are shown demonstrating the connectivity of the complete and incomplete membrane staining in the edge parts of each tile.

2.1.6 Dataset

The dataset consists of 100 WSIs of breast tumor patients which are gathered from ACIBADEM hospital and Warwick competition. The dataset from ACIBADEM hospital was acquired using a 3DHISTECH scanner while dataset from the University of Warwick was scanned using a Hamamatsu NanoZoomer C9600. Out of 52 slides from 100 WSIs were from Warwick dataset and were stained using HER2 antibody. The other 48 were data acquired from ACIBADEM Hospital. The size of the slides were about 150000×100000 pixels. The size of each tile was 512 × 512 which was automatically given to UNet architecture to get output results. The overall result was evaluated after merging tiles with corresponding neighbors (Upper, left, right, bottom, and corner if available) [38]. For membrane in edge parts, a similar approach is employed. This process is important as the membrane in the edge part would be counted as open membrane and the overall results would be wrong.

2.1.7 Implementation details

The process of training and testing of CNN is implemented on the GPU because its time efficiency is more than CPU. The graphic card is NVIDIA Geforce GTX 1028 and 2GB memory. I have used Tensorflow is used as a framework which is open-source in python. In addition, Virapath viewer from Virasoft Company is used.

3. EXPERIMENTS AND EVALUATION

The dataset and ground truth presented by Acibadem hospital are utilized for the training of the model. However, the performance of the proposed method is evaluated on two different datasets. The University of Warwick provided a dataset with FISH and HER2 IHC scores. For testing, the proposed method is applied in the Warwick dataset. The result of the proposed method and the provided results from Warwick dataset are compared in Table 3.1. The result from the proposed method matches with values from FISH in 2+ cases, in all of the 3 equivocal cases. In these 3 conflicting cases, pathologists diagnosed equivocal case where they were scored as positive by automated image analysis solution. Correct analysis of 2+ cases are the common challenging difficulty in HER2 IHC test and the assuring results show the efficacy of the methodology. The HER2 status determined by the combination of machine learning method and by pathologists confirmed the accuracy of the automated image analysis solution. There are some kinds of literature in CNN which score patches individually based on their staining intensity. In the proposed method, the algorithm learns membrane intensity as a feature to discriminate between diverse tumor cells. The high affinity between the results of automated scoring using CNN and manual scores by pathologies represents the feasibility and reliability of the HER2 scoring approaches.

In Figure 3.4, detected cell membrane and corresponding ground truth of some sample tiles from Acıbadem dataset are illustrated. Here column (a) represents the original images from different slides with various scores. Column (b) illustrates the ground truth cell membranes acquired by pathologies and confirmed with conventional image processing methods. In column (c) the uncompleted cell membrane is dissolved using morphology pruning operations. In column (d) detected cell membrane by the proposed method is shown in Figure 3.1. The performance of the method is obtained by statistical evaluation method. Dice coefficient result for 1000 tiles with 512×512 pixels among pathologists and the proposed method is 0.98 with a standard deviation

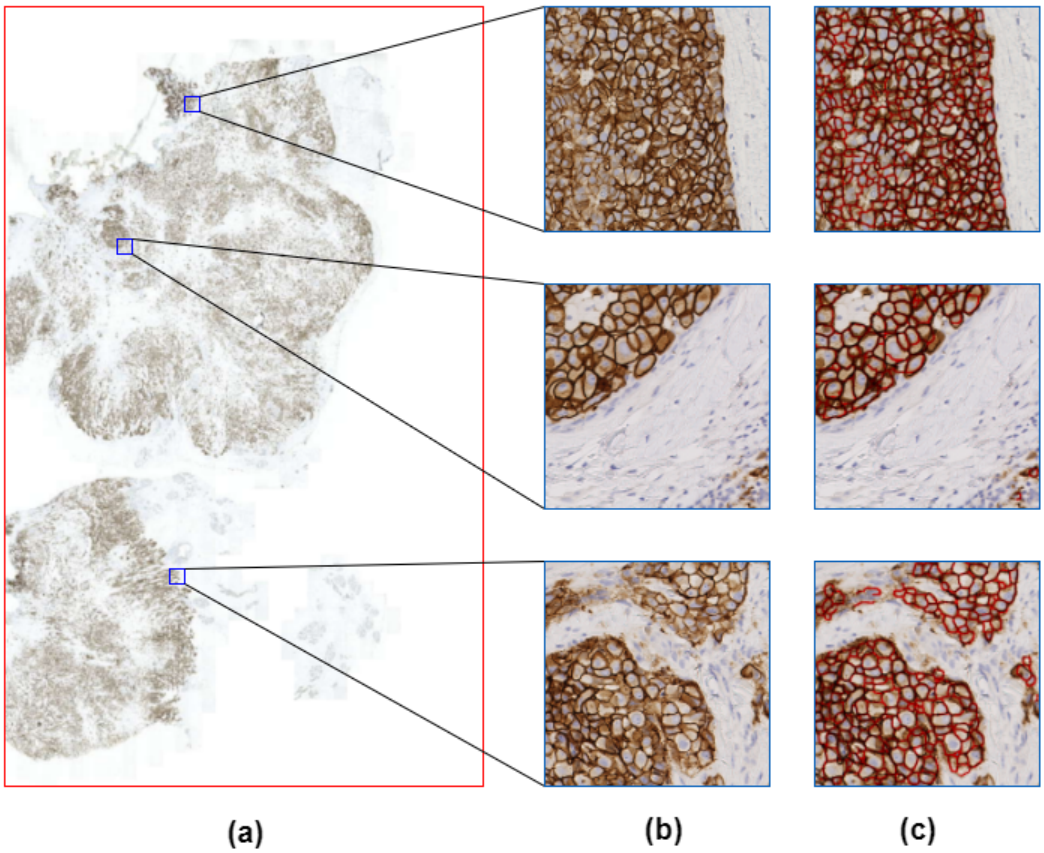

Figure 3.1 : Result images with different image characteristics from the test dataset . All images show that the membrane borders of each cell is enclosing a specific nuclei.

of 0.06 that shows a vigorous segmentation. The Dice coefficient values range between 0 (not overlapped) and 1 (perfect overlapped).

Table 3.1 : Confusion matrix compares the results of the deep learning based classification method with provided scores from Warwick dataset.

		Actual		
		0/1+	**2+**	**3+**
Predicted	**0/1+**	23	3	0
	2+	0	10	3
	3+	0	1	12

In segmentation tasks, true positive, false positive, false negative, and true negative are represented as the intersection between segmentation and ground truth, segmented parts not covering the ground truth, missed parts of the ground truth, and parts of the image beyond the union segmentation plus ground truth, respectively. To achieve higher accuracies and lower training losses we analyzed various CNN architectures were analyzed. Different combinations of kernel sizes and convolutional layers are

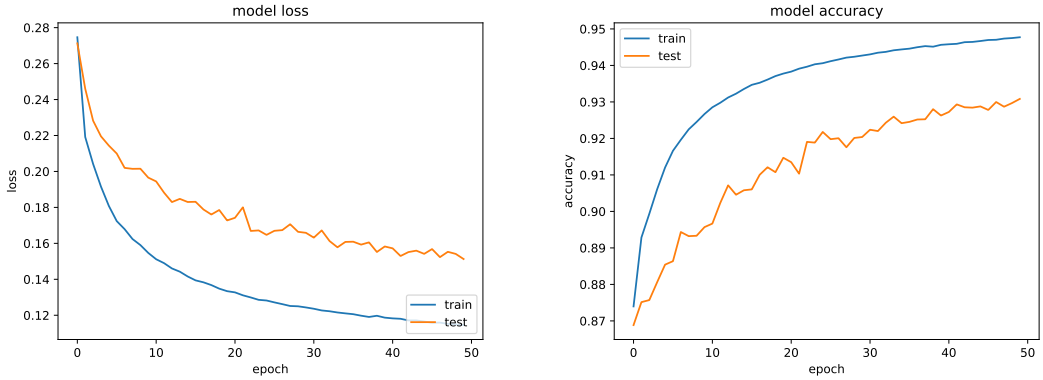

Figure 3.2 : Accuracy and learning curves for training and validation steps.

tested. For the experiments, three models were considered. In the first model, convolutional layers are used with filter sizes 3×3 and 3×3. In the second model, convolutional layers with 3×3 and 5×5 sizes are employed. In the third model, the convolutional kernels of size 5×5 are followed by 3×3. As shown in Table 3.3, an architecture with higher convolutional kernel size followed by smaller kernel size leads to better results presumably because of the importance of a larger neighborhood in pathological images.

The value of the loss function and accuracy on the training and validation sets of the proposed model has shown in the Figure 3.2. Because of data augmentation, the test accuracy is similar to the training accuracy which means that the model is generalized well and it is not overfitted. The appropriate learning rate increases the accuracy curve of training is a sustained way.

Only after 20 epochs in the training process, the model has been generated. This architecture enables the employment of CNN models with higher accuracy for scoring of IHC stained images to evaluate HER2 score. From the values of the false positive rate which wrongly indicates the membrane overlapping area and true positive rate where the model precisely predicts the membranes, the ROC curve of this model can be calculated and the proposed model provides a high area under the curve (AUC) value (88%) Figure 3.3. In order to score HER2 IHC slides, we have to consider WSI and evaluate about 3000 tiles in a short time. The computation time of an average WSI is less than 500 seconds. Table 3.2 shows the dice coefficient performance of the proposed architecture for different training and validation splits. The same test images from our dataset were used for all evaluations.

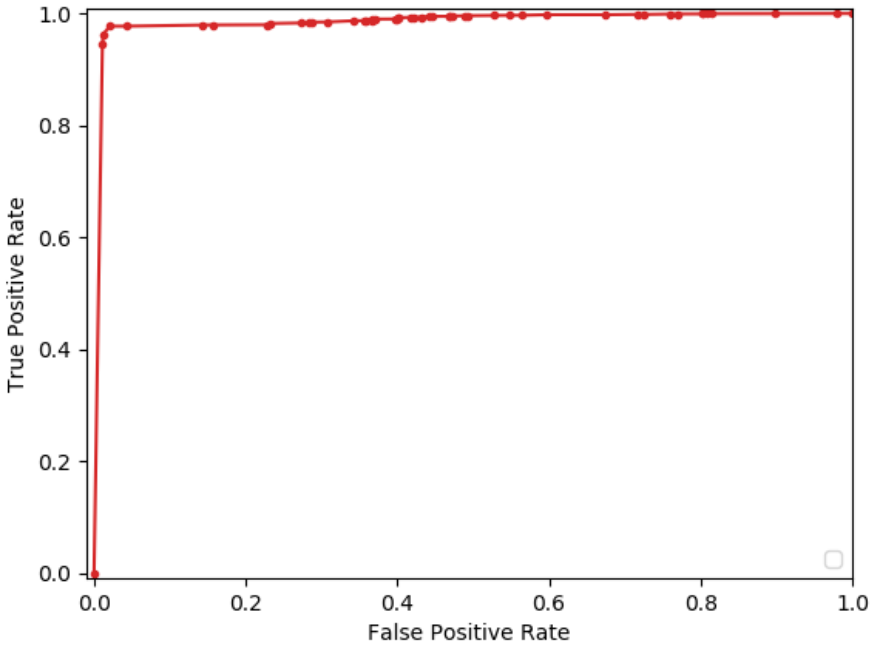

Figure 3.3 : The ROC curve shows that the proposed method can differentiate cell membranes with high sensitivity.

Table 3.2 : Comparison of results from various combination of training and testing dataset.

Training images(%)	Test images(%)	Train loss	Validation accuracy	Validation loss
75	25	0.0355	0.9700	0.0821
50	50	0.0657	0.9687	0.0950
25	75	0.4885	0.8798	0.4979

Furthermore, the results of the other models are shown in Table 3.3 which show the higher accuracy and lower loss of the proposed method. To have a better view of the results of the model, in Figure 2.5 the predicted cell membranes are overlaid on the original images. Here, alleged cell membranes are in red color. Some samples of the ground truth , the pruning of the incomplete cells and the result of the proposed method is presented in Figure 3.4.

Table 3.3 : Comparison of results from various models.

models	Test images (%)	Train accuracy	Train loss	Validation Validation
Vesselnet	0.8908	1.7403	0.8669	2.1226
U-net	0.9851	0.0355	0.9700	0.0821
SegNet	0.8428	0.1583	0.8327	0.2076

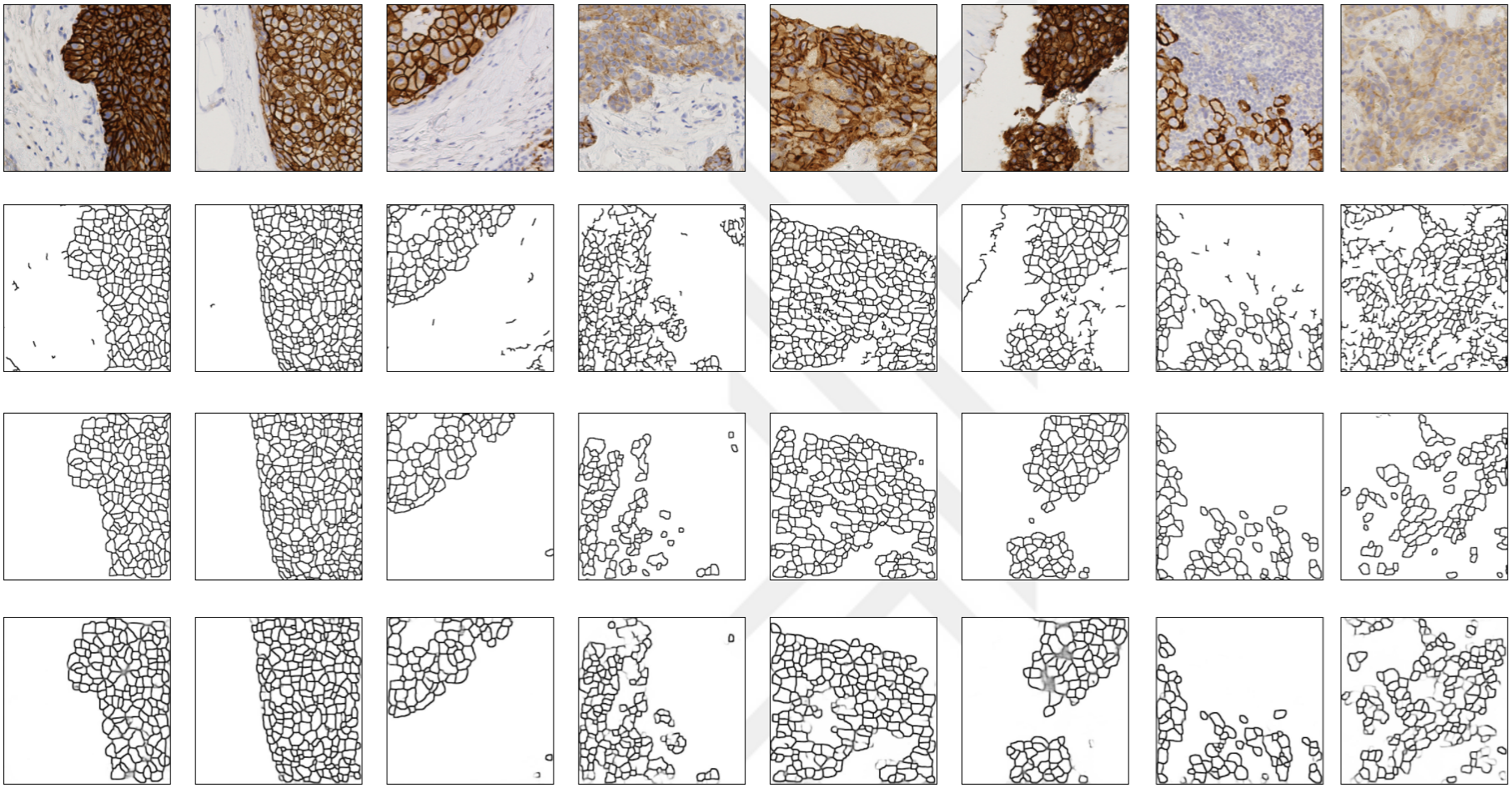

Figure 3.4 : HER2/neu image fragments and corresponding segmentation results of deep learning output compared to ground truth.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 Discussion

In this thesis, we introduced a computer-aided whole slide based deep learning method to automatically evaluate the IHC score of HER2 in breast cancer images. Because of the subjectivity of interpreting histopathology images and interobserver disagreement between pathologists, reliable methods replicable of manual annotation is necessary. In contrast to previous methods in HER2 assessment, in this research classification of HER2 IHC using deep learning-based segmentation of cell membrane in WSIs is evaluated. The analysis of results in Figure 6 indicates that the network succeeds to distinguish between membranes and cytoplasmic or wrong staining. As test dataset is not used for the training, the noticeable high accuracy indicates that the model has learned well to segment cell membranes correctly. Despite other deep learning-based methods that score and classify slides based on the patches, we assessed the WSIs considering the guideline that is accepted by the pathologists. From another perspective, cell membrane segmentation outperforms patch-based classification in explicitly considering membrane staining intensity by providing ground truth cell membranes of each image. This helps to increase the performance of the model by learning precise features related to cell membranes. The proposed methodology, which implements superpixel-based tissue classification and deep learning-based cell membrane segmentation addresses automatic HER2 assessment tasks. The high agreement between automatic assessment and the manual scoring approves the generality and acceptedly of the training data. However, the greatest discordant in our evaluation was due to various staining criteria of different laboratories. This discrepancy was the main reason of misclassifying 2+ cases as 3+ or 1+. The increment of training data from various laboratories, as well as histopathology stain-color normalization are two important steps that could easily integrate to the existing segmentation workflow to overcome these problems. Severe overlapping of

cytoplasmic staining with cell membrane gives rise to poor segmentation which causes errors in a way that some membrane staining connecting two cell are ignored. This could result in a state that inside a closed membrane more than a nucleus or no nucleus is recognized. This difficulty would directly affect the overall score in some cases, and we overcame this by simply considering these cases that doesn't have a distinct nuclei as artifacts.

4.2 Conclusion

An automatic end-to-end machine learning based framework for IHC HER2 assessment is presented in this thesis. The proposed model considers three main properties: (1) the input WSI is classified into two stromal and epithelial areas using superpixel based classification; (2) the model should be trained using patches extracted from the epithelial part, enabling the segmentation model to extract cell membrane staining pattern; (3) the scoring part of the architecture would merge the results from the tiles and transfer staining intensities and completeness to results accepted by pathologists. The HER2 scores of the model have a high correlation with the scores provided by experienced pathologists from two different and independent datasets. The generality of this methodology could be considered on other diverse membrane segmentation

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