Deep Learning in Digital Breast Pathology

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Keywords: Breast Cancer, Machine Learning, Deep Learning, Digital Pathology, Convolutional Neural Networks, Whole Slide Imaging.

Abstract: The development of scanners capable of whole slide imaging has transformed digital pathology. There have been many benefits to being able to digitize a stained-glass slide from a tissue sample, but perhaps the most impactful one has been the introduction of machine learning in digital pathology. This has the potential to revolutionize the field through increased diagnostic accuracy as well as reduced workload on pathologists. In the last few years, a wide range of machine learning techniques have been applied to various tasks in digital pathology, with deep learning and convolutional neural networks being arguably the most popular choice. Breast cancer, as one of the most common cancers among women worldwide, has been a topic of wide interest since hematoxylin and eosin-stained (H&E)-stained slides can be used for breast cancer diagnosis. This paper summarizes key advancements in digital breast pathology with a focus on whole slide image analysis and provides insight into popular methods to overcome key challenges in the industry.

1 INTRODUCTION

Advancements in whole slide imaging (WSI) have paved the way for digital pathology. This has driven the increasing demand for more research into using machine learning for whole slide image analysis. This paper provides an overview of the main aspects of deep learning in digital breast pathology. Background information is included that can be used to gain an understanding of the field. Key advancements, tools, and insight into popular methods for overcoming key challenges are discussed. While digital pathology is a large field, the focus here will be on analysis of whole slide images through deep learning techniques.

1.1 Whole Slide Imaging

Whole slide imaging shows many potential benefits compared to its glass slide counterpart. Digitized slides allow remote users to view slides for secondary or even primary diagnosis. Digitization is also useful for archiving and preserving samples, which is important since physical samples degrade over time. An additional benefit of better archiving of tissue samples is the preservation of rare specimens. Since digitized slides can be accessed remotely, WSI also provides the opportunity to make advancements in standardizing training for pathologists. It is important to consider that while WSI can also be used for diagnosis, there are still factors that can affect diagnostic accuracy from digitized images. While whole slide images are approved for diagnosis, some discrepancies still make glass slide viewing the standard for diagnosis. These issues stem from poor image quality and bad focus. Some specific microscopic details such as mitotic figures that may be needed for analysis can also be difficult to identify on the digitized images, in some cases, due to faint scanning. However, it is important to note that even glass to glass slide studies can show discrepancies due to observer variability, among other factors (Pantanowitz et al., 2015).

1.2 Digital Pathology Tasks

In histopathology image analysis, three main tasks for machine learning have emerged: classification, segmentation, and object detection. Classification involves analyzing an image and giving the image a label, sorting it into a class. There are two types of classification, binary and multi-class classification (Gupta et al., 2022b). In binary classification, there are only

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Rose, M., Geradts, J. and Herndon, N.
Deep Learning in Digital Breast Pathology.
DOI: 10.5220/0012576100003657

In *Proceedings of the 17th International Joint Conference on Biomedical Engineering Systems and Technologies (BIOSTEC 2024) - Volume 1*, pages 404-414
ISBN: 978-989-758-688-0; ISSN: 2184-4305

two possible labels or classes for an image. In contrast, multi-class labeling has three or more possible labels for a given image. A study by Araujo et al. (2017) displayed both binary and multi-class classification. They used a convolutional neural network for multi-class classification of breast biopsy images into one of four categories: normal, benign, in situ carcinoma, and invasive carcinoma. They also performed binary classification into carcinoma and non-carcinoma. More specific labelling as done in multi-class classification is often very useful in medical diagnosis. However, due to using an increased number of classes, multi-class models often require more complexity than binary models, which can impact their accuracy. For example, in the study mentioned above, the four-class model scored 65% accuracy on test data compared to the binary class model achieving 77%.

Segmentation aims to separate parts of the images – often cancerous cells vs. noncancerous cells. Object detection focuses on finding landmarks in an image, like individual cells or nuclei. In this paper, segmentation and object detection will briefly be discussed, while classification tasks will be the main focus.

2 BACKGROUND

2.1 Breast Cancer

In 2023, breast cancer accounted for 31% of all female cancers, making it one of the most common cancers among women. Breast cancer occurrence rates have been steadily increasing since the 2000s by about 0.5% per year. Improvements in treatment have seen the mortality rate for breast cancer decrease despite the increase in incidence (Siegel et al., 2023). It is well documented that early diagnosis and intervention can greatly improve survival rates in breast cancer patients. Smaller tumors have notably better long-term survival rates than larger tumors (Bhushan et al., 2021). Many techniques are used to screen for and diagnose breast cancer, including mammography and ultrasonography (Watkins, 2019). However, while these are helpful in screening and early detection of breast cancer, a breast biopsy is the only definitive method for diagnosing breast cancer (Nounou et al., 2015). Tissue samples can provide information about tumor type, grade, and biomarker status. A triple assessment is often used to evaluate patients, consisting of clinical evaluation and imaging in addition to a tissue biopsy (Alkabban and Ferguson, 2020).

Once biopsied tissue is collected, it is fixed, processed, sectioned, and stained to color different parts of the cells in the tissue. Hematoxylin and eosin (H&E) staining is considered the gold standard in breast tissue biopsies and has been around for over 100 years (Huang et al., 2023). When H&E staining is performed, different cell parts will look distinct based on which type of dye they have an affinity for. Hematoxylin is a basic dye whereas eosin is an acidic dye. Cell structures such as nuclei that have an affinity for hematoxylin appear blue after staining. Structures such as cytoplasm that have an affinity for eosin appear pink after staining. Structures with an affinity for both basic and acidic dyes will appear purple after staining (Chan, 2014; Bancroft and Layton, 2019).

2.2 Whole Slide Image Resolutions

Whole slide image scanners operate by capturing images of tissue sections tile by tile. The whole image is reconstructed at the end. These scans can be performed at multiple magnifications which increases image detail. 20x magnification is a common scan objective and is adequate for typical viewing. However, some types of slides require more detail and need higher levels of magnification such as 40x (Zarella et al., 2019).

2.3 Machine Learning Types

There are many learning types in machine learning. The two main types are supervised and unsupervised learning. These types differ based on the types of data they receive.

Supervised learning supplies a machine learning model with input data and its expected output. What this will look like can vary depending on the task being performed. In cases of classification, the output is typically a label. In segmentation tasks, often a mask of pixels is used as the ground truth label (Khened et al., 2021). In object detection, bounding boxes in certain parts of the image are typically provided (Li et al., 2019). The model will then try to predict the desired output given only the input. The revolutionary idea behind deep learning models is that they can compare their original predictions with the expected output and internally modify their configurations to see more accurate predictions during the next round of training or testing. This is done through a method called backpropagation (LeCun et al., 2015). Convolutional neural networks are a popular type of supervised machine learning model.

In contrast to supervised learning, unsupervised learning uses unlabeled data. Unsupervised learning models will group data based on patterns and similarities but are unable to provide a label (Gupta et al.,

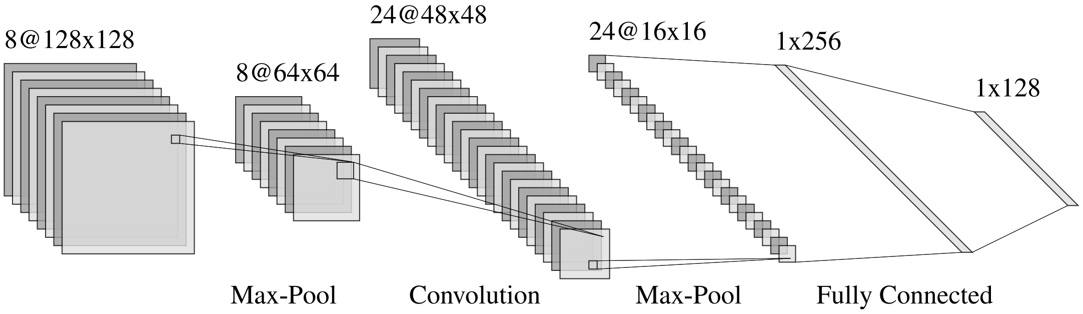


Figure 1: An example of a convolutional neural network architecture with convolutional layers, pooling layers, and fully connected layers. As the input moves through the CNN, it continues to be reduced in size. Figure generated with NN-SVG (LeNail, 2019).

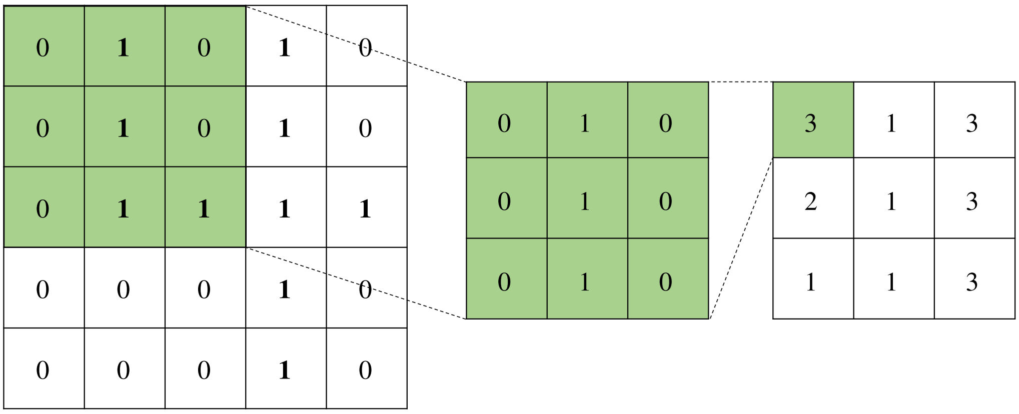
2022b). This can be useful in histopathological image analysis for detecting patterns in images that may not be currently recognized by pathologists. Additionally, unsupervised learning can be used for feature extraction on histopathologic images (Sari and Gunduz-Demir, 2019).

2.4 Deep Learning and CNNs

Deep learning is a subfield of machine learning that focuses on using nodes to form a neural network (Gupta et al., 2022a). These neural networks were originally inspired by the human brain. The nodes in neural networks are also referred to as neurons since they mimic how neurons function in a human brain (O'Shea and Nash, 2015). Deep learning has become increasingly popular for several reasons. First, these models are successful at a wide variety of tasks such as natural language processing, speech and audio processing, and digital image processing. The way deep learning algorithms extract features decreases the amount of domain knowledge and work needed by researchers (Pouyanfar et al., 2018). Convolutional neural networks (CNNs) are a type of neural network and are particularly good at image recognition tasks. CNNs take in image pixel values as input and pass these values through a series of layers while performing various operations on the images. Convolutional neural network architecture consists of three main types of layers: convolutional, pooling, and fully connected layers, which can be observed in Figure 1. In convolutional layers, two dimensional filters are applied to the image data to extract features such as edges, objects, and colors. An example of a convolution can be seen in Figure 2. These features are used to create a feature map. Convolutional layers are often paired with activation functions, such as ReLU (rectified linear units), which improve speed and performance by removing negative values after a convolution has been performed (Krizhevsky et al., 2017; Zhang et al., 2021). The pooling layer does downsampling which reduces the number of parameters used while trying to maintain the features (O'Shea and Nash, 2015; Zhang et al., 2021). Lastly, fully connected layers take inputs received by previous layers and connect them to activation units to produce output (Zhang et al., 2021).

2.5 CNN History

In 1989, Yann Lecun introduced LeNet-5, a convolutional neural network originally designed to recognize handwriting digits (LeCun et al., 1998). It became one of the first widely recognized published convolutional neural networks due to its performance with an error rate of 0.95% on the test set of 16x16 pixel handwriting digit images (Zhang et al., 2021; LeCun et al., 1998). LeNet-5 was also revolutionary for its use of backpropagation to reconfigure its own internal weights using gradient descent (LeCun et al., 1998). In 2012, convolutional neural networks surged in popularity after AlexNet won the ImageNet Large Scale Visual Recognition Challenge (ILSVRC) (Krizhevsky et al., 2017). Since then, the development of new CNN architectures rapidly expanded with the emergence of VGG16/VGG19 (Simonyan and Zisserman, 2015), Resnet (He et al., 2016) and Inception (Szegedy et al., 2015). ImageNet is a popular dataset for training and benchmarking convolutional neural networks and contains millions of annotated natural images (Deng et al., 2009). Classification and object detection are common computer vision tasks and are included in popular challenges such as ILSVRC (Russakovsky et al., 2015). Convolutional neural networks are particularly skilled at computer vision tasks and in turn have been applied to a wide range of medical imaging tasks.



0	**1**	0	**1**	0
0	**1**	0	**1**	0
0	**1**	**1**	**1**	**1**
0	0	0	**1**	0
0	0	0	**1**	0

0	1	0
0	1	0
0	1	0

3	1	3
2	1	3
1	1	3

Figure 2: An example of the convolution operation. This convolution uses a 3x3 filter (center grid) on an image of size 5x5 (left grid) with a stride of 1 and padding of 0. The result of the convolution is the rightmost grid. The filter used here is an example of a filter that detects vertical lines.

2.6 Modern CNNs

One of the most popular modern CNNs is VGG16. The Visual Geometry Group (VGG) submitted VGG16 to the ILSVRC in 2014. It proposed an increase in CNN depth and was pretrained on the ImageNet dataset. This model varied from its predecessors by using stacks of smaller 3x3 receptive fields instead of larger 11x11 or 7x7 receptive fields. This decreased the number of parameters throughout the network. Small convolutions had previously been tried but no other CNNs that used these smaller filters were as deep as VGG16, which boasted sixteen layers as its name suggests. The VGG were able to determine that a larger depth increased the classification accuracy. VGG19 was described in the same paper as VGG16 and follows a similar architecture but with nineteen layers as opposed to sixteen (Simonyan and Zisserman, 2015).

Another popular modern CNN is Inception (also known as GoogleLeNet). This neural network competed in the ILSVRC 2014 challenge and acheived high performance. Inception implements wider layers as opposed to making the entire network deeper with more layers (Szegedy et al., 2015). This network depth is why Inception is often referred to as a deep convolutional neural network, while other CNNs like VGG16 are considered shallow. This greatly reduces the computational cost of the network in comparison to other modern CNNs such as VGG16 (Szegedy et al., 2016). Like VGG16, Inception uses many smaller filters in place of larger filters to reduce the number of parameters needed. Inception was also trained on the ImageNet dataset and achieved remarkable error rates while cutting computational costs (Szegedy et al., 2015).

Additional modern CNNs that will be discussed in later sections include ResNet and MobileNet. ResNet implements residual functions to reference layer input, which was shown to make optimization easier. Additionally, this allowed for increased network depth with lower complexity and increased accuracy (He et al., 2016). MobileNet was built to be a lightweight deep neural network by utilizing depthwise separable convolutions (Howard et al., 2017).

2.7 Whole Slide Image Annotations

In whole slide imaging, there are three main annotation types. These types are patch level (sometimes called pixel level), slide level, and patient level. These three levels are illustrated in Figure 3. Each type of annotation can be useful for different tasks. These annotations can also be organized into a hierarchy of specificity.

Patch level annotation is the most specific level of annotation for whole slide images. Patch level annotations guarantee that when taking patches from WSI, every patch is fully annotated. Examples of patch level annotations include instances where each patch has its own classification label, segmentation mask, or bounding boxes (Ciga and Martel, 2021). One example of a segmentation mask would be when a pathologist identifies cancerous regions within a tissue sample and annotates all regions or pixels containing cancerous cells (Khened et al., 2021). Patch level annotations are extremely helpful for segmentation tasks as they provide the ability for high supervision. However, these annotations are much more time consuming than slide level annotations and therefore are less commonly available. Often, training will be performed at a lower annotation level such as patch level while expecting a final output at a higher level such as slide level or patient level (Dimitriou et al., 2019). Aggregation from lower to higher annotation levels is discussed in Section 4.4.

The next level of annotation is at slide level. Slide level annotations provide one label per whole slide. For example, a whole slide image with a slide level annotation may be labeled carcinoma vs non-carcinoma (Araujo et al., 2017). Slide level annotations are much less time consuming to do than pixel level annotations and are therefore more abundant. If looking at individual patches, as is common in WSI analysis, it is possible for a patch to not match its slide level annotation (Dimitriou et al., 2019; Hou et al., 2016). This is why aggregation is needed when moving from one level during training to another at prediction time.

The least specific level of annotations is patient level. Patient level annotation is similar to slide level annotations in that a single label/class is provided, however, this label is provided to a patient rather than a specific slide. Patients may have multiple images. For example, in the CAMELYON17 dataset, each patient had 5 images (Litjens et al., 2018). Patient level annotations mean that individual slides will not be provided a label, but rather the patient, which means the label applies to all slides associated with the patient. This is the least specific level of annotation because it is possible to incorrectly label a whole slide (Dimitriou et al., 2019).

3 COMMON CHALLENGES

There are many unique challenges in pathology image analysis when trying to apply deep learning. Solutions to these challenges are the basis of many works in the field.

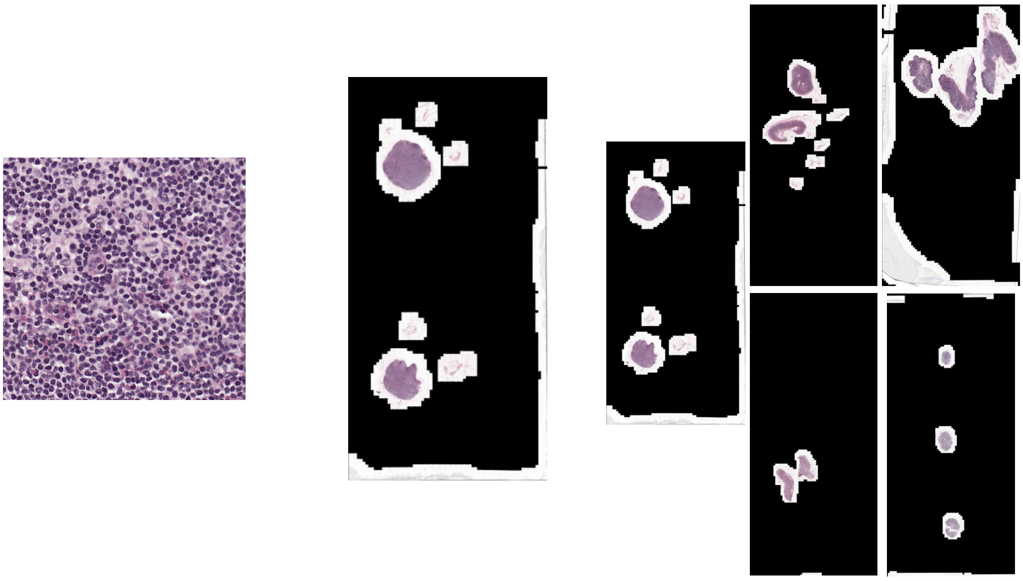
3.1 Image Size

One major issue that must be addressed when trying to apply any type of machine learning technique to histopathology image analysis is the size of whole slide images. Whole slide image scans are extremely large, typically 100,000 x 100,000 pixels each (Dimitriou et al., 2019). With images this large, they are not feasible for machine learning use without modifications. For example, CNNs usually perform best with smaller images around 224 x 224 pixels in size (Ciga et al., 2021). Image compression would certainly be helpful but also has drawbacks including reduced image quality and distortion of important markers. It has been shown that there is a significant performance decrease in benign vs. malignant breast tissue classification once compression levels increase past 32:1 (Krupinski et al., 2012). Even if extreme downsampling were performed, the image would remain too large for use in a convolutional neural network (Ciga et al., 2021). A common approach to address this issue is to split the image into smaller images that would be more suitable for use by machine learning models (Hou et al., 2016). These methods are discussed in Section 4.3.

3.2 Data Availability

A lack of well-annotated and publicly available training data is a well-known problem in digital histopathology image analysis. Even when images are available, domain knowledge is required to annotate these images to make them suitable for analysis via machine learning methods. Researchers have a few options: use a publicly available dataset, or create their own, using images provided to them by an institution or pathologist. One of the most popular datasets used in breast histopathology image analysis is the CAMELYON dataset, which is a publicly available dataset of whole slide images along with their associated pathologist annotations. This dataset was collected from Dutch hospitals and contains 1,399 unique whole slide images totaling 2.95 terabytes. Slides were scanned with three different scanners based on which hospital they came from, with the majority of hospitals using the 3DHistech Pannoramic Flash II 250 while the Hamamatsu NanoZoomer-XR C12000-01 scanner and Philips Ultrafast Scanner were both used by one hospital each. All 1,399 WSI were annotated with a slide level label. Additionally, 399 slides from CAMELYON16 and 50 slides from CAMELYON17 were also annotated at the patch level (Litjens et al., 2018).

As of the 2018 publication about the dataset, it had already been accessed by over 1000 users. Along with the dataset came the CAMELYON16 and CAMELYON17 challenges, which encouraged teams to design models to classify breast cancer metastases (Litjens et al., 2018). Although the main goal of the CAMELYON challenges is breast cancer metastases detection, the dataset is widely used by researchers interested in a variety of breast histopathology image tasks. As of December 2023, the CAMELYON17 challenge website boasts 205 submissions to the leaderboard with 1,943 total participants. The current top 10 submissions on the leaderboard all boast Cohen-Kappa scores of greater than .90 when evaluated by the CAMELYON team.



a) Image Patch b) Slide Image c) Collection of scans for a patient

Figure 3: Three main annotation types for whole slide images. (a) In patch level annotation, each image patch would have its own classification label or would have a pixel annotation boundary. (b) In slide level annotation, there would be a single classification label for the entire image. (c) In patient level annotation, there would be a single label associated with all five images. Whole slide images come from the CAMELYON17 dataset (Litjens et al., 2018).

4 COMMON APPROACHES

4.1 Transfer Learning/Pretrained Models

One downside to deep learning is the computational complexity and the amount of well annotated data needed. One technique that helps reduce model complexity as well as the amount of domain specific annotated data needed is transfer learning (Wakili et al., 2022). Transfer learning is another brain inspired technique. It comes from the idea that knowledge in one task can aid in performing a different, but somewhat related task. The use of transfer learning in convolutional neural networks is often used to pretrain a CNN with large amounts of publicly available and well annotated data, such as the ImageNet dataset. Later, the CNN can be finetuned with domain specific data (Kim et al., 2022). Ultimately, this reduces the amount of domain specific data needed since the original weights will already be pretrained. Training time is also reduced when using pretraining methods since some portion of the training is already complete (Gupta et al., 2022a). This is particularly useful in fields such as digital pathology where there may be a lack of widely available annotated data.

In a study comparing transfer learning methods in medical imaging, Kim et al. (2022) defined four types of transfer learning based on how the training is handled after the original pretraining. The feature extractor method freezes the convolutional layers and only retrains model weights in the fully connected layers. The feature extractor hybrid also freezes the convolutional layers but replaces the fully connected layers with another machine learning model, such as a support vector machine (SVM). The fine-tuning method unfreezes a few of the convolutional layers to be retrained. Finally, fine tuning from scratch completely retrains the model on the new data. After analysis of 121 publications focused on using transfer learning on convolutional neural networks with medical images, they recommended the feature extractor approach and then incrementally fine tuning the layers. Fine tuning from scratch appeared to be a prevalent method but did not show significant improvements in model accuracy despite being much more computationally expensive than other transfer learning methods (Kim et al., 2022).

4.2 Common Models

There are many pretrained convolutional neural networks available for use. Some models such as Inception have become commonly used because of their good performance. One review of medical imaging using CNNs found that most works use multiple models. However, Inception was the leading model when only one model was used (Kim et al., 2022).

While tumor detection is a common task in breast digital pathology, it is not the only task that interests researchers. One study attempted to predict early recurrence from histopathological images. Early recurrence was defined as the return of a primary tumor within three years of the original diagnosis. VGG16 pretrained on ImageNet was used in conjunction with support vector machines (SVM). This approach observed a 70.3% accuracy (67.7% sensitivity) using within-patient validation (Shi et al., 2023). Another study focused on predicting breast cancer recurrence from whole slide images used six pretrained models were used including VGG16, ResNet50, ResNet101, Inception_ResNet, EfficientB5 and Xception. Two fully connected layers were added to help reduce the computational load. Here, Xception was found to have the highest accuracy on the training data (91%) and was used for further testing where it achieved an accuracy of 87% (Phan et al., 2021).

4.3 Image Patches

Due to the enormous size of whole slide images, one common solution is to use patches of a whole slide image rather than the entire image itself. However, this adds another variable, what is the optimal patch size? There isn't a clear-cut answer and researchers select different patch sizes based on their specific needs. However, some patch sizes are more often used and are selected as default values. When selecting patch size, it is important to consider several factors. Finding the optimal patch size is important because it plays a role in how long training takes and can also impact model accuracy. Patch size often depends on the overall goal of a work. For example, works looking to perform slide level classification more often use larger patch sizes such as 512 x 512 and 1024 x 1024 pixels (Pinchaud, 2019; Khened et al., 2021; Lee et al., 2021). This allows for more information to be captured by each patch used in training and gives a better overall view of the tissue elements and cell architecture. However, other studies such as those focused on object detection and labeling individual cells and nuclei, may decide to use smaller patch sizes. Additionally, researchers need to decide whether they will use overlapping or non-overlapping patches. An example of the differences between overlapping and non-overlapping patches can be found in Figure 4.

In the CAMELYON challenge there are a wide range of approaches taken and this extends to selected patch size. The top submission on the leaderboard for CAMELYON17 uses a patch size of 704 x 704 pixels (Lee et al., 2021). However, the other submission in the top 5 all use either 512 x 512 pixel patches, 1024 x 1024 pixel patches or some combination of the two (Pinchaud, 2019; Khened et al., 2021; Lee et al., 2021).

A study focusing on segmenting whole slide images from the CAMELYON dataset tried two ensembles with different patch sizes, 256 x 256 non-overlapping patches and 1024 x 1024 overlapping patches. The ensemble that used 1024 x 1024 overlapping patches performed slightly better than the ensemble with 256 x 256 non-overlapping patches. This study is currently a top 5 score on the CAMELYON17 leaderboard (Khened et al., 2021).

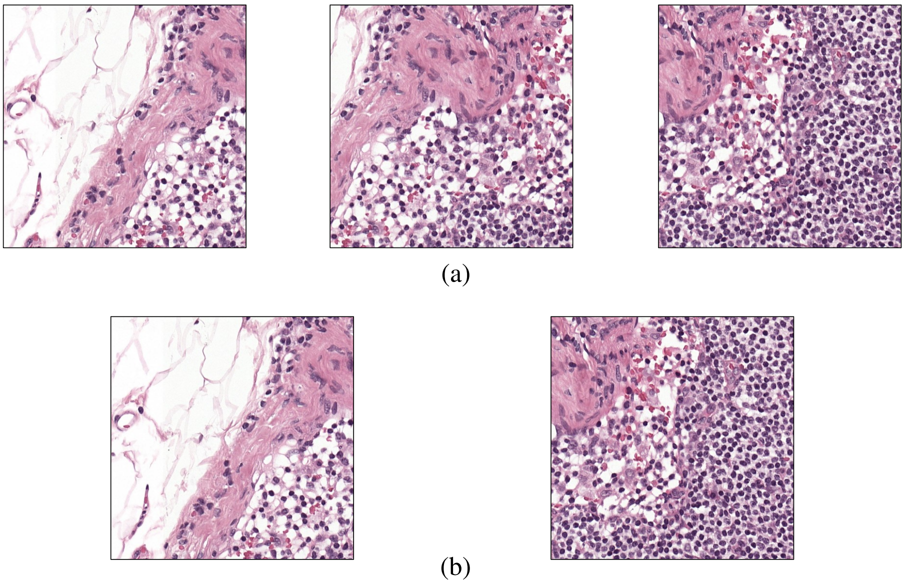
One work focused-on object detection of signet ring cells. Images from 10 different organs were used, with breast among them. Out of 127 images, each had 3 patches of size 2000 x 2000 selected for annotation with a bounding box. A total of 12,381 signet ring cells were annotated. However, due to overcrowding, some signet ring cells were not able to be annotated. This work is also a top 5 scorer on the CAMELYON17 leaderboard (Li et al., 2019).

Another work used randomly selected 1000 x 1000 pixel sized patches for the task of tumor region recognition. The patches had to be downsampled four times to 224 x 224 to satisfy the requirements of their selected model, MobileNetV2 (Huang et al., 2023).

There are many instances where a larger patch size, such as 512 x 512, is initially chosen and then cropped or resized to a smaller size like 128 x 128 to make the image better match the selected model's input size (Phan et al., 2021). One work applied this, originally selecting patch sizes of 512 x 512 before randomly cropping to 448 x 448. The 448 x 448 pixel patches then went through dimension reduction to achieve a size of 224 x 224 for training with the EfficientNet framework. This study found an improvement in results in both slide level classification and segmentation tasks with randomly cropped patches (Ciga et al., 2021).

4.4 Annotation Aggregation

Training is often performed at a lower annotation level such as patch level while expecting a final output at a higher level such as slide level or patient level. In these cases, aggregation is needed to combine the results from many patches to achieve the output for the higher level (Dimitriou et al., 2019). One study converted from patch level to slide level predictions for tumor and tumor bed detections. In this case, if one or more patches were determined to be positive or a tumor bed was detected, the entire WSI would be labeled as tumor positive (Ciga et al., 2021). An-



(a)

(b)

Figure 4: An example of overlapping (a) and non-overlapping (b) patches. These patches cover the same image region, but with overlapping, three patches are needed to cover the same area as two non-overlapping patches. The first and last patches of (a) match the patches of (b), but the middle patch of (a) is a combination of the patches from (b). Note: these patches were generated from whole slide images in the CAMELYON17 dataset (Litjens et al., 2018).

other study proposed a diffusion model for aggregating from patch level to slide level (Hou et al., 2016).

One study used patch level classification to extract features for slide level classification. 86.67% accuracy was achieved using Inception for patch level classification. An overall accuracy of 90.43% was achieved for the slide level classification of normal, benign, in situ carcinoma, and invasive carcinoma (Mi et al., 2021).

4.5 Thresholding

When working with patches in an image, there are hundreds of thousands of possible patches to be selected depending on the patch size selected. Patch selection methods vary between studies, with some studies performing random patch selection while others incorporate algorithms to select "best" patches (Hou et al., 2016). However, one thing they all have in common is avoiding irrelevant patches with no cells and only background material. In most whole slide images there are large background areas that are irrelevant for image analysis (Veta et al., 2014). With any patch selection technique, preprocessing is typically performed to eliminate irrelevant background patches. Often, thresholding is used to separate the image background from the relevant material. Thresholding is a technique that maps all image pixels into one of two groups. This technique is best used when there is high variance between an image's background and foreground. One popular method of thresholding in whole slide imaging is the Otsu thresholding technique. The Otsu threshold is determined by finding the maximum inter-class variance (Otsu, 1979; Xu et al., 2011). While the Otsu threshold is popular in whole slide image segmentation of background and foreground, there are instances where it is not as effective. For instance, in Khened et al. (2021), the Otsu threshold could not be used to segment the CAMELYON dataset due to black regions within the WSI. Instead, the black pixels were changed to white first and then a median blur filter of size 7x7 kernel was used prior to performing the Otsu thresholding. (Khened et al., 2021).

While the Otsu threshold is popular, it is not the only thresholding method used. One study used a custom threshold to segment areas without nuclei from the image as regions that lack nuclei are not relevant in tumor identification. Their thresholding removed any regions that met the following criteria: hue between 0.5 and 0.65, saturation greater than 0.1, and value between 0.5 and 0.9. These bounds were derived from experimentation with whole slide images, and patches with at least 25% foreground were included in the study (Ciga et al., 2021). Neural networks have also been applied to the segmentation task of tissue sample from its background with success (Alomari et al., 2009).

4.6 Staining Techniques

Another common issue is variability in slide images. Although hematoxylin and eosin (H&E) staining is the most commonly used staining technique, it does have some drawbacks. This staining technique does not label the nuclei and cytoplasm in cells exclusively. Sometimes other staining techniques are used such as fluorescent staining, which is more common in tissue morphology clinical research. Whole slide image datasets using H&E stained slides that are publicly available are already scarce, so these alternative staining methods have limited annotated data to be used for machine learning. One study attempted to bridge H&E stained images with fluorescent stained images. Due to color variations, cross analysis can be difficult. Through methods involving color normalization techniques for preprocessing and nuclei extraction, they were able to create a model that had 89.6% accuracy in identifying tumor regions in H&E images and 80.5% accuracy in identifying those same regions in fluorescent stained slides. Further work into cross analysis between staining methods will increase the amount of available data for all types of stained whole slide image analysis (Huang et al., 2023).

4.7 Tools for Whole Slide Image Analysis

Several approaches have produced free and open-source software to aid others conducting research in this area. One available tool is DigiPathAI. This is a generalized deep learning-based framework for histopathology tissue analysis. When creating DigiPathAI, four main problems were addressed – the large size of WSI images, minimal training samples, stain variability and extraction of clinically relevant features. Four datasets were used in training the model including CAMELYON16 and CAMELYON17 along with DigestPath (colon) and PAIP (liver). DigiPathAI used an ensemble of 3 fully convolutional networks – Deeplabv3, Inception-ResNet and DenseNet. A divide and conquer approach was taken for the WSI image size problem. Patches of the image were selected and segmented. Once all patches were segmented, they stitched together the segments to generate the whole slide image segmentation. The researchers used data augmentation to combat a lower number of training samples as well as to generalize across different staining and scanning protocols. This included horizontal/vertical flip, rotations, and Gaussian blurring and color augmentation (Khened et al., 2021).

MIA (Microscopic Image Analyzer) is another open-source tool developed for deep learning on microscopic images. MIA provides a graphical user interface for using deep learning tools for classification, segmentation, and object detection of microscopic images. By providing the graphical user interface, programming skills are not required to work with MIA. MIA simply requires training data, although the user needs to be able to select a model and hyperparameters. MIA also provides image labeling tools for annotating datasets (Körber, 2023).

The creators of the CAMELYON dataset also have created an open-source tool for visualizing and interacting with the CAMELYON dataset. This tool is called ASAP (Automated Slide Analysis Platform) and works on Linux and Windows operating systems. ASAP offers tools for both viewing and annotation (Litjens et al., 2018).

4.8 Comparing Machine Learning Approaches to Pathologist Analysis

In 2017, a study put pathologists and coding teams up to the CAMELYON16 challenge. They split pathologists into two groups and provided them with the same WSI images for two tasks – metastases identification through pixel level annotation and slide level labeling of metastases. 129 WSI were provided for annotation. The first group of pathologists was given a time constraint of two hours while the second group had no time constraint. The group without time constraint took approximately 30 hours to assess all 129 images. The challenge was open to coding teams and 32 total algorithms were submitted across 23 teams. Of the 32 algorithms, 25 were based on deep convolutional nueral networks, showing their popularity for whole slide imaging tasks. GoogleLeNet team scored 0.994 AUC on the image classification task. In comparison, the median AUC for pathologists without time constraint was 0.966 and 0.81 for pathologists with time constraint. This showed that the model outperformed pathologists with time constraint. This is more realistic since pathologists have many cases to analyze and a limited amount of time. The algorithm was comparable to the results achieved by human pathologists with unlimited time to view and classify whole slide imaging (Bejnordi et al.,). Importantly, while not perfect, CNNs can be used to predict the phenotype of breast cancers, potentially reducing the need for expensive biomarker assays (Couture et al., 2018; Su et al., 2023). Deep learning algorithms also have the potential to predict patient outcome, which is hard to achieve with pathologic evaluation of a breast cancer tissue sample (Shi et al., 2023; Fernandez et al., 2022).

5 CONCLUSION

Since the advent of whole slide imaging, research in digital pathology has surged. Computer-aided diagnosis and medical image analysis have become a focus for researchers, especially in digital pathology. While there are still many challenges when working with whole slide images, current research shows promise for finding the solutions to overcome these challenges. Deep learning in digital pathology has the potential to become a powerful tool for pathologists and assist them with the high demand of the field, which could ultimately lead to better care for breast cancer patients. This paper provides background information about breast cancer, whole slide images, and deep learning along with key challenges and the techniques employed by researchers in the field to overcome these challenges. The implementation of deep learning shows potential for incredible benefits that can both propel digital pathology forward as well as help patients.

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