Nucleus Segmentation of Breast Histology images using a PIX2PIX Adversarial Neural Network

A Thesis

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Master of Computer Science and Engineering

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by

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I hereby declare that this thesis entitled **"Nucleus Segmentation of Breast Histology images using a pix2pix Adversarial Neural Network"** contains a literature survey and original research work by the undersigned candidate, as part of his degree of Master of Computer Science and Engineering of the Department .

All information has been obtained and presented in accordance with academic rules and ethical conduct.

I also declare that, as required by these rules and conduct, I have fully referenced all materials and results that are not original to this work.

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Chapter 1

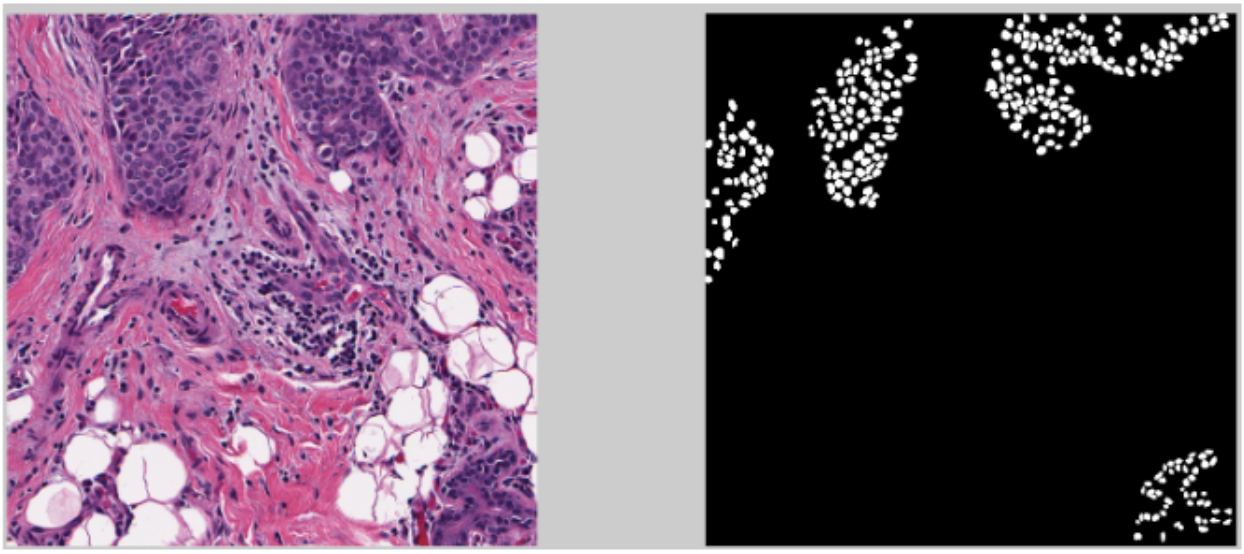
Introduction

1.1 Nucleus segmentation of histology images

Breast cancer is the most common type of women's cancer and is the second cause of women's cancer death each year. Early breast cancer diagnosis is vital for cancer patients, the survival rate of 5-year for breast cancer patients can reach 90% with early diagnosis .

Currently, breast cancer detection modalities have evolved with many types, and most of them use image-based approaches to screen and detect the tumor mass. We can name them such as electrical impedance based imaging, breast ultrasound, scintimammography, computed tomography (CT), positron emission tomography (PET), thermography, optical imaging, mammography, and magnetic resonance imaging (MRI). Breast cancer diagnosis often starts with mammography imaging analysis using Deep Learning to detect the existence of cancer tumors. However, sometimes the method comes to an undetermined conclusion, and in situations like this, the breast cancer diagnosis needs to use biopsy and H & E-stained image analysis to have a more accurate diagnosis . The ER+ BCa image is given in fig 1.1 with the manually segmented mask [1].

Hematoxylin and Eosin-stained is one of the primary techniques used for histological staining, because of its simplicity and the capability to illustrate many different tissue structures. The biopsy samples are prepared on slides, where Hematoxylin material stains the nuclei with a black colour to show the intranuclear intricacies in clear detail and Eosin material stains the cell cytoplasm and tissue fibers with pink, red, or orange colours in a variety of intensity levels. The slide photos were taken using whole-slide digital scanners and kept for breast cancer staging or cancer tumor cell detection. Traditionally, digitized histopathology images have been evaluated manually and laboriously, which has been time-consuming and limited by pathologists' skill. Thus, we need an accurate and quantitative analysis approach for histopathology images.



(a) (b)

Fig 1.1 (a) ER+ BCa images scanned at 40x (b) nuclei segmented image

Recently, the machine learning approach applied for histopathology image analysis has taken a leap, especially for Deep Learning-based methods that increase the efficiency and accuracy of histopathological diagnosis.

1.2 Applications

Apart from the complex nature of the problem, nuclei segmentation has great importance as it is required as a primary step for many other medical image segmentation problems such as,

- **Nucleus segmentation:**

  It involves cancerous nucleus segmentation from histology images using suitable techniques.

- **Cell counting:**

  It is a technique to count the total number of cancerous cells present in the histology image.

- **Cell size prediction:**

  It is a technique to verify the abnormal cell sizes present in Histology Images.

- **Medical image analysis:**

  It is used to achieve greater efficacy and efficiency in routine clinical practices and to support decision making processes.

- **Nuclei morphometric features detection:**

  It is a technique for extraction of relevant nuclei morphometric features(such as shape and chromatin texture). Such extraction is necessary for identifying nuclear pleomorphism.

1.3 challenges

Nucleus segmentation from histology images is a complex problem in the field of computer vision. The major reasons are as follows,

- **Lack of labeled data**

  The majority of AI systems need a sizable collection of high-quality training photos. Ideally, these training photos will be annotated. The region of interest (i.e., abnormalities or malignancy) must typically be manually delineated on all pictures by a pathologist. The optimum person to undertake annotation is an expert. In addition to the time commitment, human annotations also hinder the development of apps financially. Crowdsourcing could be speedier and less expensive, but it could also add noise. Working with low resolution or unclear images, slow networks, and ambiguous feature sets can make careful annotation of numerous images for pathologists not just tedious but also difficult. This strenuous effort might be made easier by active learning applied to

annotation. Right now, there are a small number of publicly available datasets that contain labeled images that can be employed for this purpose.

- **Pervasive variability**

  There are several basic types of tissue (e.g., epithelium, connective tissue, nervous tissue, and muscle). However, the actual number of patterns derived from these tissues from a computational perspective is nearly infinite if the histopathology images are to be "understood" by computer algorithms. Several tissue types build an organ that is also reflected in new textural variation of the basic tissue types. This extreme polymorphism makes recognizing tissues by image algorithms exceptionally challenging. Thus, the inherent architecture of deep AI requires many training cases for each variation. This, however, may not be readily available, especially as labeled data.

- **Dimensionality obstacle**

  WSI(Whole slide image) deals with gigapixel digital images of extremely large dimensions. Image sizes larger than 50,000 by 50,000 pixels are quite common. Deep ANNs, however, operate on much smaller image dimensions (i.e., not larger than 350 by 350 pixels). "Patching" [i.e., dividing an image into many small tiles, Fig 1.2] is a potential solution for not just AI algorithms but also for general computer vision methods. However, even for patches, one generally needs to downsample them in order to be able to feed them into a deep network. A region smaller than 1.5 μm^2 may not be suitable for many diagnostic purposes and this is, most of the time, at least 1000 by 1000 pixels. Downsampling these patches may result in loss of crucial information. On the other hand, deep nets with larger input sizes would need much deeper topology and much larger number of neurons making them even more difficult and perhaps impossible to train. Of note, patch-based ANNs have been shown to outperform image-based ANNs.

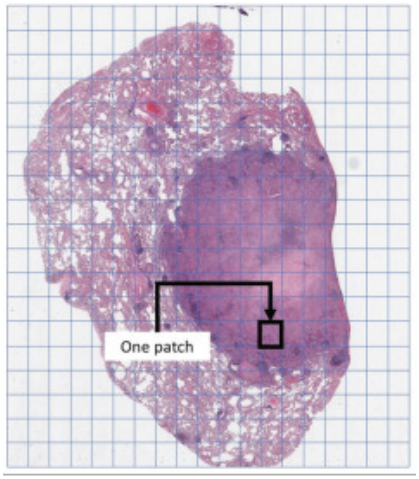


Fig 1.2 Patching is generally used to represent large scans. For instance, every patch could be a 2000 pixel ×2000 pixel image at ×40

- **Affordability of required computational expenses**

  Deep AI solutions are heavily dependent on using Graphical Processing Units (GPUs), highly specialized electronic circuits for fast processing of pixel-based data (i.e., digital images and graphics). Training and using deep solutions on ordinary computers with Central Processing Units is prohibitively slow and hence impractical. It is obvious that having access to GPU clusters is a must to deploy deep networks in practice. Pathology laboratories, however, are already under immense financial pressure to adopt WSI technology, and acquiring and storing gigapixel histopathological scans is a formidable challenge to the adoption of digital pathology. Asking for GPUs, as a prerequisite for training or using deep AI solutions.

- **Lack of transparency and interpretability**

  Deep ANNs have demonstrated several impressive success stories in object and scene recognition. However, they have not removed one of the major drawbacks of ANNs when used as classifiers, which is lack of interoperability. Some consider ANNs to

embody a “black box” after they are trained. Although researchers have started to investigate creative ways to explain the results of AI, there is at present no established way to easily explain why a specific decision was made by a network when dealing with histopathology scans. In other words, the millions of multiplications and additions performed inside a deep ANN in order to provide an output (i.e., a decision) do not provide a verifiable path to understanding the rationale behind its decisions. This is generally unacceptable in the medical community, as physicians and other experts involved in the diagnostic field typically need to justify the underlying reasons for a specific decision. The pathway to a reliable diagnosis must be transparent and fully comprehensible.

1.4 Scope of the work

The scope of the present work is to use pix2pix Generative Adversarial Network(GAN) for nucleus segmentation from the high resolution digital pathology slides images of size(2000,2000,3). For example, typical breast biopsy images scanned at 40x magnification containing billions of pixels , of which only a small percentage belong to the class of interest(i.e the nucleus) . preprocessing is required to superimpose the ground truth with respect to its original histology image to convert nuclei position pixel values with the original pixel values of the image. In the generated image post processing is done by applying gaussian filter and threshold with respect to image histogram.

1.5 Thesis Organization

The remaining part of the thesis are as follows,

Chapter 2 discusses literature survey on nucleus segmentation method for high resolution histology images, various approaches etc.

Chapter 3 contains brief discussion on pix2pix GAN(Generative adversarial network).

Chapter 4 presents methods used in this experiment.

Chapter 5 presents the result of the experiment and concludes the thesis.

Chapter 2

Past Work

In the previous chapter we have discussed the different applications and challenges of semantic segmentation of histology images .The goal for semantic segmentation is to assign a label to every pixel in an image is one of the fundamental topics in computer vision. The researchers have been addressing this topic since digital images became a thing. The algorithms have been improving over the years, starting from simple image thresholding classifying pixels into two classes to deep neural networks performing a multi-class segmentation with outstanding results.

Few of the past work on histology images for nucleus segmentation are explained below:

- Nucleus in Histology Images with Hierarchical Multilevel Thresholding [2] is a automatic segmentation of histological images is an important step for increasing throughput while maintaining high accuracy, avoiding variation from subjective bias, and reducing the costs for diagnosing human illnesses such as cancer and Alzheimer's disease. In this paper,the author presents a novel method for unsupervised segmentation of cell nuclei in stained histology tissue. Following an initial preprocessing step involving color deconvolution and image reconstruction, the segmentation step consists of multilevel thresholding and a series of morphological operations. The only parameter required for the method is the minimum region size, which is set according to the resolution of the image. Hence, the proposed method requires no training sets or parameter learning. Because the algorithm requires no assumptions or a priori information with regard to cell morphology, the automatic approach is generalizable across a wide range of tissues. Evaluation across a dataset consisting of diverse tissues, including breast, liver, gastric mucosa and bone marrow, shows superior performance over four other recent methods on the same dataset in terms of F-measure with precision and recall of 0.929 and 0.886, respectively.

- Mitosis detection in breast cancer histology images with deep neural networks[3].In this paper the author uses deep max-pooling convolutional neural networks to detect mitosis in breast histology images. The networks are trained to classify each pixel in the images, using as context a patch centered on the pixel. Simple post processing is then applied to the network output. This approach won the ICPR 2012 mitosis detection competition, outperforming other contestants by a significant margin.

- Detection of invasive ductal carcinoma in whole slide images with convolutional neural networks[4]. This paper presents a deep learning approach for automatic detection and visual analysis of invasive ductal carcinoma (IDC) tissue regions in whole slide images (WSI) of breast cancer (BCa). Deep learning approaches are learn-from-data methods involving computational modeling of the learning process. This approach is similar to how the human brain works using different interpretation levels or layers of most representative and useful features resulting in a hierarchical learned representation. These methods have been shown to outpace traditional approaches of most challenging problems in several areas such as speech recognition and object detection. Invasive breast cancer detection is a time consuming and challenging task primarily because it involves a pathologist scanning large swathes of benign regions to ultimately identify the areas of malignancy. Precise delineation of WSI is crucial to the subsequent estimation of grading tumor aggressiveness and predicting patient outcome. DL approaches are particularly adept at handling these types of problems, especially if a large number of samples are available for training, which would also ensure the generalizability of the learned features and classifier. The DL framework in this paper extends a number of convolutional neural networks (CNN) for visual semantic analysis of tumor regions for diagnosis support. CNN is trained over a large number of image patches (tissue regions) from WSI to learn a hierarchical part-based representation. The method was evaluated over a WSI dataset from 162 patients diagnosed with IDC. 113 slides were selected for training and 49 slides were held out for independent testing. Ground truth for quantitative evaluation was provided via expert delineation of the region of cancer by an expert pathologist on the digitized slides. The experimental evaluation was designed to measure classifier accuracy in detecting IDC tissue regions in WSI. This method yielded

the best quantitative results for automatic detection of IDC regions in WSI in terms of F-measure and balanced accuracy (71.80%, 84.23%), in comparison with an approach using handcrafted image features (color, texture and edges, nuclear textural and architecture), and a machine learning classifier for invasive tumor classification using a Random Forest. The best performing handcrafted features were fuzzy color histogram (67.53%, 78.74%) and RGB histogram (66.64%, 77.24%)

- A boosted Bayesian multiresolution classifier for prostate cancer detection from digitized needle biopsies[5] . In this paper, the author presents a boosted Bayesian multiresolution (BBMR) system to identify regions of prostate cancer(CaP) receding step to a Gleason grading algorithm, where their objective is to score the invasiveness and severity of the disease. In the first step, the algorithm decomposes the whole-slide image into an image pyramid comprising multiple resolution levels. Regions identified as cancer via a Bayesian classifier at lower resolution levels are subsequently examined in greater detail at higher resolution levels, thereby allowing for rapid and efficient analysis of large images. At each resolution level, ten image features are chosen from a pool of over 900 first-order statistical, second-order co-occurrence, and Gabor filter features using an AdaBoost ensemble method. The BBMR scheme, operating on 100 images obtained from 58 patients, yielded: 1) areas under the receiver operating characteristic curve (AUC) of 0.84, 0.83, and 0.76, respectively, at the lowest, intermediate, and highest resolution levels and 2) an eightfold savings in terms of computational time compared to running the algorithm directly at full (highest) resolution. The BBMR model outperformed (in terms of AUC): 1) individual features (no ensemble) and 2) a random forest classifier ensemble obtained by bagging multiple decision tree classifiers. The apparent drop-off in AUC at higher image resolutions is due to lack of fine detail in the expert annotation of CaP and is not an artifact of the classifier. The implicit feature selection done via the AdaBoost component of the BBMR classifier reveals that different classes and types of image features become more relevant for discriminating between CaP and benign areas at different image resolutions.

- Automatic Nuclei Segmentation in H&E Stained Breast Cancer Histopathology Images[6] Here the author developed an automated nuclei segmentation method that works with hematoxylin and eosin (H&E) stained breast cancer histopathology images, which represent regions of whole digital slides. The procedure can be divided into four main steps: 1) pre-processing with color unmixing and morphological operators, 2) marker-controlled watershed segmentation at multiple scales and with different markers, 3) post-processing for rejection of false regions and 4) merging of the results from multiple scales. The procedure was developed on a set of 21 breast cancer cases (subset A) and tested on a separate validation set of 18 cases (subset B). The evaluation was done in terms of both detection accuracy (sensitivity and positive predictive value) and segmentation accuracy . The mean estimated sensitivity for subset A was 0.875 (±0.092) and for subset B 0.853 (±0.077). The mean estimated positive predictive value was 0.904 (±0.075) and 0.886 (±0.069) for subsets A and B, respectively. For both subsets, the distribution of the Dice coefficients had a high peak around 0.9, with the vast majority of segmentations having values larger than 0.8.

- Mitosis detection in breast cancer pathology images by combining handcrafted and convolutional neural networks [7]. In this paper the author uses a cascaded approach for mitosis detection that intelligently combines a CNN model and handcrafted features (morphology, color, and texture features). By employing a light CNN model, the proposed approach is far less demanding computationally, and the cascaded strategy of combining handcrafted features and CNN-derived features enables the possibility of maximizing the performance by leveraging the disconnected feature sets. Evaluation on the public ICPR12 mitosis dataset that has 226 mitoses annotated on 35 HPFs by several pathologists and 15 testing HPFs yielded a measure of 0.7345. In fig 2.1 network architecture is given.

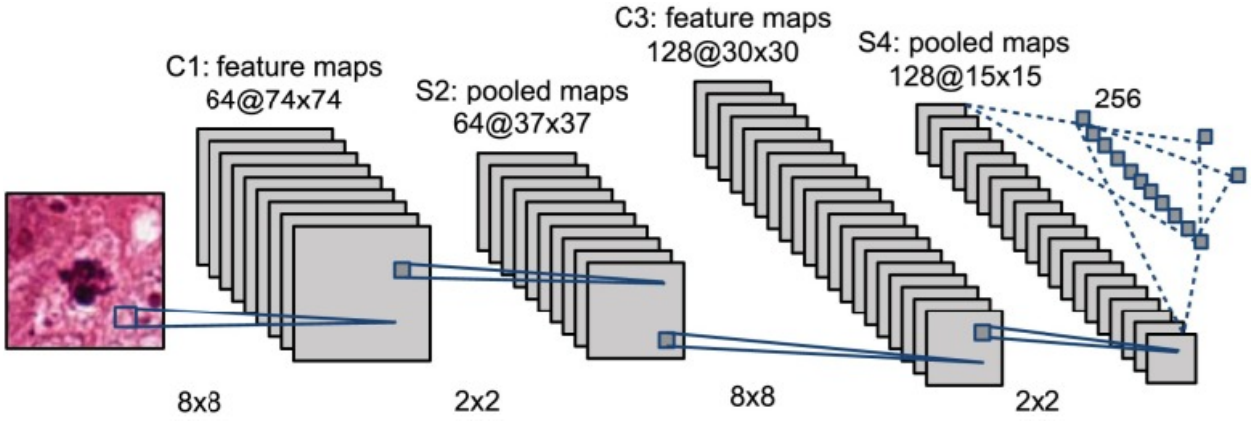


Fig. 2.1 Architecture of the CNN model. The CNN architecture comprises three layers: two consecutive convolutional-pooling layers and a fully connected classification layer.

- Stacked Sparse Autoencoder (SSAE) for Nuclei Detection on Breast Cancer Histopathology Images [8]. In this paper, a Stacked Sparse Autoencoder (SSAE), an instance of a deep learning strategy, is presented for efficient nuclei detection on high-resolution histopathological images of breast cancer. The SSAE learns high-level features from just pixel intensities alone in order to identify distinguishing features of nuclei. A sliding window operation is applied to each image in order to represent image patches via high-level features obtained via the auto-encoder, which are then subsequently fed to a classifier which categorizes each image patch as nuclear or non-nuclear. Across a cohort of 500 histopathological images (2200 × 2200) and approximately 3500 manually segmented individual nuclei serving as the groundtruth, SSAE was shown to have an improved F-measure 84.49% and an average area under Precision-Recall curve (AveP) 78.83%. The SSAE approach also out-performed nine other state of the art nuclear detection strategies. The network architecture of SSAE is given in fig. 5.

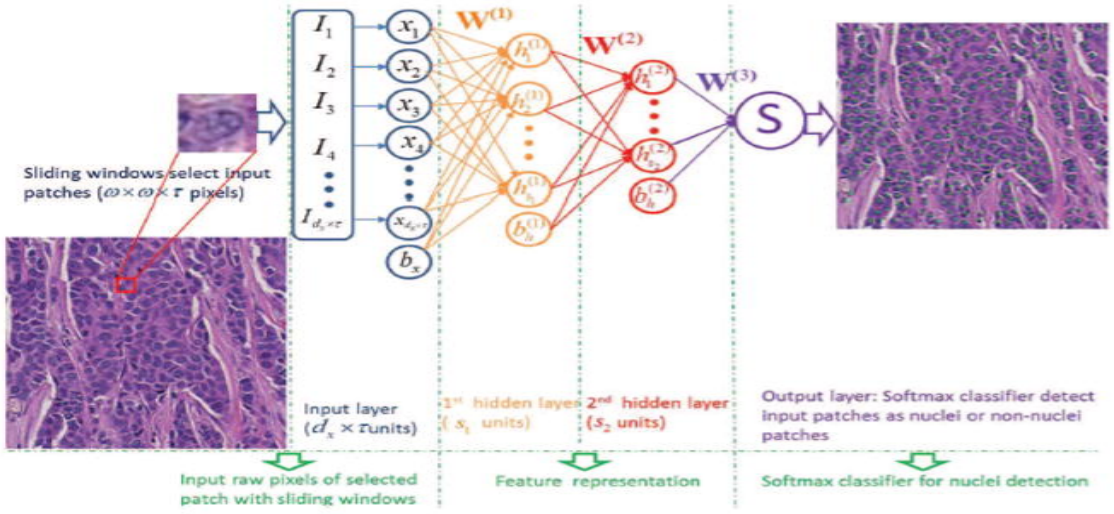


Fig. 2.2 Illustration of SSAE+SMC for nuclei detection on breast histopathology.

- The author use Resolution Adaptive Deep Hierarchical (RADHicaL) approach[9]. In this paper, the author present a resolution adaptive deep hierarchical (RADHicaL) learning scheme wherein DL networks at lower resolutions are leveraged to determine if higher levels of magnification, and thus computation, are necessary to provide precise results.here, author evaluate (RADHical) approach on a nuclear segmentation task with a cohort of 141 ER+ breast cancer images and show we can reduce computation time on average by about 85%. Expert annotations of 12,000 nuclei across these 141 images were employed for quantitative evaluation of RADHicaL. A head-to-head comparison with a naïve DL approach, operating solely at the highest magnification, yielded the following performance metrics: .9407 vs .9854 Detection Rate, .8218 vs .8489 F-score, .8061 vs .8364 true positive rate and .8822 vs 0.8932 positive predictive value. These performance indices compare favorably with state of the art nuclear segmentation approaches for digital pathology images. The network architecture of Resolution Adaptive Deep Hierarchical is given in figure 2.2.

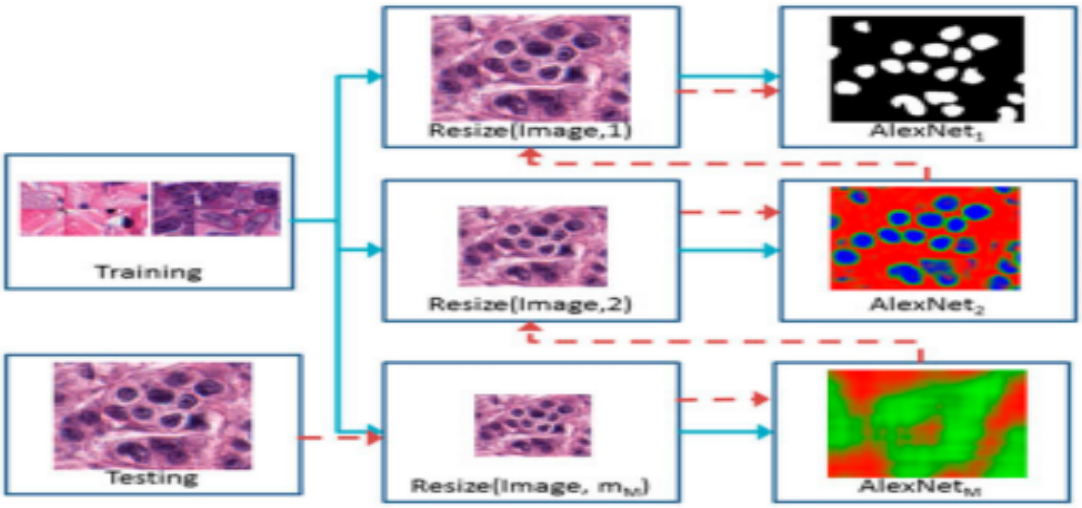


Fig 2.3. High-level flowchart illustrating RADHicaL. Training images (blue paths) are resized to train M different AlexNet networks at each of the different resolutions m.

- Most Deep Learning-based models applied for image segmentation tasks use some types of techniques related to encoder-decoder architecture. There are two categories of encoder-decoder for image segmentation problems, the general segmentation, and the medical, biomedical segmentation approaches [10]. The early work of Noh et al. [11] had proposed a network that learns the deconvolution network and contains the deconvolution and unpooling layers. The network achieved outstanding results on the PASCAL VOC 2012 dataset and produced the best accuracy on the Microsoft COCO dataset. Another paper focused on the encoder-decoder approach is the work of Badrinarayanan et al. [12] named Segnet. The novelty of the network is the upsampling layer in the decoder path, the pooling indices allow the network to reduce the need for learning to upsample

- In 2015, Olaf Ronneberger et al. [13] published the Unet paper in which they proposed a U-shape encoder-decoder architecture that contains two symmetric paths, the contracting path, and the expanding path. The network can train end-to-end using a small number of training images, and surpass the accuracy of other best methods on the

ISBI dataset with the high performance of segmentation results.Since the outstanding performance of Unet, there is a fast growth of the Unet related papers in medical and biomedical image segmentation tasks. Some of the Unet variant models such as 3D Unet, Attention Unet, Inception Unet, Residual U-Net, Dense Unet, Unet++, Adversarial Unet, etc had gained significant improvement on a specific type of Medical, Biomedical images . The work of B. Baheti et al. [14] tried to combine pre trained EfficientNet and Unet as encoder and decoder of an architecture which had ranked in first place for image segmentation in the India Driving Dataset (IDD) lite challenge.

- The problem facing when conducting experiments for breast tumor cell nuclei segmentation is the availability of labeled training datasets. To overcome the scarcity problem of training data, Lagree et al. [15] proposed the idea of multi-organ transfer learning, the neural net will be trained on the multi-organ H & E-stained cell datasets of liver, prostate, kidney, lung, colon, brain, bladder and stomach, then will be inferenced on the breast tumor cell dataset to evaluate the capability of transfer learning.

Chapter 3

A brief introduction of GAN

Generative adversarial network(GAN)[19] was developed and introduced by Ian J. goodfellow in 2014. It is a powerful class of neural networks used for unsupervised learning. GANs essentially consist of a system of two neural network models that compete with one another in order to assess, capture, and duplicate variations within a dataset.

3.1 Importance of using GAN

Most common neural networks have been found to be easily tricked into classifying objects incorrectly by getting just a tiny bit of noise into the initial data.Surprisingly, the model has higher confidence in the incorrect forecast after noise addition than it does in the accurate prediction. Due to the fact that most machine makes them vulnerable to overfitting and also the mapping between the input and the output is almost linear.Although it may appear that the boundaries separating the different classes are linear, in reality they are made up of linearities, and even a little change in a point in the feature space could result in incorrect categorization of the data.

3.2 Working principle of GAN

A generative adversarial network has two parts:

- **Generator** - It is an unsupervised learning approach. It will generate data that is fake data based on original(real) data. It is also a neural network that has hidden layers, activation, loss function. Its aim is to generate the fake image based on feedback and make the discriminator fool that it cannot predict a fake image. And when the discriminator is made a fool by the generator, the training stops and we can say that a generalized GAN model is created.The diagram below helps you understand how it works.

- **Discriminator** - It is a supervised approach means It is a simple classifier that predicts data is fake or real. It is trained on real data and provides feedback to a generator.

Both the Generator and the Discriminator are neural networks, and throughout the training phase, they compete with one another. The procedures are repeated multiple times, and each time, the Generator and Discriminator become better at what they are doing. The diagram below helps you understand how it works.

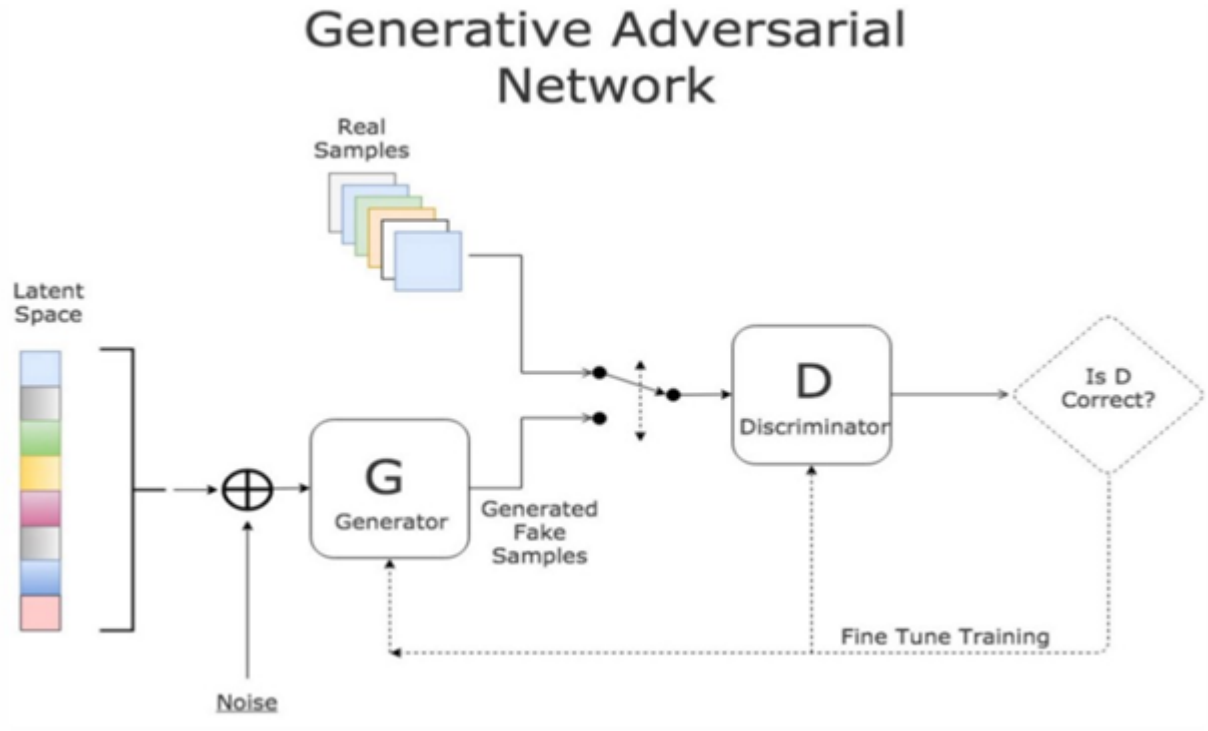


Fig 3.1. Generative adversarial networks(GAN)

Here, the generative model captures the distribution of data and is trained in such a manner that it tries to maximize the probability of the Discriminator in making a mistake.

The Discriminator, on the other hand, is based on a model that estimates the probability that the sample that it got is received from the training data and not from the Generator. The GANs are formulated as a minimax game, where the Discriminator is trying to minimize its reward V(D, G) and the Generator is trying to minimize the Discriminator's reward or in other words, maximize its loss. It can be mathematically described by the formula below:

$$\min_G \max_D V(D,G)$$

$$V(D,G) = \mathbb{E}_{x \sim p_{data}(x)}[\log D(x)] + \mathbb{E}_{z \sim p_z(z)}[\log(1 - D(G(z))]$$

Here,

G - Generator.

D - Discriminator.

P_{data} - distribution of real data.

P(z) - distribution of generator.

x - sample from $P_{data}(x)$.

z - sample from P(z).

D(x) - It is the discriminator's estimate of the probability that real data instance x is real.

G(z) - It is the generator's output when given noise z.

3.3 pix2pix generative adversarial network(GAN)

Pix2pix GANs were proposed by researchers at UC Berkeley in 2017. Pix2Pix is a conditional image-to-image translation task(i.e. converting one image to another, such as facades to buildings and Google Maps to Google Earth, etc.) architecture that uses a conditional generative adversarial network objective combined with a reconstruction loss. The conditional GAN objective for observed images $\boldsymbol{x}$, output images $\boldsymbol{y}$ and the random noise vector $\boldsymbol{z}$ is:

$$\mathcal{L}_{cGAN}(G,D) = \mathbb{E}_{x,y}[\log D(x,y)] + \mathbb{E}_{x,z}[log(1 - D(x,G(x,z))]$$

where G tries to minimize this objective against an adversarial D that tries to maximize it, and then it is augment with there construction term:

$$\mathcal{L}_{L1}(G) = \mathbb{E}_{x,y,z}[||y - G(x,z)||_1]$$

The final objective function used is,

$$G^* = \arg\min_G \max_D \mathcal{L}_{cGAN}(G,D) + \lambda \mathcal{L}_{L1}(G)$$

3.3.1 Generator

In generator UNet is used. This architecture is able to localize, i.e. it is able to find the object of interest pixel by pixel. Furthermore, UNet also allows the network to propagate context information from lower resolution to higher resolution layers. This allows the network to generate high-resolution samples.

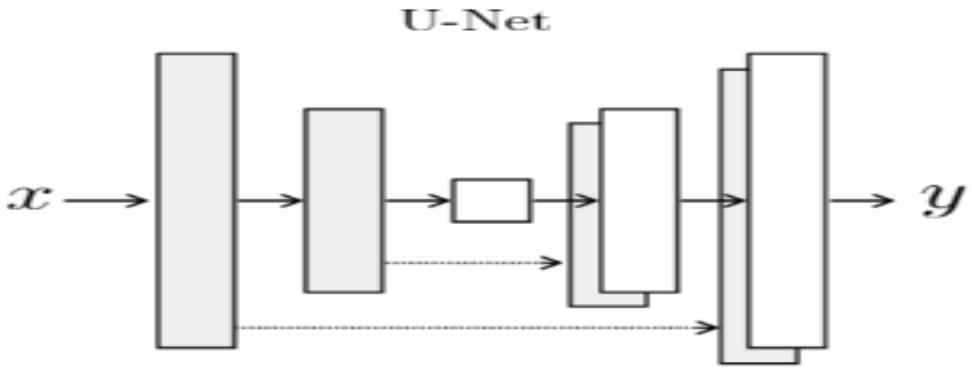


Fig 3.2 Generator architecture (U- Net)

3.3.2 Discriminator

The discriminator employs the Patch GAN architecture. Several transpose convolutional blocks are present in this design. It examines a NxN portion of the image to determine whether it is authentic or false here N might be any size. It can be scaled down from the original image and yet generate results that are of a good quality. Convolutionally, the discriminator is applied to the entire image. Additionally, because the discriminator is smaller, that is it has fewer parameters than the generator so, it is really faster.

Chapter 4

The proposed work

This chapter describes the methods used for the nuclei segmentation from the histology images.

Nucleus segmentation of histology images is needed for various applications, such as cancer cell type classification , cancer grading and cancer subtype classification . Major challenges posed by this problem are colour variation in histology images , the overlapping and clumped nuclei and the ambiguous boundary between different cell nuclei . Further to that ,it is much laborious and time consuming to identify each nucleus by a human operator. Our main aim is to design a generative adversarial network(GAN) to segment nuclei in histology images. It requires training the network with histology images in which the nuclear segments are properly labeled. The original labeling of histology images is usually performed with a binary mask. In such masked images ,pixels of nuclear segments are represented with gray scale value 1 and all other pixels are represented with a gray value 0.

To prepare the training data for the pix2pix network , three separate masks are generated for R,G and B channels of the data separately.The pixel value of nucleus segments in each such images mask are keep unchanged compared to the original image , where as the pixel values of all other pixel positions is made Zero. Thus three separate image masks are generated to label nucleus segments for three channels of data (i.e Red ,Green and Blue).

During training of pix2pix GAN the original histology images of shape (2048,2048,3) are given as input to the generator and the output generated from the generator of shape(2048,2048,3) and coloured masked images of same shape as original images are given as input to the discriminator .This model architecture is given in fig 4.1. The optimizer used in pix2pix GAN is Adam optimizer and the model is trained for 50 epochs at 21 epochs we got the optimum accuracy . After completion of training two steps of post processing is done on the generated RGB images (1). The generated RGB images are converted to gray scale images by using Gaussian filter. (2). Image histogram of the gray images is created, to visually adjust the brightness value of each pixel to make nuclei more prominent than the background .

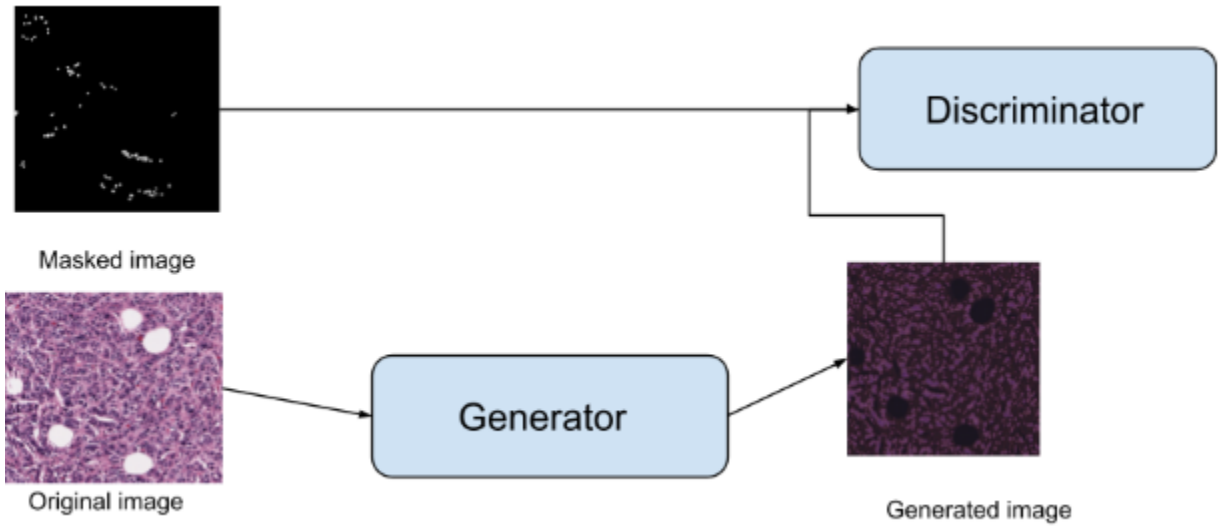


Fig 4.1. proposed model Architecture

Chapter 5

Experimental Results & Discussion

5.1 Dataset used

In this study the dataset used is publicly available digital histology images of breast cancer given by Andrew Janowczyk in 2014[].The dataset consists of 141 images of estrogen receptor positive (ER+) breast cancer (BCa) images scanned at 40x. Each image is 2,000 x 2,000. Across these images there are about 12,000 nuclei manually segmented. Dataset is randomly split into 80% for training (121 images), and remaining 20% for testing (20 images) .

5.2. Performance Metrics

In this section, the performance metrics highlighted are used in the current work. Before defining metrics, true positives, true negatives, false positives, and false negatives are defined.

True Positives (TP) are the cases when the actual class of the data point was 1 (True) and the predicted is also 1 (True).
True Negatives (TN) are the cases when the actual class of the data point was 0 (False) and the predicted is also 0 (False).
False Positives (FP) are the cases when the actual class of the data point was 0 (False) and the predicted is 1 (True).
False Negatives (FN) are the cases when the actual class of the data point was 1 (True) and the predicted is 0 (False).

Accuracy is the most common performance metric used for classification algorithms. It may be defined as the number of correct predictions made as a ratio of all predictions made. It can be represented mathematically by equation (5.1).

$$Accuracy = \frac{TP+TN}{TP+FP+FN+TN} \qquad (5.1)$$

Precision is the ratio of the number of correctly predicted items belonging to a class to the total number of items predicted belonging to the same class. It can be represented mathematically by equation (5.2).

$$Precision = \frac{TP}{TP+FP} \tag{5.2}$$

Recall is the ratio of the number of correctly predicted items belonging to a class to the total number of items belonging to the same class. It can be represented mathematically by equation (5.3).

$$Recall = \frac{TP}{TP+FN} \tag{5.3}$$

F1-Score is the harmonic mean of precision and recall. It can be represented mathematically by equation (5.4).

$$F1\ Score = 2\ \frac{Precision \times Recall}{Precision + Recall} \tag{5.4}$$

For semantic segmented images of nuclei we prepared our metric based on per nucleus basis.Algorithm is written below .

ALGORITHM

1. Move an image patch of size 8x8 simultaneously over the generated masked image and the ground truth image.
2. If the number of positive class in the ground truth image patch is at least 1 then do the following on the masked image patch.

   2.1 Find $\sum_{patch\ i}$ TP.

   2.2 Find $\sum_{patch\ i}$ TN.

   2.2 Find $\sum_{patch\ i}$ FP.

   2.4 Find $\sum_{patch\ i}$ FN.

3. Find Accuracy,F- score,precision and recall.

4. Repeat step 1 -2 for all image patches.
5. stop

5.3 Result and discussion

In this study,training of pix2pix GAN the original histology images of shape (2048,2048,3) are given as input to the generator and the output generated from the generator of shape(2048,2048,3) and coloured masked images of same shape as original images are given as input to the discriminator .The optimizer used in pix2pix GAN is Adam optimizer and the model is trained for 50 epochs at 21 epochs we got the optimum accuracy .After post - processing the generated RGB image to binary image and applying a threshold to the binary image to make the nuclei more prominent compared with the background . Output imaage and post process images are given in figure 5.1.

Certain problems remain even after a nucleus segmentation of breast histology images using a pix2pix GAN. Due to over segmentation in some cases, the lone nuclei in the image may get coalesced into a single nucleus. This has a high chance of affecting the diagnosis. The total number of nuclei in a histology image is an important parameter for successful diagnosis of the image . To deal with this problem a morphological erosion operator has been applied here to further segment such coalesced nuclei as far as possible . The method is found to be effective to work on large size clusters of nuclei with a high overlapped area. To solve this problem, a deep neural network may be trained fresh for nucleus edge detection in future.

	Accuracy	**F1 - Score**	**Precision**	**Recall**
Naive DL	98.54	84.89	83.64	89.32
Resolution adaptive DL[9]	94.07	82.18	83.64	88.22
Pix2pix GAN	97.89	93.07	92.42	94.14

Table 5.1 comparison metric table of our approach (pix2pix GAN) with respect to resolution adaptive DL and Naive DL

The present approach to nucleus segmentation of breast histology images is tested on a benchmark dataset Estrogen Receptor positive(ER+) breast cancer images scanned at40x. The results obtained from these experiments are shown in Table 5.1. Since the entire ground truths for each image are not available. The performance metrics in the table are computed for those nuclei for which ground truths are available .so, all the performance index are computed here in terms of pixels covered by the ground truth.

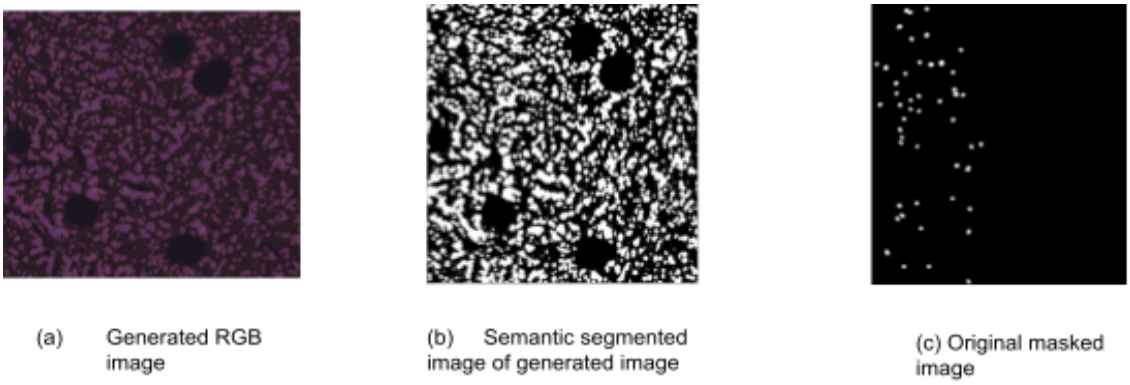


Fig 5.1 generated image(a) is converted to gray scale image (b) and the original masked image ©.

As evident from Table 5.1, the present method based on pix2pix GAN , has performed much better than the contemporary approaches in terms of F1-score,precision and recall. In terms of Accuracy, it slightly lags behind the Naive DL. For nuclear segmentation F1-score,precision and recall which measure the power of predicting positive samples are more important compared to accuracy.In the respect, our present method has performed fairly well. But nuclear segmentation is a part of the entire problem here separating ,overlapping and clumped nuclei is also a critical problem as the number of nuclei is an important parameter for cancer grading, subtype classification etc. To address this problem , a morphological operator erosion is applied on nucleus segmented images .Figure 5.2 shows the performance of this method. The method although successfully separates touching nuclei, it fails to work on overlapped nuclei.
To explore a better method, another pix2pix GAN may be trained to generate nucleus boundaries. In doing this the manual annotation of training images as described in the work of Yuxin cui[].

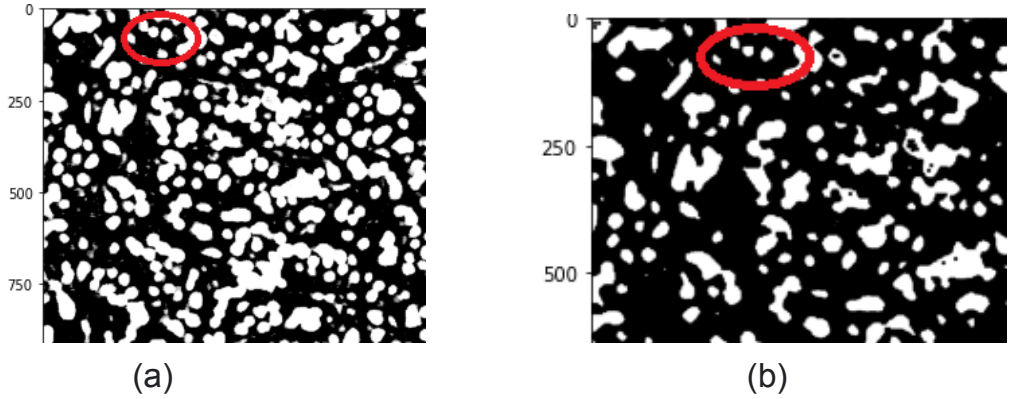


Fig. 5.2 (a) generated masked image (b) after applying an erosion morphological operator .

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