

CELL SEGMENTATION IN CANCER HISTOPATHOLOGY IMAGES USING
CONVOLUTIONAL NEURAL NETWORKS

by

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Presented to the Faculty of the Graduate School of
The University of Texas at Arlington in Partial Fulfillment
of the Requirements
for the Degree of

MASTER OF SCIENCE IN COMPUTER SCIENCE AND ENGINEERING

THE UNIVERSITY OF TEXAS AT ARLINGTON
December 2016

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To my parents and my sisters.

ACKNOWLEDGEMENTS

I wish to express my sincere thanks to my supervising professor, Dr. Junzhou Huang who encouraged me to do this thesis without whom this thesis would not have been possible. His irreversible fortitude and constant motivation are the main reasons for the successful outcomes of my research. I sincerely express my gratitude to Dr. Jeff (Yu) Lei and Dr. Jia Rao for spending their valuable time by serving on my committee.

I would like to thank Mr. Zheng Xu, my P.hd mentor for his continuous assistance in this thesis and also I wish to mention special thanks to Mr. Jiawen Yao, Mr. Sheng Wang, Mr. Ashwin Raju and other friends in my lab for constantly motivating me to achieve success and sharing their suggestions on every step in this project.

I would fail if I forget to express my gratitude to my friends Mr. Vivek Arvind Balaji, Mr. Vivek Sundararajan, Mr. Deepak Raj Srinivasan, Mr. Eshwar Ravindran, Mr. Sree Rathan Chadalavada, Mr. Srinivas Varadharajan, Mr. Gokul Manivendhan and all my dear friends at Arlington and India for their boundless belief in my abilities and endless encouragement for my success.

Above all I express my earnest thanks to my dear parents, my sisters, my cousins, far and near family for all their blessings and sacrifices they had done to help chase my dreams and live my passion. Finally, I thank God for all the opportunities he creates for me.

November 18,2016

ABSTRACT

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Cancer, the second most dreadful disease causing large scale deaths in humans is characterized by uncontrolled growth of cells in the human body and the ability of those cells to migrate from the original site and spread to distant sites. The major proportion of deaths in cancer is due to improper primary diagnosis that raises the need for Computer Aided Diagnosis (CAD). Digital Pathology is a technique that acts as second set of eyes to radiologists in delivering expert level preliminary diagnosis for cancer patients. Cell segmentation is a challenging step in digital pathology that identifies cell regions from micro-slide images and is fundamental for further process like classifying sub-type of tumors or survival prediction. Current techniques of cell segmentation rely on hand crafted features that are dependable on factors like image intensity, shape features, etc. Such computer vision based approaches have two main drawbacks: 1) these techniques might require several manual parameters to be set for accurate segmentation that puts burden on the radiologists. 2) Techniques based on shape or morphological features cannot be generalized as different types of cancer cells are highly asymmetric and irregular.

In this thesis, Convolutional Networks, a supervised learning technique recently gaining attention in the field of machine learning for vision perception tasks is investigated to perform end-to-end automated cell segmentation. Three popular convolutional network models namely U-NET, Seg-Net and FCN are chosen and transformed to accomplish cell segmentation and the results are analyzed. A predicament in applying supervised learning models to cell segmentation is the requirement of huge labeled dataset for training our network models. To surmount the absence of labeled data set for cancer cell segmentation task, a simple labeling tool called SMILE-Annotate was developed to easily mark and label multiple cells in image patches in lung cancer histopathology images. Also, an open source crowd sourced based labeled dataset for cell segmentation from Beck Labs; Harvard University is used to lay empirical evaluations for automated cell segmentation using convolution network models. The result from experiments indicates Seg-Net to be most effectively performing architecture for cell segmentation and also proves it has scope to generalize between different datasets only with minimum efforts involved.

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CHAPTER 1

INTRODUCTION

1.1 CANCER

Cancer is a progressive disease that remains to be one among the most dreadful diseases posing threat to human life. Cells are building units of any living being and vital structures in living organisms are associated with cells. An important property of cells is its natural style of reproduction by diving through a process called cell division. Normal cells execute process of cell division when they are near to worn out or damaged. When cells start to grow out of control or sudden rapid growth of cells with same mutation is termed as cancer. Cancer cells are related to its malfunction of gene mutation and cell division process, this leads cells to be in collective structure or as huge masses. A misleading term when discussing about cancer is tumor. The word tumor and cancer are used interchangeably in many situations but as a fact tumor and cancer are different terms with distinguished meaning. Tumor is an abnormal mass of tissue that can either be cancerous (malignant tumor), non-cancerous (benign) or pre-cancerous (pre-malignant). Tumors occur due to abnormal growth of tissues by mass and needs to be treated but they are not necessarily life threatening in nature unless malignant. Benign tumors are slow in growth, does not affect surrounding tissues and generally do not recur once removed from body. Cancer ¹ is regarded as a group of several complex diseases with wide range of possible causes. Though cancer was initially assumed to be a wasting disease meaning removal of too many cells during process of cell division, in later years it was clear that abnormal proliferation

¹<http://www.cancer.org/cancer/>

of cells leads to cancer. Cancer cells do spread to different parts of body as an instance infected cells in lung can go to bones and start growing there but will still be a part of lung cancer. Hence the origin of cancer cells is vital to be identified as propagation of cancer cells does not certainly indicate infected cells of particular region of migration. A medical practitioner suggests treatments for diseases initially based on symptoms prior to exhaustive diagnosis. But when cancer begins, it invariably produces no symptoms. The initial growth of cells yields no signs or symptoms that leads in difficulty of preliminary diagnosis. But as cells continues to increase in mass or ulcerates there occurs few systematic symptoms. Few symptoms are specific in nature but are in common with many other diseases due to repetition of genetic nature. This gave cancer a name as great imitator. It is uncommon for people with cancer to be diagnosed and treated for other diseases which were assumed to be causes for initial symptoms. There exists several type of cancers based on their origin with few properties being alike and also properties that are completely different. Alike properties of cancer are its ability to reproduce continually without responding to signals from body through programmed cell death that takes responsibility to fade out feeble cells by replication of old cells information through cell division. Cancer cells act with power of being uncontrollable in terms of crowding out normal cells also without performing tasks intended to be performed by unaffected cells. While the common properties have been highlighted there does exist distinct characteristics based on type of cancer such as some cancers grow and spread ultimately fast while other types grow in a slow fashion leading to more complications. Treatment for cancer also extensively depends on classes of cancer as they respond in different ways. Few cancers are best treated by means of surgery while others respond to usage of drugs on a regular, long term process. Doctors do recommend combination of

treatments to ensure accurate identification of origin and understand patients nature of responsiveness.

1.2 LUNG CANCER

The presence of malignant tumors, cancer cells in either or both lungs of a human is termed as lung cancer. Lung cancer better known as lung carcinoma among medical community has proven as an effect of smoking or other means of tobacco usage. Although around 10% - 15% of registered cases occur in persons who have never used tobacco, major cause is still associated with usage of tobacco products. When growth of abnormal cells increases in lung tissues, huge masses are formed but remain nonfunctional with respect to activities needed to be carried out by healthy lung cells, hence it jeopardizes the essential purpose of lungs in our body. Lungs are responsible to bring in oxygen during inhaling and release carbon dioxide when exhaling. Respiration is key for survival of lives and disturbance in this due to aberrant cancer cells accounts to major deaths due to lung cancer. Lung cancer progresses through several stages, in later stages the cancer cells travel away from original tumor area to different sites this is termed as metastasis. The new affected sites are called as metastases. Diagnosis of lung cancer is done by means of biopsy, where a small piece of lung tissue is examined under microscope to look for cancer cells. The doctors take help from pathologists (person who identifies disease by examining cell structure and tissues under microscope). Several stages of lung cancer and large size of lungs that accommodates unnatural cell growth during early stages such as stage one or stage two emanate difficulties in identifying lung cancer. Two major classification of lung cancer cells can be given as non-small lung cancer cells (NSCLC) and small cell lung cancer (SCLC). Small cell lung cancer contributes as main reason in a very few cases and are effectively treated by means of chemotherapy. In contrary non-small lung

cancer cells are vital cause of lung cancer and imperative to be enumerated clearly.

Non-small cancer are classified into three main categories as

1. Adenocarcinoma, most common form of lung cancer is a kind neoplasm from epithelial tissues that has glandular characteristics, i.e. responsible for secretion of important body fluids such as mucus.
2. Squamous cell carcinoma that accounts for 25 percent of lung cancers are found in middle region of lungs. Also called epidermoid carcinoma this type of cancer begins in squamous cells that are thin, flat look like fish cells when seen under microscope.
3. Large cell carcinoma are named after its appearance as large round cells when examined under microscope. They occur mostly in outer region of lungs, grows rapidly when compared to other forms of non-small cell lung cancer. This type accounts only to ten percent of cancer cases.

1.3 COMPUTER AIDED DIAGNOSIS

Diagnosis of cancer is strenuous task and might require several phases of tests, evaluations. In most cases unlike other diseases, cancer is not effortlessly diagnosed by doctors with help of symptoms. Inaccurate treatments based on symptoms, delay in appropriate examinations being conducted aids cancer cells to multiply and advance to further stages. Aforementioned reason leads doctors to prescribe computer aided diagnosis for cancer. If lung cancer is suspected, several screening procedures are subjected to the individual before finalizing the presence of malignant tumor cells. Medical imaging techniques are advocated by physicians to diagnose lung cancer. Imaging techniques provide guidance in looking at suspicious regions, learn staging of cancer, validating effects of treatments and look out signs indicating return if cancer cells [1]. There are several imaging based screening tests that can be used the

popular ones being Chest radiograph or better known as chest X-rays (CXR), Computed Tomography and Positron Emission Tomography (PET). When symptoms are not closely related to lung cancer or a smoker patient is concerned about his wellness doctors recommend chest x ray for diagnosis. Chest radiographs reveal large masses of nodules but may not be actually significant in identifying abnormalities. Chest X rays often miss out potentially small lung cancer tumors but identify large benign tumors that ends performing several other tests in most of patients. However advancement in technology, research being conducted over the years brought new hope by means of computed tomography (CT) scans or spiral CT scans which benefits by providing high resolution images identifying small cell and non-small cell lung cancer. Recent studies around United States of America suggest although high resolution scan images discover stage one lung cancer their credibility in answering questions related to staging of cancer, type of tumor is uncertain. This raises questions among researchers if computed tomography imaging can indeed save lives. But CT is widely used in practice even in present generation. Positron emission tomography is a test conducted by injecting a form of radioactive sugar, to observe cell behavior. Tracers help in identifying the difference between normal cells and cancer cells, fluorodeoxyglucose (FDG) is most used tracer in PET. PET images are visualized in three dimension to get distinct information on absorbing behavior by cell tissues. PET are in general combined with CT scan for accurate information. Despite its effectiveness major concern about Positron Emission Tomography is related to radiotracers harmfulness to health of an individual. Radiotracers are administered in very small amounts but as cancer treatment is subjected to regular checkups continuous use of such nuclear substances might lead to adverse effects over long term. Furthermore nuclear radiotracers maybe time consuming as it takes several hours for radiotracers to accumulate in region of interest, it may take few more hours after that to conduct imaging process. There

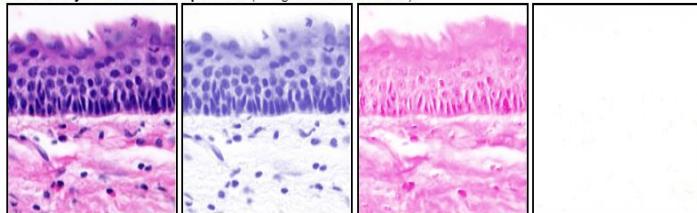
occurs need for automated diagnosis to address aforementioned drawbacks of various techniques.

1.3.1 HISTOPATHOLOGY

Histopathology is the study of tissues in terms of finding or diagnosing a particular disease with help of microscopy slide images. In lung cancer imaging histopathology aids automatic computer diagnosis in predicting survival analysis [7] for a patient and also in identifying type of malignant tumors. Histopathological images are backed up by principle of histology, the study of microscopic anatomy of tissues in living organisms. It is commonly performed by examining cells and tissues under a light microscope or electron microscope, which have been sectioned, stained and mounted on a microscope slide. Histological studies may be conducted using tissue culture, where live human or animal cells are isolated and maintained in an artificial environment for various research projects. Lung cancer being reported as most malignant tumors in men and second next to women only behind breast cancer intensifies the need for a robust, accurate prognosis technique. Recent advances in Whole Slide Imaging (WSI) assists pathologists, enhances digital pathology in recognizing diseases. Pathologists plays a central role in therapeutic decision making because such diagnosis with help of pathology images supervised by experts remains most favored method in cancer diagnosis. Humans are in general prone to errors and it takes huge toll on pathologists to present cancer prediction based on pathology images as infinitesimal mistake leads to severe effects as it deals with human lives. Advancements in microscopy techniques support computer aided diagnosis by generating high quality but large dimensional images of tissues. The growth of technology and its usage in microscopy paves way for different types of microscopy being used in medical imaging. Each microscopy has its own principles and instrumentation engineers and pathologists are trained well to

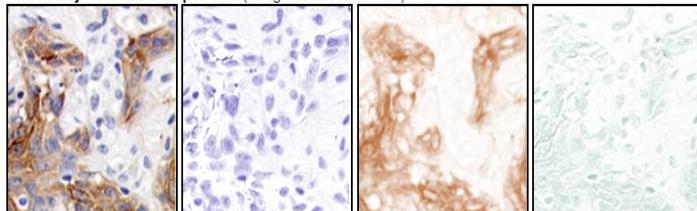
choose appropriate techniques of microscopy according to problem statement. With respect to computer scientists, the structure of image is that matters and effects of microscopy types plays significant role in the type of data being analyzed. Interference contrast microscopy is used when specimens are studied being alive that contrasts based on spatial difference in cellular composition rather than staining the entire image. Absorption microscopy is where stains absorbs light passing through specimens and represent dark objects on a clear background. Stains [5] are again of different types where in cell pathology, Haematoxylin and Eosin (H&E) stain used to generate histopathology images that are widely used in cell pathology. The widely used stain is Haematoxylin and Diaminobenzidine (H DAB) also forms a part of histology images. The colour deconvolution with respect to above two stains can be shown in an image between the two mentioned can be illustrated using the figure given below.

Haematoxylin and Eosin separation (using the built-in vectors).



From left to right: original, Haematoxylin, Eosin, virtually empty 3rd (complementary) component (showing that the vectors match the image quite well).

Haematoxylin and DAB separation (using the built-in vectors).



From left to right: original, Haematoxylin, DAB, 3rd component (the vectors did not perfectly matched the stains in this image, so they should be determined again from single-stained samples).

Figure 1.1. H&E Stain Separation vs H DAB Stain Separation.

1.4 CONVOLUTIONAL NEURAL NETWORKS

Convolutional Networks fondly known as ConvNets is biologically inspired form of artificial neural network with local connections and shared weights [2]. Conv Nets are most important tool of machine learning in the current generation with a wide application to Image recognition tasks in the field of Computer Vision. Convolutional Networks trace backs to 1990s when Professor Le Cun, attempted to train back propagation algorithm for recognizing handwritten digits. Later in 1998, Yan Le Cun et al came up an architecture called Le-Net [3], which still forms the basis for several state of the art ConvNet architectures. These networks lead to some of the most influential innovations in recent years, one among which was 2012 ImageNet competition that was won by Alex Krizhevsky using the concept of ConvNets. In that competition Alex's architecture [35] brought down the classification error rate from 26% to 15%, an astonishing improvement at that point of time. As a first step to answer the question of what is meant by Convolution? Convolution is a mathematical concept used heavily in Digital Signal Processing when dealing with signals that take the form of a time series. In naive terms, convolution is a mechanism to combine or blend two functions in a coherent manner. For a discrete domain of two variables, it can mathematically be described as:

$$(f * g)(x, y) = \sum_{u=-\infty}^{\infty} \sum_{v=-\infty}^{\infty} f(u, v).g(x - u, y - v) \quad (1.1)$$

A ConvNet is a stack of layers, and every layer of a ConvNet transforms a set of activations to another through a differentiable function. There are three main types of layers to build ConvNet: Convolutional Layer, Pooling Layer, and Fully-Connected Layer. We can list out different components in a convolution network as follows:

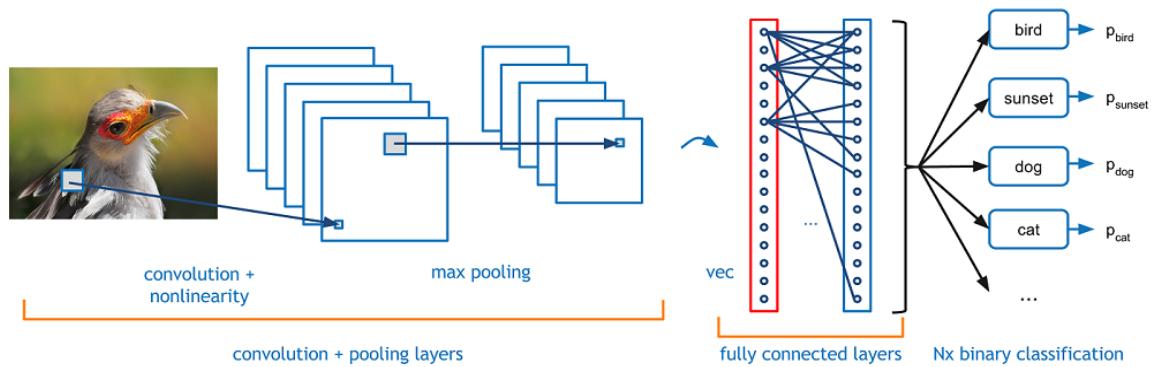


Figure 1.2. Components of a Convolution Network.

1. INPUT: An array to hold input features, in case of an image the shape of the array will be [height, width, number of channels]. For example, an RGB image of size 32 X 32 will have the shape of input array as [32, 32, and 3]. The 3 corresponds to number of channels namely R, G, and B.
2. CONVOLUTION: computes the output of neurons that are connected to local receptive field of the input, computing a dot product between weights and local region of input volume.
3. ACTIVATION: to apply element wise activation function such as ReLU.
4. POOLING: to perform down sampling operation along the spatial dimensions of the input.
5. FULLY CONNECTED: This is similar to a neural network where each neuron is connected to all neurons in previous layers. This layer helps to estimate class scores.

Figure 1.2 ² gives a visual representation of components in convolution network as listed above.

²<https://adeshpande3.github.io/assets/Cover.png>

1.5 GOAL OF THESIS

In this thesis we aim to transform three leading convolutional network architectures for semantic segmentation to perform completely automated end to end cell segmentation in cancer histopathology images. Three three architectures are namely:

1. Fully Convolutional Network (FCN)
2. U-NET
3. Seg-Net

Cell segmentation widely differs from typical object segmentation by means of varying size, shape of cells in a single image. Also the number of cells in an image is very high compared to simple object segmentation tasks which have one to ten objects per image. To incorporate and overcome the mentioned challenges and also other unseen challenges, modifications to original architectures have been employed in this thesis. Cell segmentation tasks is considered highly challenging due to unavailability of annotated data, to overcome that in this thesis we develop SMILE-ANNOTATE, a matlab based cell labelling tool to help researchers manually label cells using a very simple interface. An empirical analysis of three models for cell segmentation is laid out by testing the approaches on two different datasets.

CHAPTER 2

CELL SEGMENTATION & CLASSICAL METHODS

Cell segmentation is described as an effort to distinctly recognize cell structures in histopathology images by differentiating cell objects from tissue layer and remaining background. In Automated diagnosis of cancer, identifying cells accounts as a preliminary yet most significant task, cell segmentation can be visualized as determining accurate region of cells present in a given image. In the pipeline of automated histopathological image analysis automated segmentation of cells is regarded as a major hurdle and it continues to be major focus of research in this field. An effective cell segmentation combined with efficient cell detection [4][6] can boost later steps such as sub-type prediction and image based survival analysis [7] of cancer in the pipeline of CAD. Cell segmentation of real histopathology images is a difficult domain-specific problem (Baochuan Pang et al, 2010) and often requires an immense amount of prior knowledge in order to produce adequate results. This is usually due to the fact that histopathology images can contain digital noise to vast extent, vary greatly in intensity and can contain many artifacts and unwanted objects. In traditional low level vision tasks, the prior knowledge was mostly used in the initial choice of the algorithm through modification of the algorithm (gradient direction, boundary smoothness, etc.) or by parameter tuning for 'optimum' performance. However, due to the complex structures and inherent variability found in biological specimens, the manual choosing and tuning of the hand designed algorithms become so complex and time consuming and impractical. Cell segmentation can be observed as a challenging task for several reasons including but not limited to the following: first, formation

of cell compartments as well as inter-cell and intra-cell variability engenders non-homogeneous marker distributions across cells bringing about inadmissible features like intensity gradient. Second, cells in general occurs in clusters and especially cancer causes huge lumps of continually dividing cells that results in dense, overlapping cell regions making it arduous to assign specific cell features in spatially close cell objects. Third, most image segmentation approaches are model based but cells especially tumor cells in particular are of heterogeneous shapes, does not maintain a consistent size neither any morphology can be clearly defined thus defining accurate models becomes near impossible. Histology incorporates several immunohisto chemistry techniques as well as staining chemicals to generate histopathology images this leads to varying imaging modalities further increasing complexity in bringing about one unique algorithm suitable for any computer aided diagnosis. This tunes aim of challenge to design methods that are sufficiently generic and easily trainable for wide range of applications while achieving high sensitivity and specificity in every individual case. As outlined by E. Meijering [9] Rather than converging to a robust, unified solution, it thus seems that the field is diverging, and by now almost as many cell segmentation methods have been developed as there exists cell analysis problems.' There exists a plethora of cell segmentation techniques out of which few are referred as conventional with proven success in clinical analysis for cancer diagnosis but still having a scope of improvement with some defect in each algorithm. In related work chapter we will review most widely recognized cell segmentation approaches.

2.1 THRESHOLDING

2.1.1 INTENSITY THRESHOLDING

Thresholding is the most straightforward and earliest method for nuclei / cell segmentation. Thresholding is a technique to convert gray-scale images into binary images with objects classified into background and foreground. This technique makes assumption that nuclei or cells (foreground) are adequately distinct from the background in terms of intensity. While this assumption is not necessarily true because there exists a plethora of imaging modalities and different staining techniques to obtain histopathology images, in general thresholding techniques requires images to be converted from RGB channels to gray-scale. Thresholding creates binary images from grey-level images by turning all pixels below some threshold to zero and all pixels above that threshold to one.

If $g(x, y)$ is a thresholded version of $f(x, y)$ at some global threshold T ,

$$g(x, y) = \begin{cases} 1, & f(x, y) \geq T \\ 0, & \text{Otherwise} \end{cases} \quad (2.1)$$

The threshold, T can be either a global value that applies to the entire image or a local threshold can be applied to different regions of image. Local thresholding requires an additional parameter to define local region of the size. There exists a strategy of dividing the whole images into several small images and binarize each small image based on a specific local threshold value. Local thresholding to an extent deals with non-uniform illumination in microscopic images. The selection of optimum threshold is a challenge and with respect to cell segmentation they can use one of the two following methods namely:

- Optimal Thresholding Method (Histogram Based)
- Otsu's Thresholding (Clustering Based)

2.1.2 OPTIMAL THRESHOLDING METHOD

This technique is based on approximation of the histogram of an image using a weighted sum of two or more probability densities with normal distribution.

The threshold is set as the closest gray level corresponding to the minimum probability between the maxima of two or more normal distributions, which results in minimum error segmentation. The image given below ¹ establishes an idea about probability distribution for determining optimal threshold.

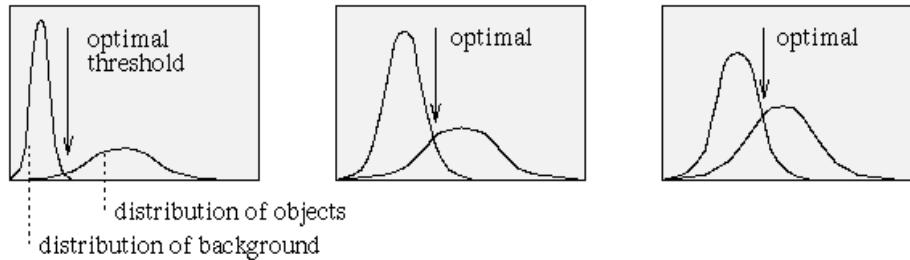


Figure 2.1. Probability Distributions of Background and Foreground.

2.1.3 OTSU THRESHOLDING METHOD

Otsu's method of thresholding performs statistical discriminant analysis and chooses a threshold value by minimizing the inter-class variance which is also proved to be the same as maximizing the intra-class variance. This method is simple and utilizes only the zeroth and first order level of cumulative moments of the gray-level histograms. This method assumes uniform illumination in the gray-scale image such that a bimodal distribution is maintained. This assumption helps to find an optimal threshold by iterating through all the possible threshold values and calculating a measure of spread for the pixel levels each side of the threshold (background or foreground). Certain type of staining in microscopic images yields bright foreground

¹http://www.mcs.csueastbay.edu/~grewe/CS6825/Mat/Segmentation/Seg_thresh.html

objects in a relatively uniform dark background such that a bimodal distribution or near bimodal is achieved and a single threshold can be used as a step for nuclei cell segmentation. Zhonghua et al. 2011, proposes a K-L transformation be applied to each channel of RGB image and apply Otsu's thresholding method to each channel separately to find an optimum threshold for image thus segmenting cells from background. Callau et al. 2014 [10] try to segment epithelial cell areas of cytokeratin-19 breast cancer TMA images by first converting three channel images to gray level images and performing a thresholding to obtain initial epithelial segmentation and has additional processing for splitting cell areas. Nguyen et al. [8] propose a novel method to extract cytological feature to detect, segment cancer cell regions also in addition to the novel feature conventional image features have been considered. They compare the method to techniques involving thresholding as a part of segmentation algorithm and contribute evaluation of their technique with respect to Otsu optimal thresholding for segmentation. The Figure 2.2 taken from [8] shows a simple Otsu thresholding of cancer cell histopathology images which happens to be coarse segmentation and might prove in-efficient in practical scenarios when the segmentation has to be completely disjoint with epithelial layer for medication, also not all staining techniques yields brightly stained cell foreground to use a single threshold to segment cell images.

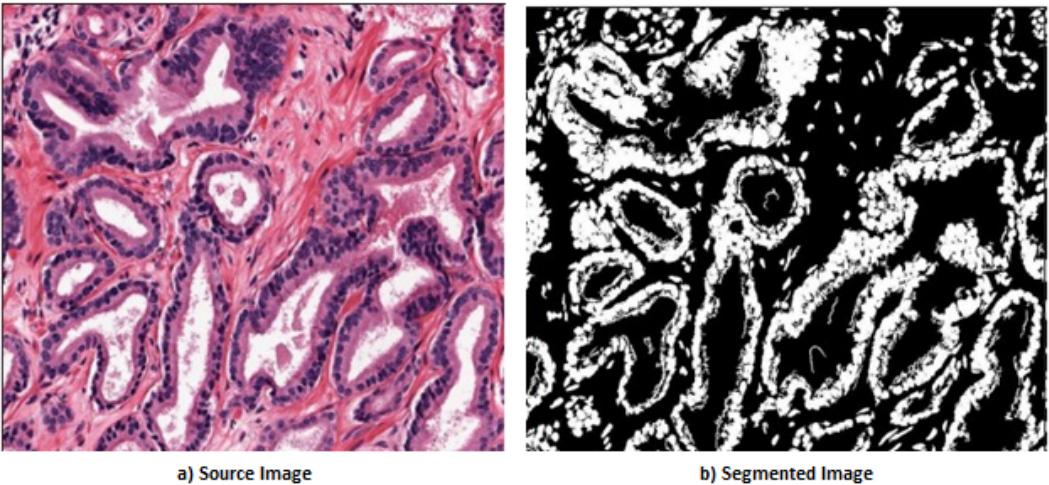


Figure 2.2. Cell Segmentation Using Otsu's Thresholding.

2.2 WATERSHED SEGMENTATION

Watershed transformation being the popular region growing method has proved its effectiveness in several applications with respect to Image segmentation. The transformation starts from specific points also known as seed points, iteratively accumulates pixels tending to be connected and creates labelled regions [9]. In watershed transformation the image is viewed as landscape where the intensity of represents symbolizes elevation, this transformation floods the images into different regions, analogous to identifying areas of low elevation or drainage regions of landscape and builds dams to avert merging of water in such regions with catchment basins. In a gradient magnitude image there exists intensity variations between foreground objects and background pixels, thus applying watershed transformation directly to a gradient magnitude image results flooding from local minima in specific region to cause over segmentation over the image. The cause of such an over segmentation can also be attributed to noise or irregularities in the specific region of image. To avoid such over segmentation in images, a technique called marker-controlled watershed

segmentations was introduced where the flooding is controlled with help of plotting markers over foreground and background several nuclei segmentation algorithms use this technique [11] [12]. “Figure 2.3 ² is an example of Electrophoresis image highlighting over segmentation by use of typical watershed transformation also shows makers for foreground objects and outline of marker controlled water shed segmentation.”

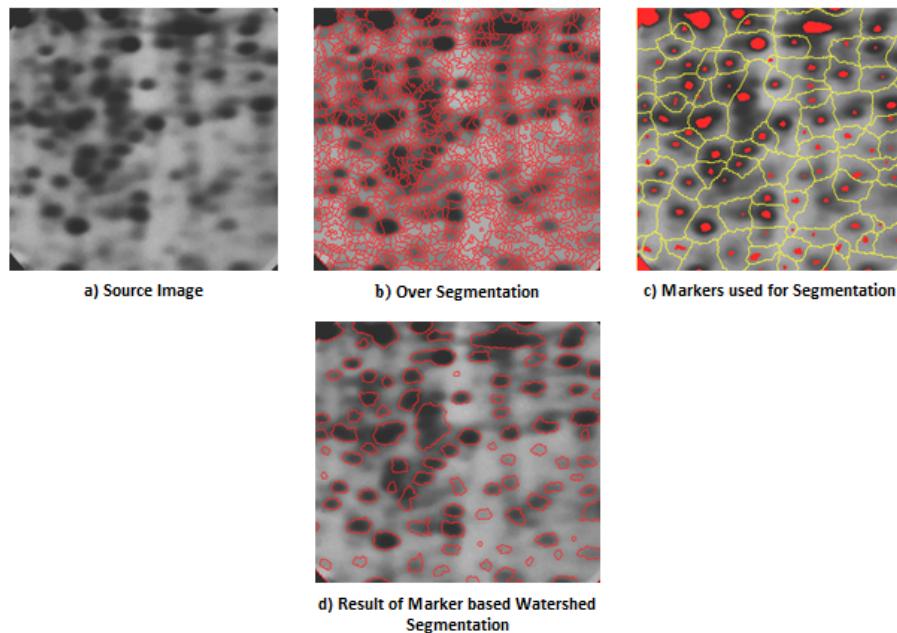


Figure 2.3. Cell Segmentation Using Marker-based Watershed.

2.2.1 MARKER-CONTROLLED WATERSHED SEGMENTATION

To alleviate the effects of over segmentation in simple watershed transformation, a marker to represent interested object or point of interest in foreground is provided and constrained to be regional minima from where flooding occurs for segmentation. Marker-controlled watershed transformation helps reducing over segmentation but

²<http://cmm.ensmp.fr/~beucher/wtshed.html>

constitutes for a new problem of finding appropriate Markers. There exists several variation to this technique in terms identifying markers which can broadly be classified into two categories manual marker generation and automatic marker generation. Numerous techniques and literature's propose various kinds of marker based segmentation for nuclei segmentation. Manual marker based cell segmentation is discussed by N. Beliz-Osorio et al. [13] obtains compelling segmentation results, this does not scale well for huge dataset nor whole slide imaging. Manual labelled markers can be considered semi-supervised approach but still accounts for errors by human intervention and allows way for complete computer aided diagnosis. Amongst various automated maker establishments for nucleus segmentation, one widely used technique is to employ a successful automatic cell detection algorithm based on distance methods [14] [15] to images as a result to obtain point of interests, i.e. the cell or nucleus and employ these points as markers to segmentation. Using approaches similar to morphology [16] [17], Hough transforms [18] or symmetry voting are other different techniques for cell segmentation using marker-controlled watershed transformation.

2.3 HYBRID METHODS

Cell segmentation literature encompasses plenty of techniques of which most of the successful algorithms and articles with numerous citations in general blend ideas from various computer vision or image processing approaches in order to evolve a hybrid model. Such approaches are of great potent as advantages of diverse techniques are associated into a single algorithm. In this section we review such hybrid models proposed for cell or nucleus segmentation.

2.3.1 CELL SEGMENTATION USING GRAPH-CUT

BINARIZATION AND LAPLACIAN-OF-GAUSSIAN (LOG) FILTERING

The technique proposed by Yousef Al-Kofahi et al. [19] claims robust cell segmentation using combination of basic ideas. The fundamental workflow follows automatic binarization of images using a hybrid graph-cut approach followed by extraction of seed points using Laplacian of Gaussian (LoG) constrained by distance map based adaptive selection. The seed points extracted here are comparable to extraction of markers discussed in previous section. The complete workflow as outlined in the paper can be presented as a flowchart [19]. The author sketches initial step in nucleus segmentation, binarization of images and lists various methods used as thresholding (discussed in earlier section), clustering based methods, graph-cuts [20] and level-set approaches [21]. The general method listed above requires a good initialization or training process, hence this paper proposes a hybrid graph-cut based automatic binarization of images. Initially a normalized histogram is computed that is modeled by Poisson distribution [22], this had been chosen rather than commonly used mixture of Gaussian's model based on empirical evaluation [23]. The histogram uses the Poisson-distribution-based minimum error thresholding algorithm and further this is standardized by the use Graph-Cut approach as an implementation of fast max-flow / min-cut algorithm [24]. This method is claimed to get more accurate binarization results. This binarization extracts nuclei clusters that are intended to be separated into separate nuclei.

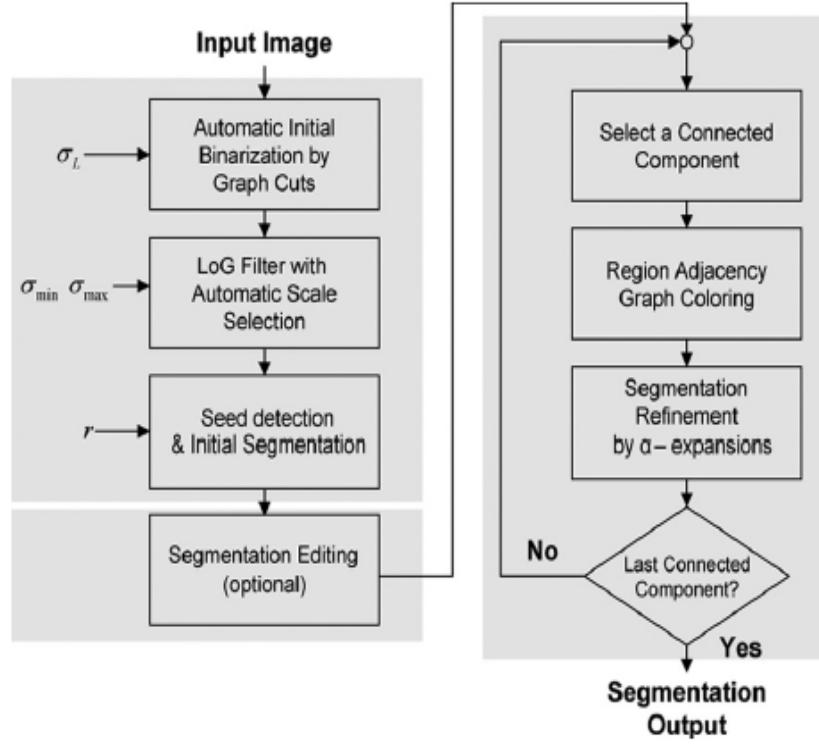


Figure 2.4. Workflow of Graph-Cut based Cell Segmentation.

The process of splitting clusters into individual nuclei requires markers that identify points of interests or individual nuclei. In this work the authors had used multiscale LoG filter with claim that these filters are robust to chromatin texture and identify nuclear blobs clearly. But there seems to be problems when clusters of different sizes or shapes are formed, to overcome which Euclidean distance map based on size and shape cues are taken into consideration. Based on experiments, the algorithm claims to be faster than typical marker based watershed transformation because here only the foreground marker is considered for segmentation. Refinement of segmentation results while using multiple labels is achieved with the help of Graph coloring.

2.4 CELL SEGMENTATION: MINIMUM-MODEL APPROACH

Stephan Wienert et al [24] highlighted the problem involved in model-based approaches for cell detection and cell segmentation as model-based approach relies heavily on a-priori information of shape features to achieve segmentation that might introduce a bias of segmenting nuclei with only certain properties. This paper introduced a new contour based technique to achieve cell segmentation irrespective of cell shape also with use of minimum a-priori information. The use of nuclei shape features, staining features for cell segmentation are considered disagreeable because differences of cell nucleus shapes in cancer cells, the use of different microscopy methods or staining techniques is apparent in the real world. Minimum model approach lists out six steps to achieve accurate segmentation and establish results that claim to assist pathologists effectively in contrast to other cell segmentation techniques. The six steps for cell segmentation using minimum-model approach are listed as follows:

1. **Detection of all possible closed contours:** The first step, primary segmentation aims to detect all possible contours in the image regardless of contour belongingness to a cell or nucleus. The technique uses a contour detection algorithm for binary images but slightly modified to adapt it for gray-scale images.
2. **Contour Evaluation:** Step 1 proposes possibility of all contours in the image which is generally over-defined. Step 2 aims at choosing valid contours for further processing. This is achieved by introducing two new factors, mean gradient and gradient fit. A contour value is established as a product of these two factors to decide the importance of a contour.
3. **Generation of non-overlapping segmentation:** A labeling analogy is defined and performed in sorted order based on previously obtained contour value. The labeling analogy uses a 2D map of the same size as corresponding image to label the area within each contour with a unique identifier. By avoiding

overwriting of already defined labels non-overlapping contours are expected to be returned.

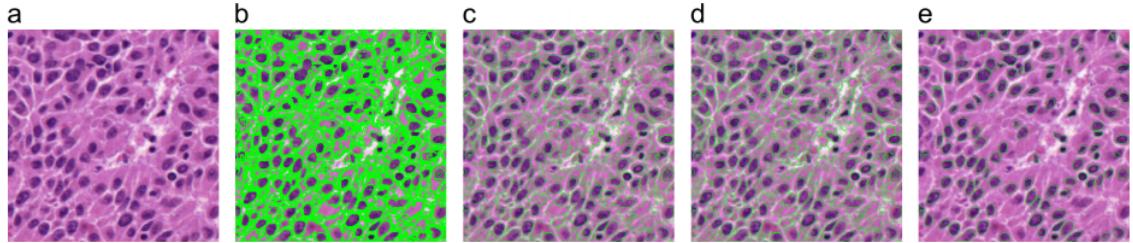


Figure 2.5. Minimum Model based Cell Segmentation a) Source Image b) Primary Segmentation c) Non-Overlapping Segmentation d) Contour Optimization e) Concave object separation.

4. **Contour optimization:** Step 3 establishes continuous labeling that might result in some part of contour actually not being among object. This step aims to establish a simple optimization technique based on distance measure (Manhattan distance) to find compact pixels using a specific relation of distance. The identified non-compact pixels are finally removed to obtain improved contours.
5. **Concave object separation:** In order to manage cells forming clusters, this step aims at separating objects at concave borders by removing object pixels between two concavities based on following criteria: the contour concavities are located opposite to each other and length of cutting line is minimal in comparison with depth of concavities.

6. Classification into cell nuclei and other objects: Minimum information of color based on staining is employed to achieve accurate classification of objects. This step employs color deconvolution technique [25] to extract Hematoxylin signals. Eventually, staining intensity for each pixel is calculated and pixels below certain threshold are eliminated. Additionally, tiny objects or artifacts that are less than 50 pixels in area are eliminated.

CHAPTER 3

CONVOLUTIONAL NEURAL NETWORKS FOR SEGMENTATION

Convolutional neural networks are powerful deep learning based approaches for visual recognition tasks. As discussed in Section 1, ConvNets yield robust hierarchies of features unlike computer vision approaches that rely on hand crafted features from images and videos. It is being observed that there is huge success for deep learning based methods in categorization of whole images, detecting objects in images or video frames [26] [27], speech recognition [28]. The accomplishments of deep learning approaches in object classification tasks drives researchers to explore its feature learning capabilities for structured prediction such as segmentation. Semantic segmentation tasks modeled as pixel-wise classification problems inherently raises the need for convolution networks to keep hold of both semantic information and positional localization. Deep feature hierarchies keeps hold of global information that yields what (semantics) and local information that yields where (location) in the form of non-linear global to local pyramid. In convolution networks for object classification tasks, there exists fully connected layer which have fixed dimensions of output and discard spatial information. But, such fully connected layers can be viewed as convolutions with kernels covering entire input region. Such a change aids the network to output classification maps. This method is just an intuition to convert typical ConvNets to fully convolutional networks for pixel wise segmentations. But there have also been experiments to apply networks designed for object categorization for segmentation [29] [30]. There also exist plenty of techniques for object segmentation with help of region proposals or bounding boxes. R-CNN [31] is one such approach

to use bottom-up region proposals for effective segmentation. In [32] a bounding-box annotation based segmentation of objects is proposed that reduces time involved in labeling process but such techniques involving bounding-boxes or region proposals are not suitable for medical imaging tasks in general. Especially tasks like cell segmentation or tumor segmentation in computer aided diagnosis must be practically accurate to each pixel and hence raises the need for End-to-End Segmentation approaches. Ciresan et al [33] won EM segmentation challenge at ISBI 2012 with a huge margin using a network trained in a sliding window setup to predict probability of a pixel being a membrane, using as input the image intensities in a square window centered on the pixel itself. An image is then segmented by classifying all of its pixels. The drawbacks of this model are, first, the network is quite slow as network must run separately on each square window (patch) and chances of redundancy in terms of overlapping pixels do exist. Second, change in patch size affects localization by max-pooling, for example smaller patches with less max-pooling allows only tiny information to be observed by feature hierarchies while use of large patch size requires more pooling layers accounting to the loss of information. Analogous to previously discussed techniques this deep network for EM brain segmentation also highlights need for complete end to end trainable network for semantic segmentation. In the following section we consider three fully convolutional network models that can be trained image to image and its adaptability with respect to cell segmentation.

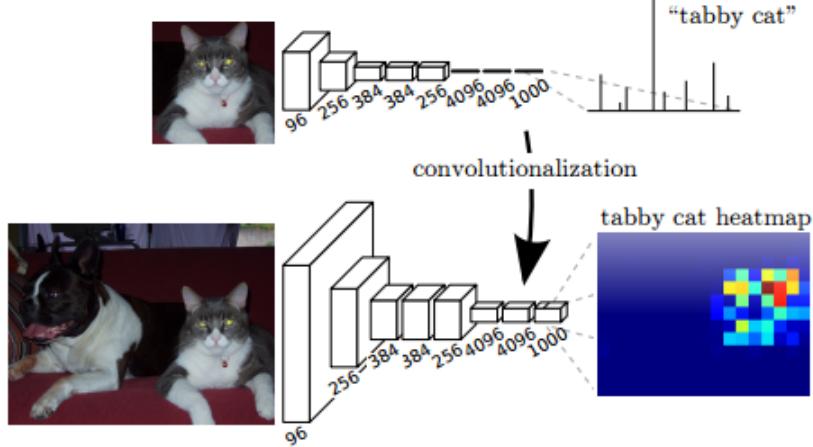


Figure 3.1. Convolutanalizing to Fully Convolutional Network.

3.1 FULLY CONVOLUTIONAL NETWORKS (FCN) FOR SEMANTIC SEGMENTATION

The network Fully Convolution Network (FCN) [34] was trained end-to-end, pixels-to-pixels on semantic segmentation and did not employ any machinery in terms of label or during training process. This paper claims to be the first work to train a FCN for pixel wise classification task. Fully convolution network are trained from input image of size $h \times w \times d$ with each layer followed by convolutions, activations, or pooling layers. This method employs the intuition discussed in previous section to convert a typical ConvNet into fully convolutional network by converting the fully connected layers of convolution network with corresponding trainable convolution filters with kernel size covering over the entire input region. Such architecture yields output maps for any input size but size of output is subsampled due to pooling layers involved. But for a semantic segmentation task it is expected that output be a probability map of classification scores for different classes at each pixel level, i.e. same size of the input. So, it implies that coarse prediction from a typical ConvNet is not acceptable for segmentation tasks as it requires more dense output with spatial

information lost during pooling being restored. The paper for object segmentation discusses a technique of Shift-and-stitch approach to densify output map but is not employed and just included as a part of discussion. In our implementation of FCN for Cell Segmentation we do not include Shift-and-Stitch and adhere to upsampling through backwards strided convolution.

3.1.1 LEARNED UPSAMPLING

Interpolation is a process to reconstruct lost signals in sampling process with help of interpolation function that helps smoothing the data samples. This process can be applied to connect coarse outputs to dense pixels. Convolution can be related to upsampling by some integral factor F , i.e. a convolution with stride of $1/F$ is analogous to upsampling by factor of F . Hence, the information lost in ConvNet for classification due to sub sampling that accounts for coarse output can be dealt in terms of backwards convolution also called deconvolution [36]. This network proposes the idea of using a learnable deconvolution through backpropagation instead of a fixed upsampling. Thus upsampling is performed in-network for end-to-end learning by backpropagation from the pixel wise loss.

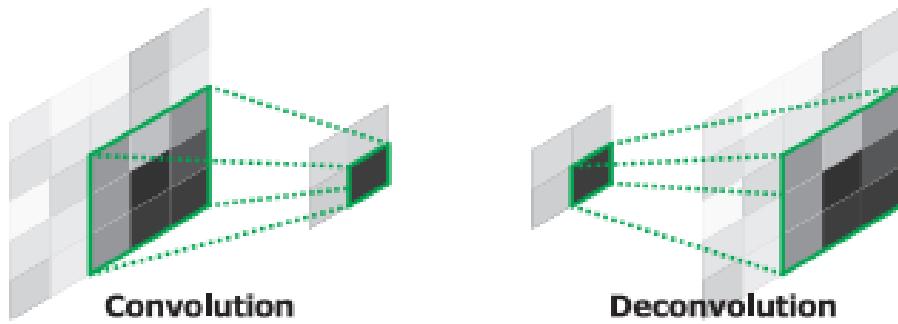


Figure 3.2. Illustration of Convolution-Pooling - Deconvolution-UnPooling.

3.1.2 FCN ARCHITECTURE

The Fully Convolution Network applied to cell segmentation in histopathological images uses convolutionalized model of AlexNet [36] to predict a probability map of size equivalent to input image as output. All the layers of AlexNet are employed but the fully connected layers are betrayed and 1X1 convolution with channel 2 (Only Two Classes Background and Foreground) is appended to predict scores for respective class. The original architecture for object segmentation also employs VGG-16 as another type of architecture for classifier. The output of this classifier is followed by a learnable deconvolution layer that stores in memory the switches during pooling and corresponding switches are unpooled for upsampling purpose and weights are learned. In this network since deconvolution part has learning process the number of parameters are generally higher. But such architecture with one level of 32X upsampling based learnable deconvolution filter still gives segmentation output to be coarse and boundaries are delineated. To avoid craggy boundaries as a part of segmentation output, skips are introduced in the network. The skips can be visualized from figure. These skips help combine final prediction layer with lower layers with finer strides. Further as a refinement to establish clear segmentation boundaries we add learned upsampling through deconvolutions as a part of previous layer, i.e. instead of a 1 X 32x upsampling, we first divide output prediction from 16 pixel stride layer. Then 1X1 convolutions are appended and this layer is fused with previously obtained convolutionalized 32 stride layer and this is further upsampled to size of the original image, this is termed FCN-16s. We do perform all operations required for FCN-16s but from a previous layer and respective fusing of corresponding 2x and 4x from convolutions of stride 16 and 32 respectively. This probability map is upsampled to size of original image and it is called FCN- 8s. In general FCN-8s have very good dense segmentation output with a clear boundary.

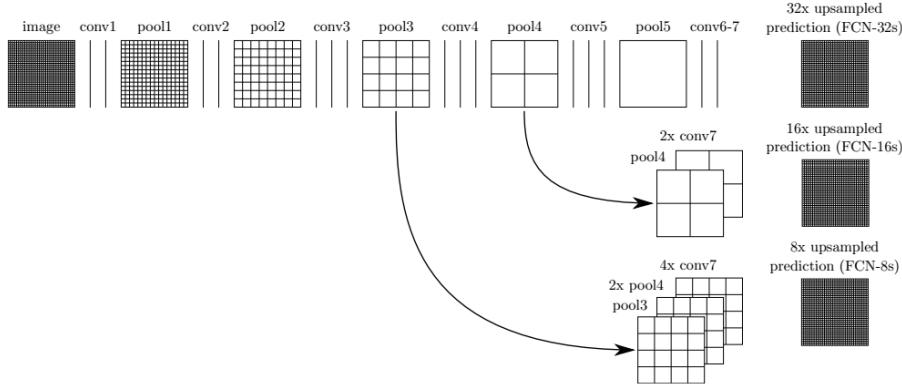


Figure 3.3. FCN Skip Architecture.

3.2 U-NET: CONVOLUTIONAL NETWORK FOR BIO-MEDICAL IMAGE SEGMENTATION

Olaf Ronneberger et al [40] proposed convolutional network architecture that won ISBI cell tracking challenge¹ 2015. The objective of this challenge was to track moving cells in time-lapse video sequences; it involved evaluating state-of-the-art whole cell and nucleus tracking methods for both 2D and 3D time-lapse microscopy videos of labeled cells and nuclei. As a typical medical imaging challenge the size of data was small compared to other object segmentation datasets this laid the importance of encompassing the use of data augmentations by the author in this network. The network is compared with sliding-window ConvNet approach that won ISBI challenge for Electron Microscopy in 2012 [33] and proves to have achieved much effective segmentation results. But unlike the compared method U-Net employs a Fully Convolutional approach for segmentation that aids in training model end-to-end and resulting in a pixel-to-pixel level classification. U-Net architecture has a contracting

¹www.codelorzano.com/celltrackingchallenge/Cell_Tracking_Challenge/Welcome.html

path to capture context and expanding path to enable precise localization. As in fully convolutional model there are no fully connected layers in this architecture.

3.2.1 U-NET ARCHITECTURE

The contracting path in U-Net architecture is similar to typical ConvNet that consists of alternating convolution layers and pooling layers with ReLU activation. With specific to U-Net the contracting path has convolution-pooling pair that consists of two convolution filters with non-linear activation (ReLU) followed by max pooling that down samples the data and provides translation invariance. The U-Net architecture has four successive convolution pooling pairs as described in the contracting path. When the input image is passed down through these successful filters the subject of image is summarized by generating feature maps at each level of convolution. Though feature maps have context information summarized in them they are reduced in size due to pooling operations. Also a direct upsampling of such a feature map may raise distorted boundaries which cannot be appreciated in a segmentation task. The contracting path is followed by two convolutions to surround entire region of feature maps to augment fully connected layers in a typical classification type ConvNet and makes U-Net a type of fully convolutional network. The goal of our task lies at predicting possibility of a pixel belonging to a cell or background and achieve this for all pixels in the input image which raises the need for our output probability map to be same size of input. Hence the contracting path of our architecture is followed by an expanding path that aims to learn localized information from image lost by pooling during contracting path and also boost the resolution of output feature maps to match input resolution. As discussed earlier segmentation architectures have very similar encoding or contracting paths which is same in this case and difference lies in the type of expanding path. U-Net uses a technique resembling decoder part of

SegNet architecture, to use convolutions followed by upsampling instead of learned deconvolutions as employed in FCN architecture but with a significant difference in terms of how the upsampling is attained. The expanding path is retained very similar to contracting path in terms of convolutions but replacing pooling layers with corresponding upsampling layers that provides this model U shaped structure. In U-NET feature maps obtained at contracting level is stored in memory and used to concatenate with upsampled feature map during the expanding path. This corresponding concatenated, upsampled feature acts input for corresponding convolutions followed by ReLU and acts as deconvolution process in this architecture. The intent to concatenate feature maps from lower level layers with corresponding increased resolution feature maps in higher layer is to provide capability for convolution filters in expanding path to gain localized information from summarized feature maps to assemble more precise output. Convolutions in expanding path also has large number of feature channels making it even more symmetric to contracting path yielding support for U-Shaped architecture. The number of convolutions or hierarchy levels for both contracting path and expanding path is to be maintained constant. At the final layer a 1X1 convolution is used to map each channel in last feature map of expanding path to classify at pixel level probability of pixel being a cell. Figure 3.4 visualizes the architecture of U-Net

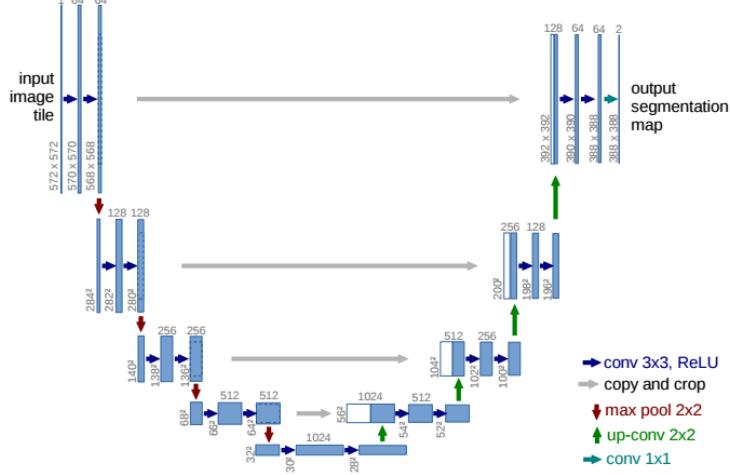


Figure 3.4. U-NET Architecture.

3.3 SEGNET: A DEEP CONVOLUTIONAL ENCODER-DECODER ARCHITECTURE FOR SEGMENTATION

SegNet [38] is a deep fully convolutional neural network for semantic pixel-wise segmentation that can be trained in an end-to-end fashion. The core trainable engine has an encoder network, corresponding decoder network followed by a pixel classification layer. SegNet claims to fully convolutional as it adopts the principle of convolutionalized means of fully connected layers in the encoding architecture. Typically, each encoder consists of one or more convolutional layers with batch normalization and a ReLU non-linearity, followed by non-overlapping max-pooling and sub-sampling. The sparse encoding due to the pooling process is upsampled in the decoder using the max-pooling indices in the encoding sequence. One key ingredient of the SegNet is the use of max-pooling indices in the decoders to perform upsampling of low resolution feature maps. This has the important advantages of retaining high frequency details in the segmented images and also reducing the total number of trainable parameters in the decoders.

3.3.1 SEGNET ARCHITECTURE

SegNet follows hierarchy of two different networks namely Encoder network and Decoder Network. Encoder network is responsible for learning non-linear coarse classifier from the input image similar to initial layers of any ConvNet (convolution layers prior to fully connected layers) and fully connected layer replaced by series of convolutions with a span of whole input region. This encoder returns a coarse map of classifier scores as an output. Therefore decoder is in charge to encompass spatial information in order to make pixel wise predictions much denser and return an output with size equivalent to input image. The original SegNet architecture was designed for road scene labeling and incorporates layers VGG-16 as a part of encoder. But for our purpose of Cell Segmentation, this specific architecture seems to be too complex with many parameters and we employ different variations of encoder and decoder network.

3.3.2 ENCODER NETWORK AND ITS VARIANTS

The variants of encoder network differ only with respect to the number of encoders used. Irrespective of variant each encoder has a convolution with a specific size of filters to produce a set of feature maps. These features maps are batch normalized, batch normalization [39] helps to provide any layer in a Neural Network with inputs that are zero mean/unit variance. Following batch normalization an element-wise rectified non-linearity (ReLU) is applied followed by max pooling with a stride of 2X2, this operation down samples corresponding feature maps by a factor of 2. Max-pooling is used to achieve translation invariance over small spatial shifts in the input image. Though several layers of sub-sampling can achieve more translation invariance there is a loss of spatial resolution of the feature maps. The increasingly lossy (boundary detail) image representation is not beneficial for segmentation where

boundary delineation is vital. Therefore, it is necessary to capture and store boundary information in the encoder feature maps before sub-sampling is performed. In case of unrestricted memory, which is not usually the scenario in practical applications all the encoder feature maps can be stored in memory to be used in upsampling. SegNet provides a memory efficient idea by storing only max-pooling indices, i.e. positions of the maximum feature in each pooling window. For 2X2 pooling that is used as a part of our network this is achieved with use of 2 bits for each window. Encoders produce coarse or sparse feature maps as classifier scores which in turn during are made dense by corresponding decoders. The pooling indices stored in memory by each encoder are used by corresponding decoder. For the purpose of Cell Segmentation considering the nature of data, available number of samples we experiment with 2, 4 and 6 encoders as a part of encoder network. Each encoder has a corresponding decoder this implies the number of encoder-decoder pairs are same for each variant.

3.3.3 DECODER NETWORK

Similar to FCN network adapted previously for cell segmentation, SegNet also has almost similar encoder features. Segmentation architectures mainly differ by means of decoding technique used. In FCN network we saw that upsampling was learned through deconvolutions, in contrary SegNet does not have learning phase during upsampling. SegNet uses the statically upsampled feature maps to be convolved with trainable multi-channel decoder filter to densify the sparse input feature maps. This significantly reduces number of trainable parameters in decoding network for SegmentationNetwork. The deconvolution process here is represented as upsampling followed by regular convolution instead of employing a backward convolution process. Dimensionality reduction of the encoder feature maps, say of 16 channels, is performed by convolving them with $1 \ 1 \ 16 \ 2$ trainable filters, where 2 represents

the number of classes in cell segmentation task. The compressed feature maps are the input to the decoder network. In a decoder of this network, upsampling is performed by inverse convolution using a fixed or trainable multi-channel upsampling kernel. A decoder is applied for every corresponding encoder in all the variants namely SegNet-2, SegNet-Basic and SegNet-6 for segmenting cells in histopathology images. Batch Normalization is also applied during decoder process in decoder network of SegNet. The feature map from final decoder is then fed as input to a sigmoid classifier layer. In Cell segmentation task sigmoid classifier is employed rather than softmax classifier as used in original architecture because there occurs to be only two classes in our segmentation task. The final classifier output is a probability map of confidence scores with size exactly same as input size. The figure below represents a visualization of original VGG based 13 layer of SegNet Encoder-Decoder.

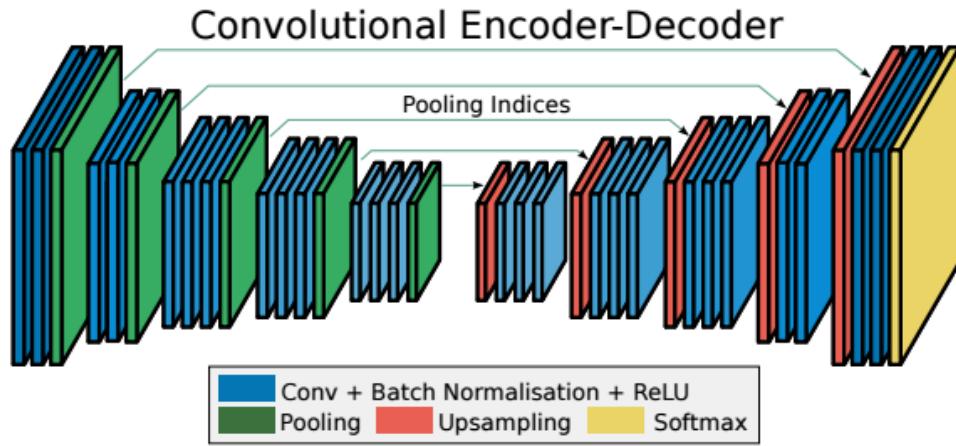


Figure 3.5. SEG-NET Architecture.

CHAPTER 4

EXPERIMENTS AND RESULTS

4.1 DATASET OVERVIEW

4.1.1 DATASET-1 : LUNG CANCER HISTOPATHOLOGY IMAGES

Lung cancer images are obtained from TCGA data portal¹ for Lung Squamous cancer carcinoma. TCGA represents a large-scale initiative funded by the National Cancer Institute and National Human Genome Research Institute. To bring out this dataset TCGA researchers claims to have examined tissue samples from 178 patients with untreated lung squamous cell carcinomas. Notably, 96 percent of the patients in this study had a history of tobacco use. From the data repository of TCGA, Whole Slide Images (WSI) for lung cancer were collected and extracted to 512 X 512 pixels at 40x magnification. Unfortunately, the data available at this repository lacks segmentation ground truth to perform experiments. Hence, SMILE-Annotate was developed as tool to manually label histopathology patch images for cell segmentation. 150 Images were carefully labeled and gold standard data for annotations was obtained to be used for experiments in this thesis. To evaluate performance of convolutional networks on this data 100 images are used for training and the remaining 50 images are applied for testing.

¹<https://gdc-portal.nci.nih.gov>

4.1.2 DATASET-2 : CROWD SOURCING LABELED CANCER HISTOPATHOLOGY IMAGES

The images from this dataset [41] come from WSIs of kidney renal clear cell carcinoma (KIRC) from the TCGA data portal. TCGA represents a major resource for projects in computational pathology aiming at linking morphological, molecular, and clinical characteristics of disease. 10 KIRC whole slide images (WSI) from the TCGA data portal were selected representing a range of histologic grades of KIRC. From these WSIs, we identified nucleus-rich ROIs and extracted 400 × 400 pixel size images (98.24 m × 98.24 m) for each ROI at 40 magnification. Out of 810 totally collected images, crowdsourced labeling was performed at three different levels namely Pathologist Labeled (64 Images), Expert research members labeled (421 Images), on-line contributors labeled (810 Images). Of these three annotation categories we consider only pathologist and expert labeled data for our experiments. The combined labeled dataset by pathologists and experts yield 485 Images of which 350 images were used for training, 35 images for validation and remaining 100 images for testing.

4.2 SMILE-ANNOTATE

From the dataset description we see that there happens to be no annotations for dataset-1 and in dataset-2 the authors employ crowd-sourcing based labeled annotation. Even though a plethora of image labelling tools for object segmentations do exist they are not widely suitable for the task of cell segmentation. In general the tools either allow users to label a single object per image or restrict size of contours with a minimum and maximum range. Some tools are so complicated with too much options that what actually the user wants and it brings additional pain to user rather than being supportive. To overcome these problems, SMILE-ANNOTATE a Matlab

based application was developed for the purpose of this thesis to obtain manually labeled ground truth for lung cancer image data. The user draws a contour around each cell and pixels within the contour are labeled as foreground while all other objects are labeled background. We provide a screenshot to help visualize the use of our tool. This is not only limited to cell annotations but can also be used for any multi-object based segmentation tasks to generate near ground-truth images.

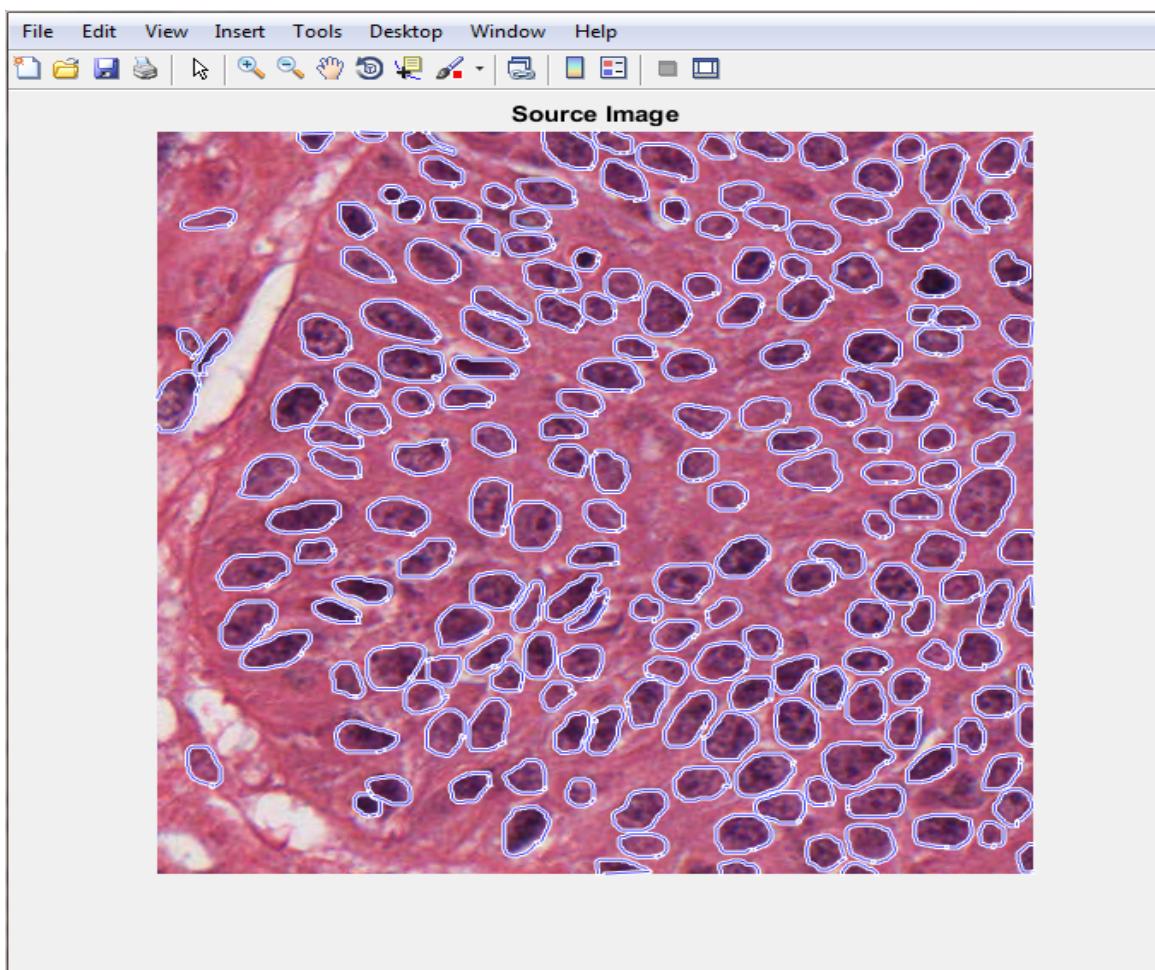


Figure 4.1. SMILE-ANNOTATE : Marking Contours by User.

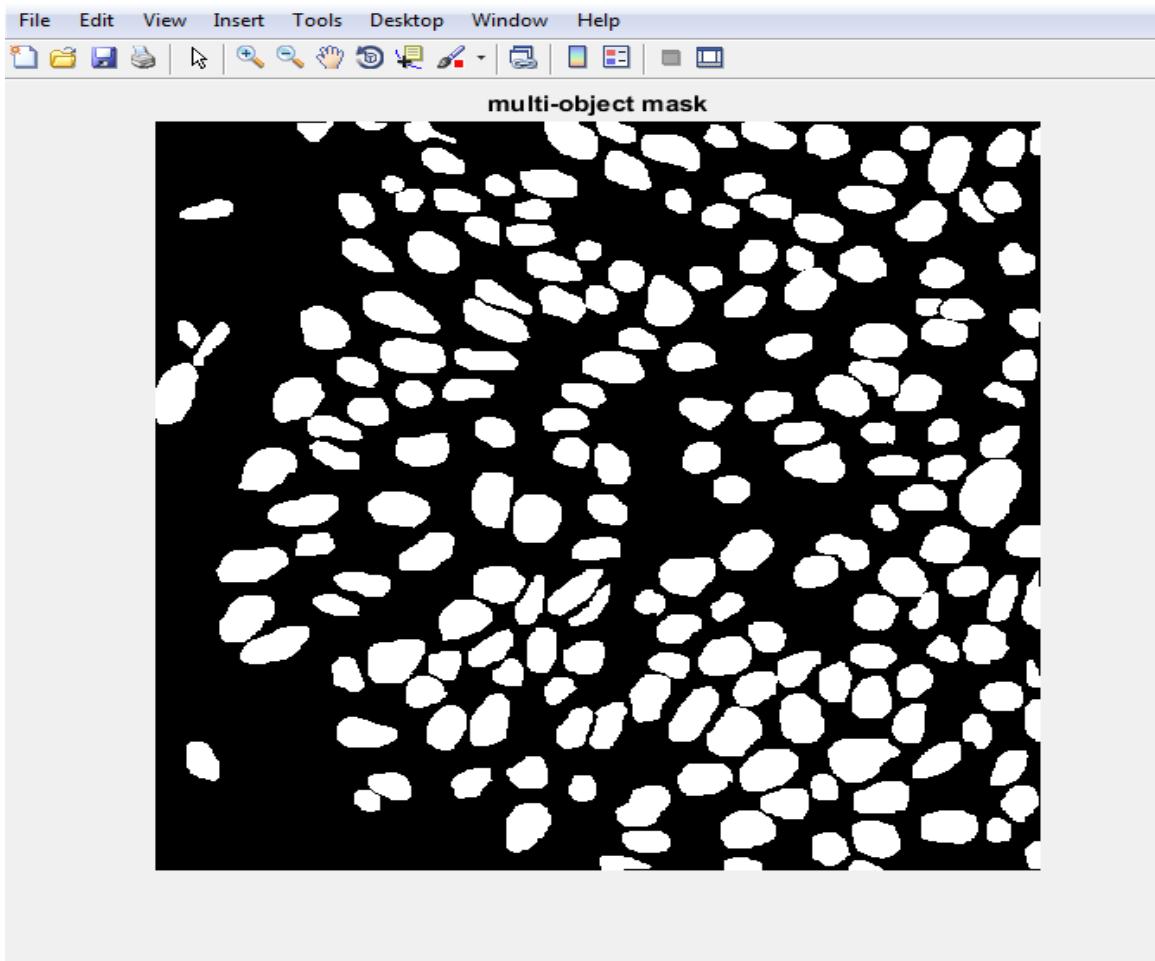


Figure 4.2. SMILE-ANNOTATE : Near Ground Truth image generated based on Marked Contours.

4.3 EXPERIMENTAL SETUP

To successfully perform experiments on both the data sets following requirements were setup at SMILE Lab. The hardware configurations of the system performing experiments were:

1. 3.4GHz Intel core i7 4770 CPU with a RAM of memory size 16 GB.
2. 12 GB of Nvidia K40 GPU
3. 12 GB of NVIDIA GeForce Titan X GPU

The software requirements setup for experiments were

1. OS : Ubuntu 14.04 and Windows 7 64 bit
2. Programming Languages: Python 2.7
3. Deep Learning libraries : Keras, Tensorflow (v0.10)
4. Scientific Toolbox: Matlab R2015a

4.4 EVALUATION METRICS

4.4.1 DICE SIMILARITY

The Dice coefficient² D is one of a number of measures of the extent of spatial overlap between two binary images. It is commonly used in reporting performance of segmentation and gives more weighting to instances where the two images agree. Its values range between 0 (no overlap) and 1 (perfect agreement). Considering X and Y as two binary vectors (segmentation images) of ground truth and automatic segmentation the Dice coefficient, D is obtained using the following Equation :

$$D = 2 * \frac{|X| \cap |Y|}{|X| + |Y|} \quad (4.1)$$

Dice coefficient, D is multiplied by 100 to present it in terms of percentage

4.4.2 ROC CURVES

The ROC³ curve helps in the creation of a full and detailed sensitivity vs 1-specificity report. The true positive rate or sensitivity is plotted against the function of the false positive rate or specificity. The sensitivity is the probability of a test

²https://en.wikipedia.org/wiki/Dice_coefficient

³<https://www.medcalc.org/manual/roc-curves.php>

outcome being positive when they are truly positive and specificity is the probability of the test outcomes being negative truly as such.

$$Sensitivity \text{ or } True \text{ Positive Rate } (TPR) = \frac{TP}{(TP + FN)} \quad (4.2)$$

$$Specificity \text{ or } True \text{ Negative Rate } (TNR) = \frac{TN}{(TN + FP)} \quad (4.3)$$

where TP denotes True Postive, TN denotes True Negative,
 FP is False Positive, FN is False Negative,
 P is Positive and N is Negative

4.5 EXPERIMENTS

4.5.1 DATA AUGMENTATIONS

Convolutional Networks makes use of pooling layers that help the model to be translation invariant, in order to make our models even more robust with rotation invariance and to gain additional training data we make use of data augmentations in the training process. The data augmentation techniques we employ in this thesis are rotations by two angles (60 Degrees and 120 Degrees), horizontal flips and vertical flips. Such a technique is generally used in convolutional networks to escalate the size of training data which in turns yields sturdy model.

4.5.2 FULLY CONVOLUTIONAL NETWORKS FOR CELL SEGMENTATION

4.5.2.1 TRAINING

The fully convolutional network is implemented with Alex-Net architecture and also as seen before has a learned upsampling means of deconvolution. This architecture consists of around 8M trainable parameters. The model is trained with ADAM

optimizer, learning rate of 1e-3, batch size is 5. A standard image data normalization is achieved to keep the values in pixels within a specific range and maintain unit variance and standard-deviation.

4.5.2.2 ROC CURVE

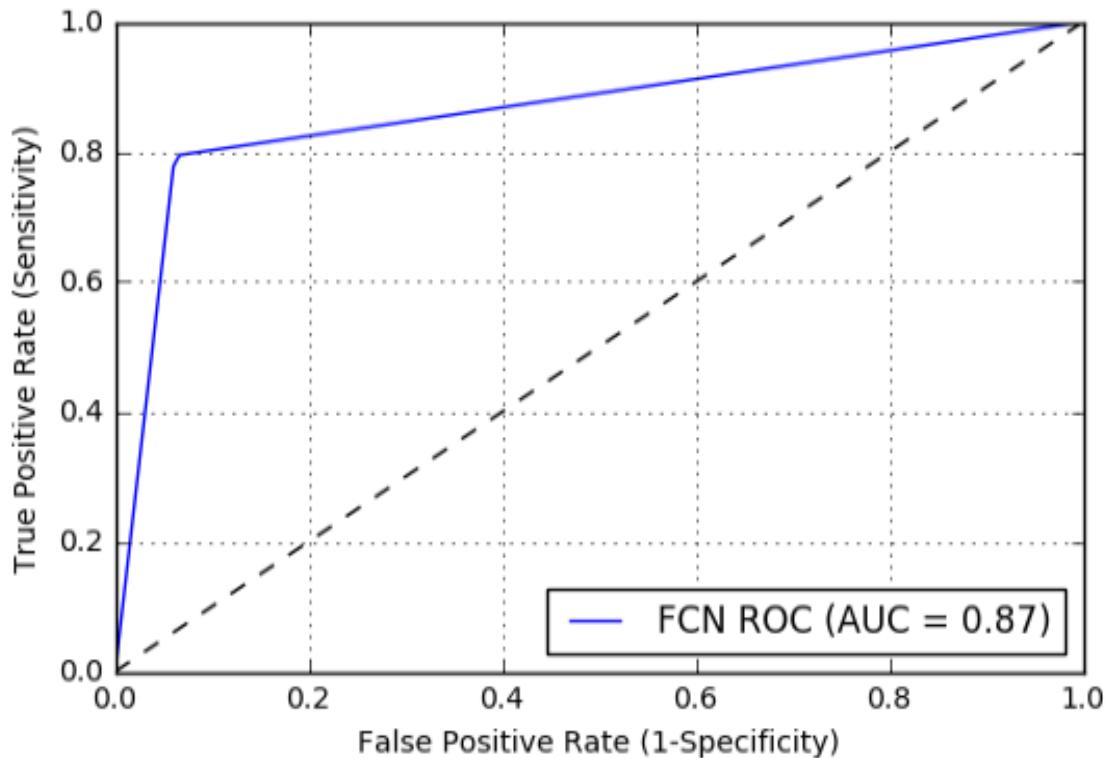


Figure 4.3. ROC Curve for FCN prediction on Dataset-2.

4.5.2.3 U-NET RESULTS VISUALIZATION

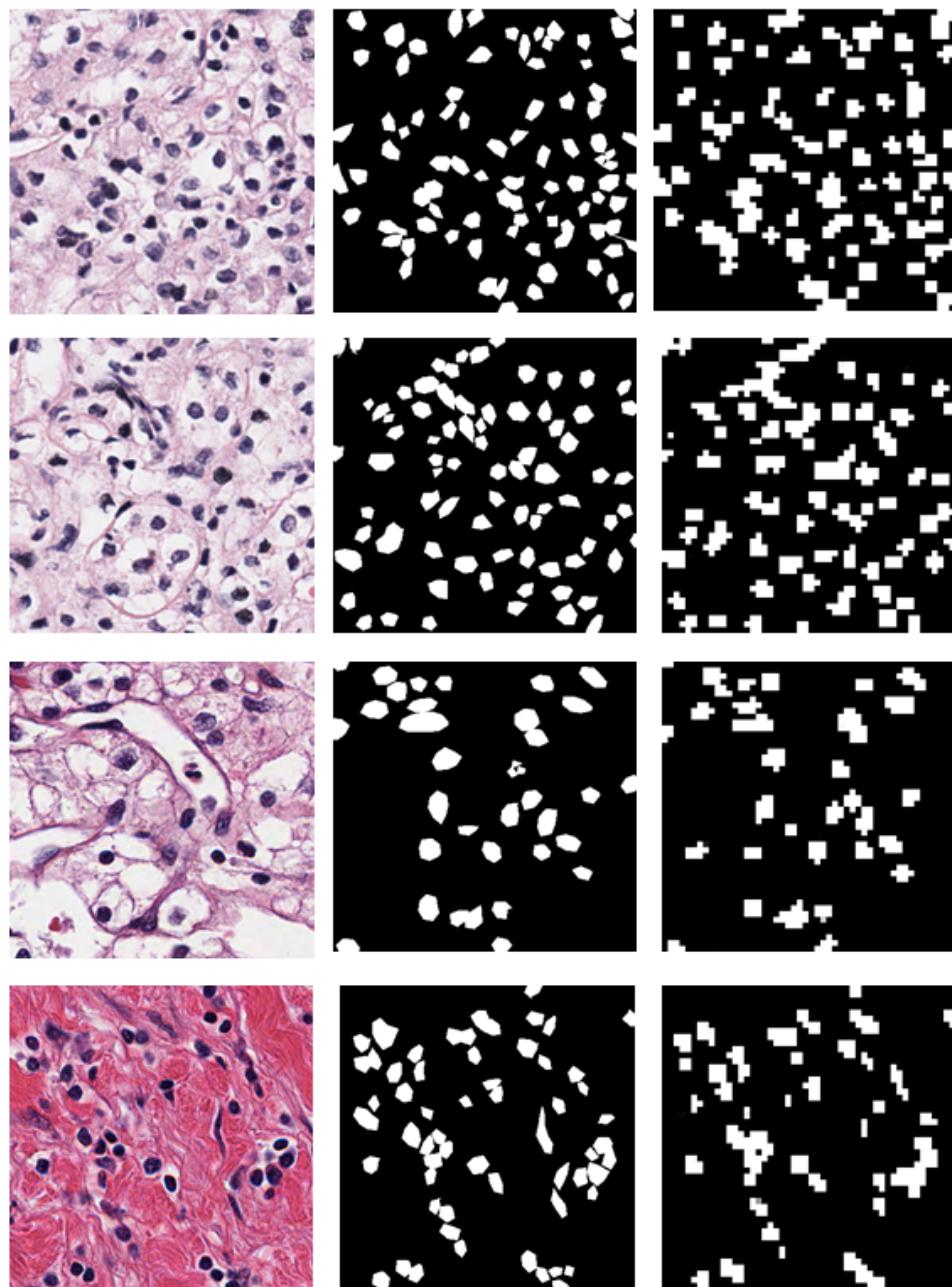


Figure 4.4. FCN CELL SEGMENTATION VISUALIZATIONS (Column A: Source Images ; Column B: Expert Labeled Annotations; Column C: Automated FCN SEGMENTATION).

4.5.3 U-NET FOR CELL SEGMENTATION

4.5.3.1 TRAINING

We employ the model architecture of U-Net with minor changes to apply for the task of cell segmentation. The network consists of 19 Convolution layers with a total of 7.8M trainable parameters. The training process is executed with the help of ADAM optimizer at a learning rate of 1e-5 and using dice_coef as the loss function. The model is trained continually on a GPU with batch size 1 until convergence is achieved. A standard image data normalization is achieved to keep the values in pixels within a specific range and maintain unit variance and standard-deviation.

4.5.3.2 ROC CURVE

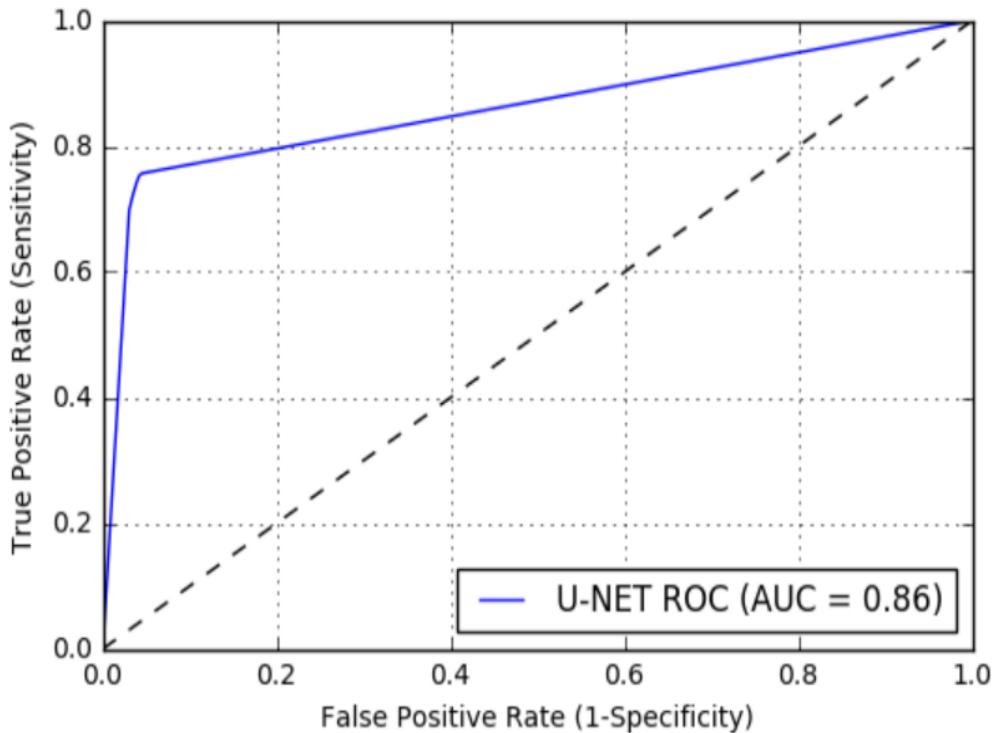


Figure 4.5. ROC Curve for U-NET prediction on Dataset-2.

4.5.3.3 U-NET RESULTS VISUALIZATION

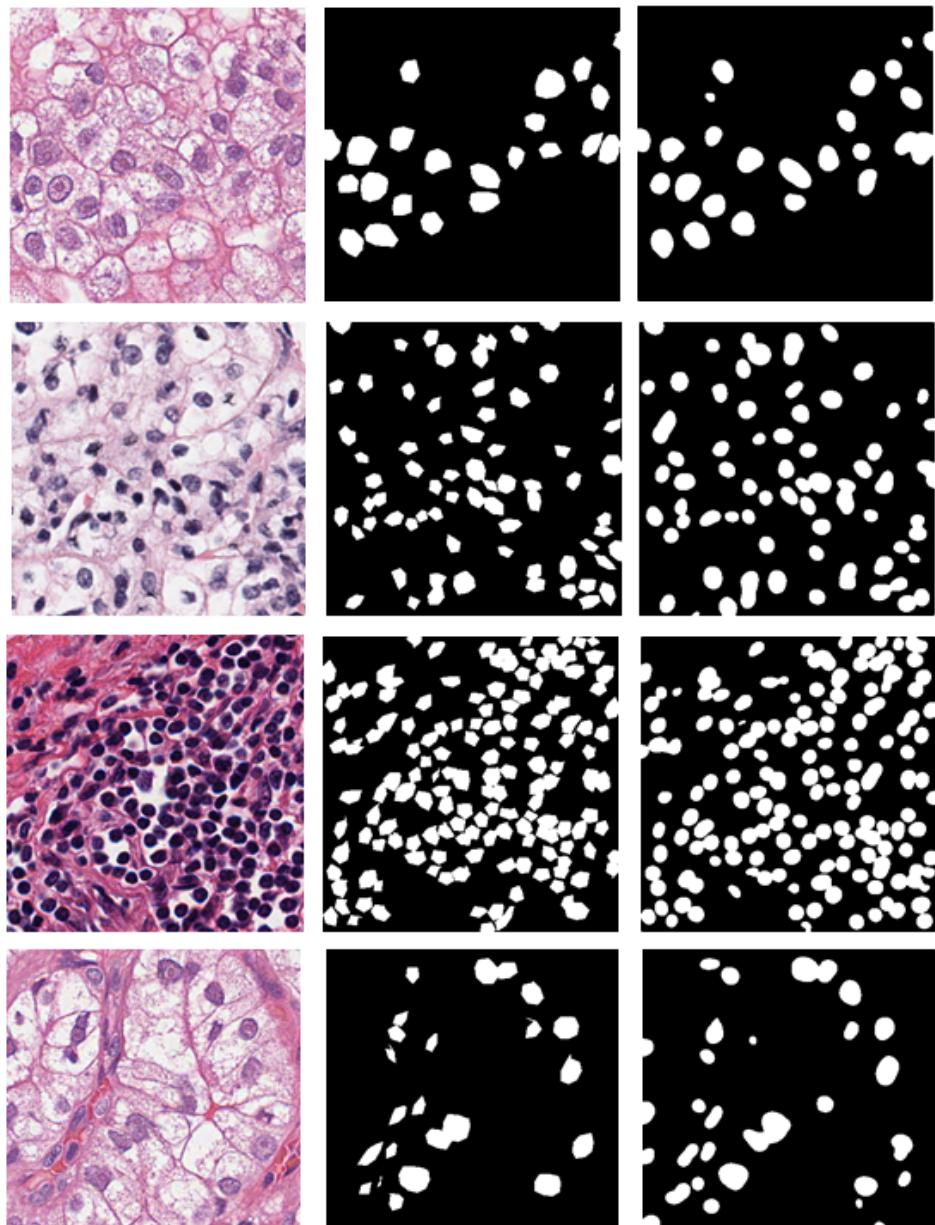


Figure 4.6. U-NET CELL SEGMENTATION VISUALIZATIONS (Column A: Source Images ; Column B: Expert Labeled Annotations; Column C: Automated U-NET SEGMENTATION).

4.5.4 SEG-NET FOR CELL SEGMENTATION

4.5.4.1 TRAINING

We employ the model architecture of SEG-Net with four encoders and four decoders to apply for the task of cell segmentation. The network consists of a total of 5.8M trainable parameters. The training process is executed with the help of ADAM optimizer at a learning rate of 1e-5 and using dice_coef as the loss function. The model is trained continually on a GPU with batch size 1 until convergence is achieved.

4.5.4.2 ROC CURVE

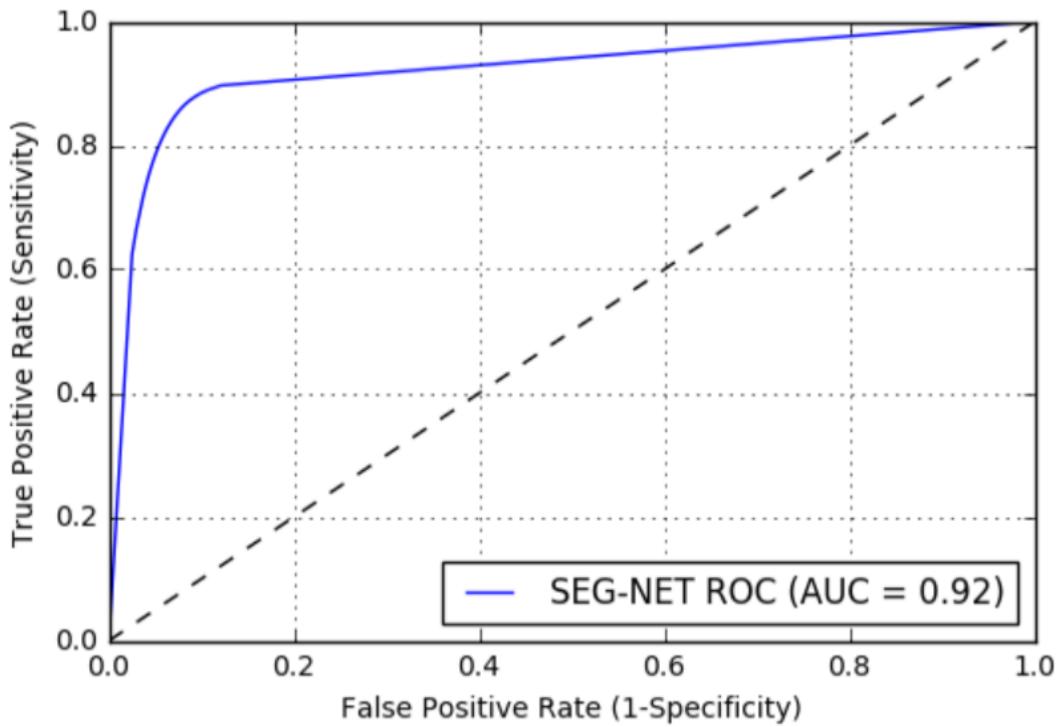


Figure 4.7. ROC Curve for SEG-NET prediction on Dataset-2.

4.5.4.3 SEG-NET RESULTS VISUALIZATION

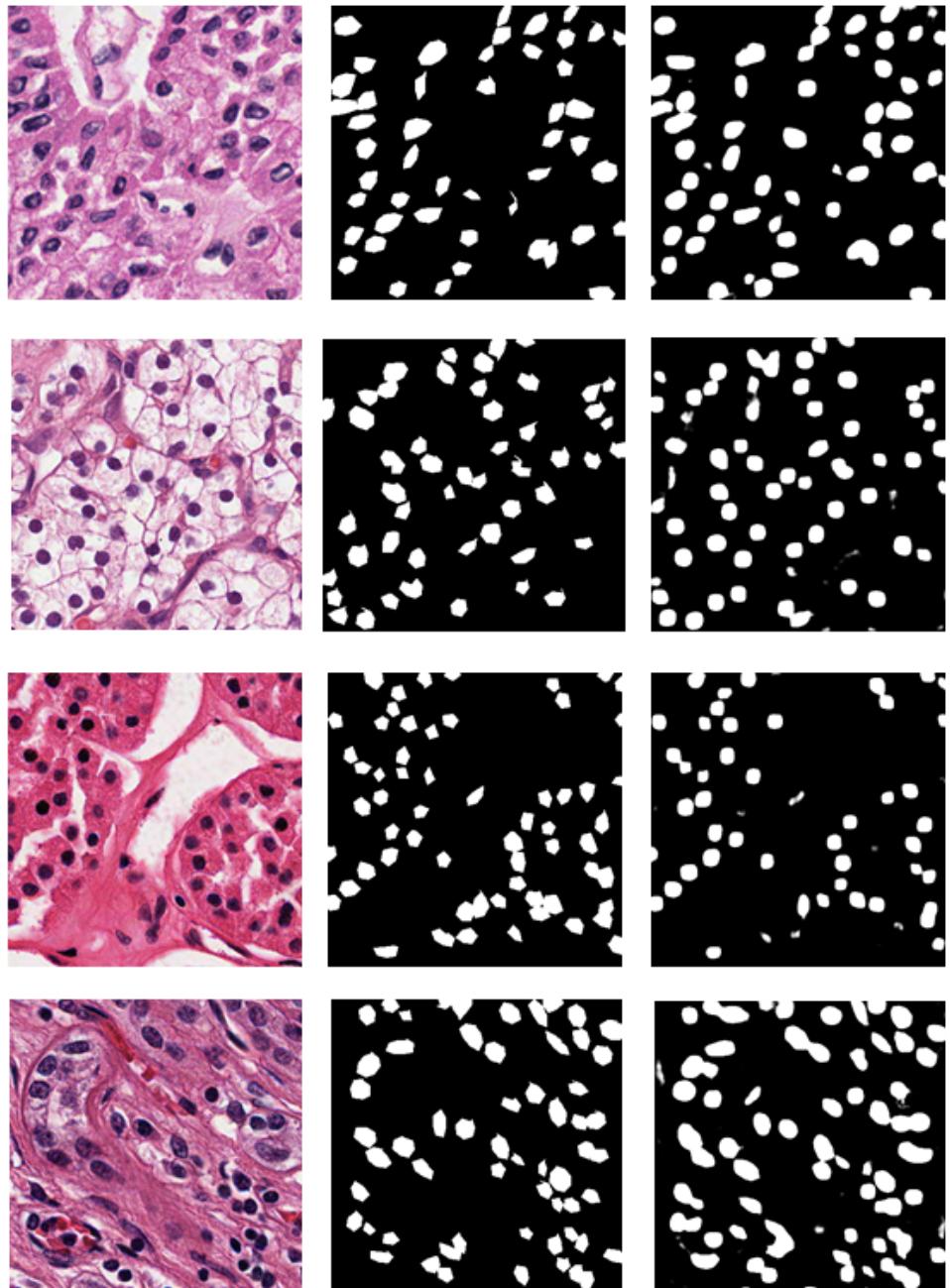


Figure 4.8. SEG-NET CELL SEGMENTATION VISUALIZATIONS (Column A: Source Images ; Column B: Expert Labeled Annotations; Column C: Automated U-NET SEGMENTATION).

4.5.5 CLASSICAL METHODS VS SEG-NET FOR CELL SEGMENTATION

4.5.5.1 ROC CURVE

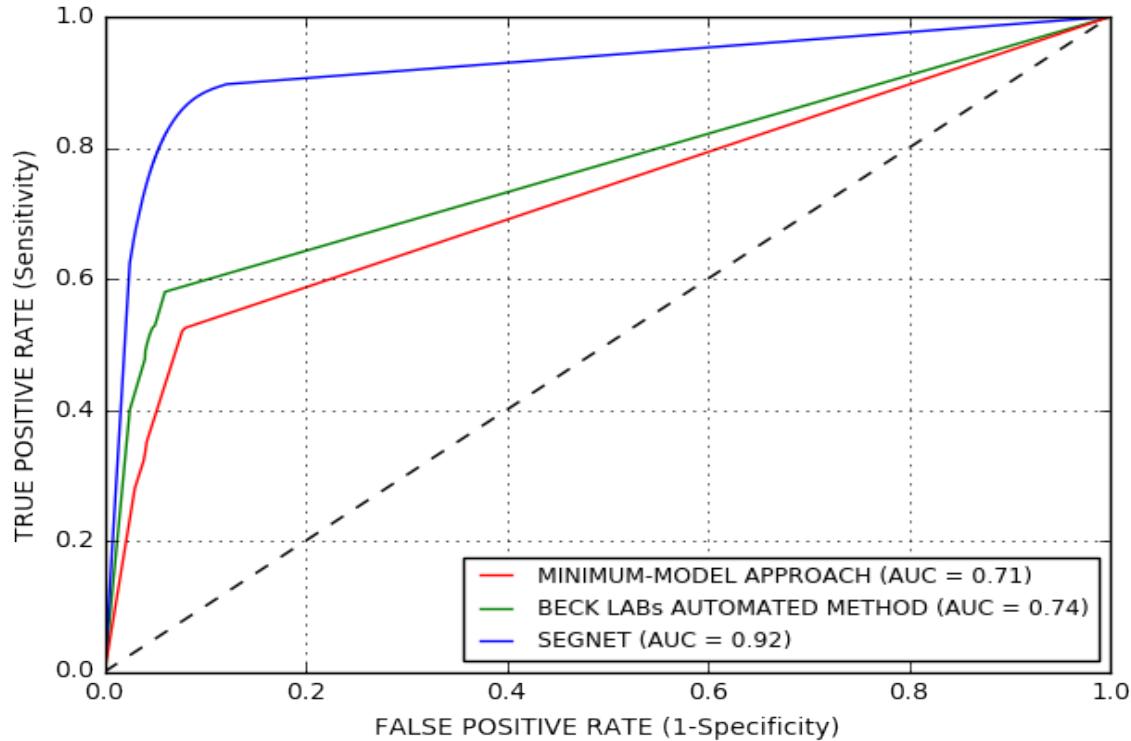


Figure 4.9. ROC Curve for Classical Methods vs Seg-Net prediction on Dataset-2.

4.5.6 RESULTS FOR LUNG CANCER DATA (DATASET-1)

The lung cancer images did not come with segmentation annotations and as a part of this thesis we had manually labeled 150 images after carefully observing several cell or non-cell examples in cancer histopathology images. Even with very few images our approach of convolutional networks work well by learning effective features from our manually generated labels. This illustrates SMILE-ANNOTATE software achieve its purpose of generating gold standard images and also highlights that data

augmentation can be strongly relied on while using deep learning approaches for cell segmentation. We present the performance comparison between U-net and Seg-Net on Dataset-1.

4.5.6.1 ROC CURVE

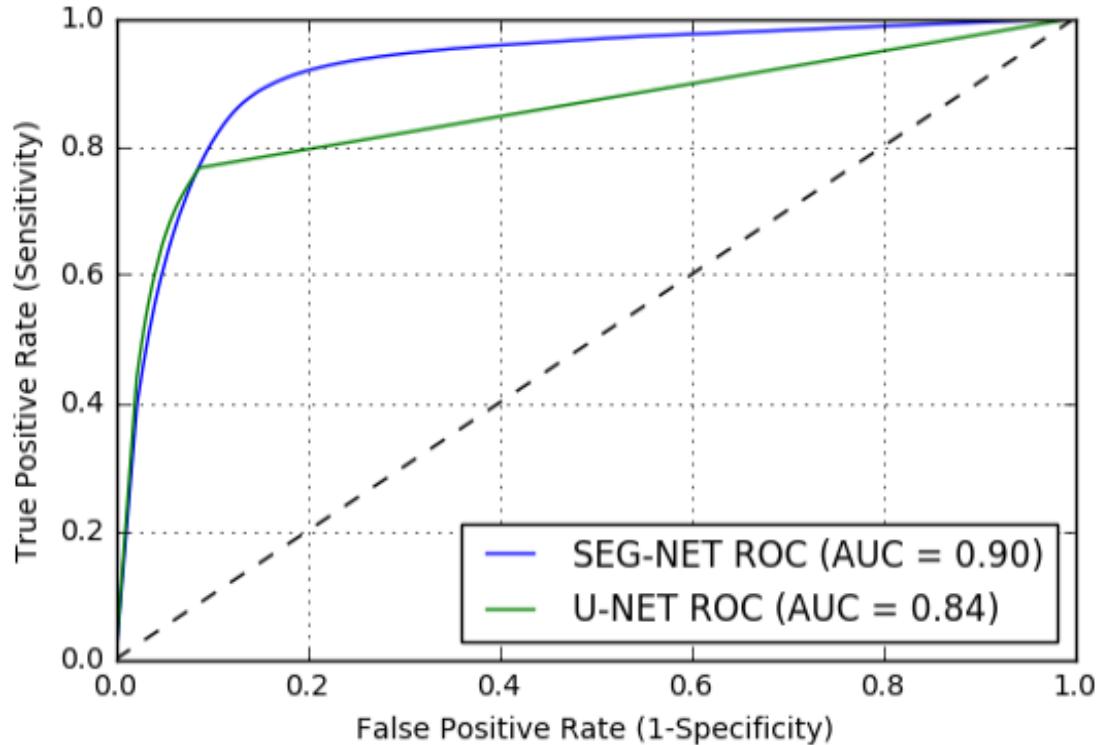


Figure 4.10. ROC Curve for SEG-NET vs U-NET on DATASET-1.

4.6 RESULT ANALYSIS AND CONCLUSION

Table. 4.1 lists certain attributes of comparison for deep-learning methods vs classical approaches for dataset 2. Also, the Average dice co-efficient which measures the image similarity or overlap measure between Annotation ground truth and Automatically Segmented Image for different methods is provided.

Comparison Attribute	Minimum Model Approach	Beck Lab Automated Method	FCN	U-NET	SEG-NET
Number of Training Images	-	-	1750	1750	1750
Number of Test Images	100	100	100	100	100
Testing Time for 100 Images	280 Seconds	-	18 Seconds	25 Seconds	6 Seconds
Average Dice-Coefficient for 100 Test Images	64 %	67 %	70 %	74 %	79 %

Table 4.1. Experimental Comparison on Dataset - 2

The next table. 4.2 lists experimental comparison between U-Net and Seg-Net for lung cancer data.

Comparison Attribute	U-NET	SEG-NET
Number of Training Images	500	500
Number of Test Images	50	50
Testing Time for 100 Images	14 Seconds	3.8 Seconds
Average Dice-Coefficient for 100 Test Images	71 %	74.6 %

Table 4.2. Experimental Comparison on Dataset - 1

From the above tables it is observed that FCN and U-NET models take much longer time for prediction of test images than SEG-NET which can be justified based on the number of parameters in the model. The average dice coefficient is a measure of overlap index which tends to be higher for SEG-NET but it does not necessarily mean

that all test images of SEG-NET performed exceptionally well than other models in terms of segmentation. Segmentation tasks give much importance to boundary delineation which obviously is better in U-NET and SEG-NET compared to FCN. But, segmentation results for few closely bounded cells it is observed that U-NET performs better because the entire feature map of lower level convolutions is concatenated in the expansion path that presumably helps U-NET to establish much clear boundaries of cells more than SEG-NET. SEG-NET has the capability to learn features which help in accurate prediction of true positives which leads to high dice-coefficient and AUC score.

CHAPTER 5

CONCLUSION AND FUTURE WORK

The aim of this thesis was to evaluate the feasibility of applying Convolutional Networks for the purpose of Cell Segmentation from histopathology images. The importance of cell segmentation in digital pathology is realised by the fact that cancer being a disease proliferating by means of cells, accurate preliminary of localization of cells in digital images aid radiologists to accelerate preliminary diagnosis of patients [9]. Currently convolutional neural networks outrun all previous methods in many vision perception applications like classification by winning challenges over huge margin. The onset of ConvNets success influences researchers to apply its techniques for structured prediction tasks like pixel level classification. Off late numerous researchers experiment effectiveness of ConvNets in segmentation approaches with respect to different applications. This curiosity fuels the goal of our thesis to evaluate effectiveness of deep learning approaches in cancer diagnosis. Due to the fact that large annotated dataset is missing for cancer histopathological images and ConvNets can be highly exploited only in the presence of huge labelled datasets there are very few research tasks being performed in cell segmentation using deep learning. We took this as a challenge and successfully evaluated three deep learning approaches and its efficacy for effective cell segmentation by resorting to techniques like use of data augmentations and development of simple image labelling tool to generate gold standard annotation images.

From the experiments performed on two different datasets, we conclude that convolutional networks with appropriate loss functions and training methods can very

efficiently segment cancer cells from micro-slide images. We also prove that crowd-sourcing labeling process seems effective to train our models as convolution networks inherently have the ability to understand data in a coherent manner for the application it is being trained. Despite the results that shows CNN's have power to generalize between different type of cells tumor or non-tumor with little data there exists a huge arena to optimize the performance of convolution networks for cell segmentation. Based on our evaluations a future research direction could be to apply training on whole open source dataset of kidney cancer images and fine-tune it on TCGA's lung cancer data to deliver more detailed report on generalization capability of convolution networks. Also another suggestion for future research direction to boost accuracy of cell segmentation would be the use of Ensemble methods for cancer diagnosis using deep learning.

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BIOGRAPHICAL STATEMENT

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