Review Article

Computer-based image analysis in breast pathology

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Abstract

Whole slide imaging (WSI) has the potential to be utilized in telepathology, teleconsultation, quality assurance, clinical education, and digital image analysis to aid pathologists. In this paper, the potential added benefits of computer-assisted image analysis in breast pathology are reviewed and discussed. One of the major advantages of WSI systems is the possibility of doing computer-based image analysis on the digital slides. The purpose of computer-assisted analysis of breast virtual slides can be (i) segmentation of desired regions or objects such as diagnostically relevant areas, epithelial nuclei, lymphocyte cells, tubules, and mitotic figures, (ii) classification of breast slides based on breast cancer (BCa) grades, the invasive potential of tumors, or cancer subtypes, (iii) prognosis of BCa, or (iv) immunohistochemical quantification. While encouraging results have been achieved in this area, further progress is still required to make computer-based image analysis of breast virtual slides acceptable for clinical practice.

Key words: Breast pathology, breast virtual slides, image analysis, whole slide imaging

INTRODUCTION

Whole slide imaging (WSI) has the potential to be utilized in telepathology, clinical education, and digital image analysis to aid pathologists. As different types of specimen have different specifications, comprehensive studies should be carried out in each pathology subspecialty to assess the extent of added benefits of WSI in that field. A large proportion of the pathology slides are related to breast tissue; for example, in the United States, 1.6 million breast biopsies are assessed by the pathologists each year.¹ Recent studies suggested that WSI can be adopted in breast pathology as pathologists’ performance in reading breast slides while using WSI platforms was comparable to conventional microscopy in breast pathology.²

One of the major advantages of WSI systems compared to conventional microscopy is the possibility of doing computer-based image analysis on the digital slides. Recently, many researchers and slide scanner vendors have started developing automated methods to facilitate pathologists’ tasks in breast pathology. This review is restricted to the computer-based image analysis in breast pathology and aimed at discussing the previous studies, summarizing their results, and identifying the remaining challenges and areas where further studies are required.

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It should be noted that image analysis done in other pathology subspecialties or animals' tissue might be extendable to breast pathology; however, discussion about the potentials for extending these ideas to breast pathology is out of the scope of this review. For a broader review on digital pathology in general, please refer to the studies by Pantanowitz et al. and Ghaznavi et al.\textsuperscript{[3,4]}

**SEARCH STRATEGY**

Three different databases, namely Scopus, PubMed, and IEEEExplore, were searched to find relevant studies published after 1995. Our overall search strategy included terms for digital slides (e.g., whole slide, digital pathology, virtual slide) and breast and was limited to English-language, original, human studies. We also searched references of the retrieved articles. The exact search statement for each database can be found in Appendix 1. For studies where the methodology evolved in two or more papers with considerable amount of overlap, only the most expanded version was included in the review.

Studies focusing on the application of WSI or computer-aided analysis to multispectral images or quantification of biomarkers other than four clinically important immunohistochemical (IHC) stains (i.e., estrogen receptor (ER), progesterone receptor (PR), Ki-67, and human epidermal growth factor receptor [HER2]) have been excluded as they are not currently widely used in the clinical practice.

**CLASSIFICATION OF THE REVIEWED STUDIES**

The primary purpose of the reviewed studies can be classified into four categories: (i) segmentation of desired regions or objects in the slide, (ii) classification of breast slides, (iii) prognosis of breast cancer (BCa), and (iv) IHC quantification. Most of the reviewed studies had a block diagram similar to the one shown in Figure 1. Some of the methods may not include one or more of the steps illustrated in Figure 1. Moreover, multiple steps may have been merged in some studies. As shown, features could be extracted from a segmented object or tissue texture. Each group of studies is discussed in this section.

Before processing the slides, preprocessing steps can be performed to eliminate the background,\textsuperscript{[9]} segment diagnostically relevant area (DRA),\textsuperscript{[6,7]} standardize the color,\textsuperscript{[8]} or separate stain.\textsuperscript{[8]} Color deconvolution is a commonly used preprocessing step to separate the H channel. Ruifrok and Johnston\textsuperscript{[9]} proposed a formulation based on the Beer–Lambert law to map the red, green, and blue (RGB) color space to a set of three stains using color deconvolution. It should be noted that color deconvolution needs prior knowledge about the color vector of each stain (stain matrix). Standard stain matrix for a wide range of stain combinations is provided in a study by Ruifrok and Johnston.\textsuperscript{[9]} However, use of image-specific stain matrix is more accurate. This motivated the development of an image-specific stain normalization algorithm to automatically estimate stain matrix for each slide, such as the one presented in a study by Khan et al.\textsuperscript{[10]} As an alternative solution to overcome color variations due to dyeing, in a study by Ali et al.,\textsuperscript{[11]} stain separation was done adaptively in cyan, magenta, and yellow color space rather than RGB. In addition, the RGB color space is not perceptually uniform. To overcome this problem, in the studies by Dundar et al.\textsuperscript{[12]} and Basavanhally et al.,\textsuperscript{[13]} lab color space which is a perceptually uniform color space was used. Finally, the color deconvolution assumes that the relation between spectral absorbance of a stain mixture and the concentrations of the pure stains is linear. This assumption is valid under monochromatic conditions; however, it introduces an error under nonmonochromatic conditions.\textsuperscript{[14]}

**Segmentation of Desired Regions or Objects in the Slide**

A wide range of image processing methods has been used for segmenting objects in breast virtual slides. Accurate segmentation is important as it is an intermediate step of studies with various purposes. Because of its

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**Figure 1: The common steps in the reviewed studies**

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importance, only proposing a segmentation method to handle difficulties in breast slides has been main subject of 25 reviewed studies. The studies are summarized in Table 1. In this section, methods proposed for segmenting DRAs,[31-34] epithelial nuclei,[15,19,35] lymphocyte cells,[7,13,20] tubule,[21-25] and mitotic figures[6,8,26-30] are discussed.

Table 1: Summary of the studies aimed at segmentation of structures in breast virtual slides

| Reference | Initialization/seed detection | Segmentation | Features; classifier | Result |
|-----------|-------------------------------|--------------|---------------------|--------|
| **Epithelial nuclei** |
| [15] | Color deconvolution, morphological operation; fast radial symmetry transform | Multi-scale marker-controlled watershed | Morphology; rule-based discarding | TPR: 0.86, Specificity: 0.89 |
| [16] | Adaptive thresholding of sequentially filtered image; distance transform of overlapped cells | Gaussian mixture modeling of distance transform; cluster validation; occluded contour reconstruction | - | TPR: 0.97 (combined for cervical and BCa cells) |
| [17] | Morphological reconstruction; adaptive thresholding | Marker extraction based on optimal H-minima transform; Marker-controlled watershed | - | ACC: 0.96 (combined for cervical and BCa cells) |
| [5] | Background removal by graphcuts-based binarization; distance-constrained multiscale LoG filtering | Graphcuts-based method with combination of alpha expansion and graph coloring | - | ACC>0.94 |
| [18] | Color deconvolution, Calculating local features based on laws’ texture decomposition | Probability map generation; ACM including shape priors | - | - |
| [19] | Color deconvolution by singular value decomposition | Clustering; ACM | Intensity, morphology, texture; AdaBoost | ACC: 0.95 |
| **Lymphocyte** |
| [20] | Constructing the shape priors | watersheds; ACM combined with shape priors | Morphology; SVM | TPR: 0.86, PPV: 0.67 |
| [7] | Expectation maximization based segmentation of object classes | Geodesic ACM; Concavity detection; edge-path algorithm | Intensity, k-means clustering | TPR: 0.91, PPV: 0.78 |
| [13] | Thresholding luminance channel | Region growing | Size, luminance, proximity; Bayesian modeling and Markov random field | ACC: 0.9 |
| **Tubule** |
| [21] | Color swatch definition by the user | Normalized cuts on weighted mean shift reduced color space; Color gradient based geodesic ACM | AF based on O’Callaghan neighborhood; RF | TPR: 0.86, PPV: 0.80 |
| [22] | Color deconvolution | Lumen detection by hierarchical normalized cut initialized color gradient based ACM | Surrounding cell evenness; rule-based discarding | ACC: 0.9 |
| [23] | K-means; identifying the nuclei nearer to each white region | Contour detection of the nuclei near-lumen using level set | Morphology, texture, surrounding nuclei distribution | ACC: 0.91 |
| [24] | Nuclei detection by radial symmetry based method; classification of nuclei as normal/tumor | Lumen detection by thresholding | - | TPR: 0.88, Specificity: 0.92 |
| [25] | Super pixel generation; forming spatio-color-texture map using texton representation | Normalized graph cuts | - | - |

*Contd...*
Diagnostically relevant areas

In pathology slides, large areas are empty. As stated, segmenting DRAs could be used as a preprocessing step to reduce the computational cost \(^6,7\) or to avoid storage of non-DRAs with high magnification.\(^{16}\) Due to its significance, there are studies aimed only at improving the accuracy of DRAs segmentation.

In the earliest method,\(^\text{14}\) thresholding of gray-level image was used to segment DRAs. However, many important features of breast tissues are coded in the color and texture. Therefore, in a study by Mercan et al.,\(^\text{32}\) a texton-based approach was proposed to distinguish between DRAs and irrelevant patches. In another study by Khan et al.,\(^\text{31}\) Gabor-based texture features were used to differentiate hypocellular from hypercellular stroma. Gabor filters are extensively used in image analysis as they resemble the human visual system.

In a study by Peikari et al.,\(^\text{33}\) the areas that attracted pathologists’ attention were found using eye tracking data obtained from pathologists while assessing digital breast slides. The visual bag-of-words model with texture and color features was used to describe DRAs and train a logistic regression and a support vector machine (SVM) to predict DRAs in testing slides.

Epithelial cells

As shown in Figure 1, the segmentation procedure of epithelial cells included seed detection, initial segmentation, splitting, and false positive (FP) reduction. In one of the earliest studies by Dalle et al.,\(^\text{17}\) gamma-corrected red channel was used to segment the epithelial cells. K-means clustering in RGB space was also utilized to detect cells.\(^\text{18}\) However, in more recent studies, epithelial cells were mostly segmented from the H channel.\(^\text{15,35,39,40}\) Thresholding the H channel followed by morphological operation is a low-computational cost approach to detect epithelial cells. Nonetheless, thresholding is sensitive to variations of stain and cannot handle overlapping cells. Hough transform as well as Laplacian of Gaussian (LoG) filtering\(^\text{41}\) and its approximation, which is difference of Gaussian (DoG), are also popular tools to detect blob-like objects and are utilized to detect nuclei in breast tissue. They are more robust to the staining variations; however, the Hough transform is a computationally expensive approach and DoG should be deployed in a multi-resolution scheme to address cells with different sizes. The fast radial symmetry transform, which is a computationally efficient, noniterative procedure for localizing radial symmetry objects, has also been utilized for candidate nuclei locations detection.\(^\text{15}\)
The initial seeds could be used for further fine segmentation using active contour models (ACMs)\[15,19,37,39,40\] or marker-controlled watershed.\[15,35\] Conventionally, the ACM relies on gray-level image, but breast slides are colored. To address this issue, in a study by Basavanahally et al.,\[39\] color gradient-based ACM was used. In addition, ACM cannot handle overlapping cells as they rely only on intensity information and do not incorporate knowledge about the nucleus shape. In a study by Veillard et al.,\[18\] a nucleic shape prior was included to deal with this issue. In a study by Al-Kofahi et al.,\[5\] Graphcuts could partially handle the segmentation the overlapping cells when combined with distance map constrained multi-scale LoG for initial seed detection. However, Graphcuts led to over-segmentation in enlarged highly textured nuclei and under-segmentation in partially broken or weakly stained touching cells. Marker-controlled watershed is a robust approach for separating the overlapping cells when the initial seeds are correctly localized. However, in case of severely overlapping cells, spurious initial seeds are inevitable. The adaptive H-minima transform\[17\] and rule-based discarding was used to eliminate FPs. In a study by Nguyen et al.,\[25\] nuclear arrangement in tubules could be with a lumen or in solid islands without a lumen. Belsare et al.,\[25\] proposed a novel integrated spatio-color-texture-based graph partitioning method to address this issue and achieved a correct classification rate (CCR) of 92% for segmentation.

**Lymphocytes**

Lymphocytic infiltration is a prognostic indicator; therefore, recently, researchers worked on the automatic segmentation of lymphocytes. In a study by Basavanahally et al.,\[13\] lymphocytes were initially segmented using region growing, which resulted in a large number of epithelial nuclei being detected as well. Bayesian modeling of size and luminance of lymphocytes and proximity modeling using Markov random field were used to eliminate nuclei. However, region growing cannot handle overlapping cells. To address this, a concavity detection scheme was proposed in a study by Fatakdawala.\[71\] The expectation maximization-based method was used to initialize the ACM and then overlapping cells were split. Despite achieving a true positive rate (TPR) of 86%, PPV was only 64%. In a study by Ali and Madabhushi,\[20\] shape priors were incorporated in ACM to handle overlapped cells and the watershed algorithm was used to initialize the ACM. Similarly, high TPR (86%) and low PPV (67%) were achieved. Therefore, it seems that adding an FP reduction module is required to eliminate epithelial nuclei.

**Tabules**

Tubules are characterized by a white region called lumen, surrounded by a single layer of nuclei in normal breast histopathology. Dalle et al.\[42\] utilized thresholding followed by morphological operations to segment lumen areas. However, thresholding was not robust to stain variation. Xu et al.\[21\] proposed a color gradient-based geodesic ACM which was initialized by weighted mean shift clustering and normalized cuts for lumen segmentation. Later, in a study by Basavanahally et al.,\[22\] domain knowledge was incorporated into method proposed in a study by Xu et al.\[21\] and each segmented area was classified either as true or false based on architectural features and an accuracy of 86% in detection of true lumen was achieved. Maqlin et al.\[23\] used heuristic rules based on evenness and closeness of strings of surrounding nuclei to eliminate lumen-like areas and achieved an accuracy of 90%.

The above-mentioned methods associated only the closest nuclei to the lumen. However, it could be surrounded by multiple layers of nuclei. Therefore, in a study by Nguyen et al.,\[24\] the global distribution of the nuclei and lumina were considered. Furthermore, a comprehensive feature set containing architectural, morphological, intensity-based, and textural features were used to distinguish true lumina from artifacts. Finally, the discussed methods focus on lumen detection; however, nuclear arrangement in tubules could be with a lumen or in solid islands without a lumen. Belsare et al.,\[25\] proposed a multi-resolution image analysis strategy for detection of mitotic figures based on Graph-based regularization. The method was analyzed WSI at different levels and segmented the relevant areas and detected the mitoses in the highest magnification, and it was completely unsupervised. However, the detection rate was 70% and no FP reduction step was adopted; hence, further improvement was required for deploying the method in a clinical setting.

In a study by Khan et al.,\[6\] the pixel intensities of mitotic and nonmitotic areas were modeled by a Gamma-Gaussian mixture. A set of textural and intensity-based features were extracted from each region labeled as mitosis by the first module. The features fed
into an SVM classifier which detects FP instances. The obtained TPR and PPV were 72% and 70%. Irshad et al. detected the candidate region in the blue ratio image using thresholding followed by morphological operations and extracted a patch of size 80 pixel × 80 pixel from blue ratio and red and blue channels of RGB color space. A wide range of textural features including Haralick textural, gray-level run length, scale invariant feature transform, and Gabor-based features was extracted from each patch. The principal component analysis was used for dimension reduction. The features were fed into decision tree, linear SVM, and nonlinear SVM. It was shown that a decision tree achieved the highest performance with a TPR of 76% and a PPV of 75%. Later, Irshad investigated the added value of morphological features and features from other color spaces to the FP reduction step, but the result did not improve.

One of the difficulties in the detection of mitotic figure is its wide range of appearances. To handle this issue, in the studies by Cireşan et al. and Malon and Cosatto, the learned features extracted by convolutional neural networks were utilized. Malon and Cosatto achieved a TPR of 59% and a PPV of 75% while in a study by Cireşan et al., the PPV and TPR were improved to 80% and 70% using an optimized approach for sampling nonmitosis pixels in the training set. Further investigations are still required in mitotic detection filed to improve TPR and PVP and also deal with the wide range of variability in the appearance of mitotic figures.

Classification
Computer-based image analysis of breast slides may aim at classifying the virtual slide into different categories. The classification could be done based on the grade of BCa, the invasive potential of tumors, or cancer subtypes. Table 2 summarizes the purposes and methods of the reviewed studies aimed at classification of breast slides.

Cancer grading
Scarff-Bloom-Richardson grading system is a well-known grading system relying on magnitude of tubule formation, nuclear pleomorphism, and mitotic count. Segmenting the mitotic figures (which leads to the mitotic count) has been discussed in sections 1–5. The discussed studies in this section aimed at classifying the slides according to nuclear pleomorphism or all three factors. The earliest reviewed study by Weyn et al. applied wavelet transform in four levels on the segmented nuclei and the energy of filtered images in each scale was calculated. In addition to wavelet-based, Haralick, intensity-based, and morphological features were extracted and fed into a K-nearest neighbor classifier to separate individual nuclei and also each case in four categories (normal, nuclear atypia Grade I, II, III). A CCR of 64% for classification of individual nuclei and a CCR of 79% for case-based classification were observed. It was shown that textural features (wavelet-based and Haralick features) had a high additive value to intensity-based features. The dataset used in the study was highly imbalance (21 normal vs. eight Grade III cases) and the segmentation method was required further refinement.

In a study by Doyle et al., the centers of nuclei were manually segmented and a range of intensity-based and textural (Gabor-based and Haralick) features were extracted from each nucleus. The mean, standard deviation, minimum-to-maximum ratio, and mode of these features over all cells in each slide were calculated and formed the feature vector. The architectural features were also extracted from Voronoi diagram, Delaunay triangulation, minimum spanning tree, and nuclei density function. The architectural features resulted in the highest CCR for low-versus high-grade classification while the textural features resulted in a significantly lower CCR (73 vs. 93%). Despite the encouraging CCR, the fact that the segmentation was done manually limits generalizability of the study. In a study by Dalle et al., the nuclei segmentation was done automatically using polar transform, and area, compactness, and mean intensity were extracted from each segmented nuclei. A high value for CCR (92%) was achieved for scoring nuclear pleomorphism of 2396 region of interests (ROIs). However, the result could be biased as the dataset contained images from only six patients and did not include any patient with Grade I.

Pathologists implicitly integrate features from multiple field-of-views (FOVs) of different sizes when grading BCa. However, automatically selecting an optimal FOV size is not straightforward. In a study by Basavanhally et al., architectural and textural features were extracted from a multi-FOV of varying sizes and important features at different FOV sizes were identified to distinguish low/high-, low/intermediate-, and intermediate/high-grade patients. Unsurprisingly, the highest performance was obtained when distinguishing low from high-grade patients. Similar to results obtained in a study by Doyle et al., architectural features performed better than textural ones. It was also observed that the most discriminating architectural features were different in FOVs with various sizes while contrast played a dominant role among textural features. It was also shown that the multi-FOV classifier outperformed multi-scale classifier.

All of the above-mentioned methods extracted features from segmented nuclei only while pathologists rely on features from other structures such as tubules. To overcome this limitation, in a study by Petushi et al., features were also extracted from the tubule and showed that the density of tubule and number of Grade III would be useful parameters for BCa grading.
Benign versus malignant classification and distinguishing lesion subtypes

A pathologist usually inspects the breast tissue to determine if it is a benign or malignant lesion is present and also to identify the cancer type (if appropriate). Computer-aided detection (CAD) tools could help the pathologists in this task and make the results less susceptible to observer variation.

In the earliest CAD system,\(^{[44]}\) it was shown that textural features (wavelet-based and Haralick) outperformed morphological and intensity-based features in differentiating benign from malignant cells. Later, in a study by Doyle et al.,\(^{[45]}\) Gabor-based features, which are also a textural feature, achieved higher CCR compared to architectural features, and the diagnostic importance of nuclear texture in differentiating normal

| References | Purpose | Processing/segmentation | Features; classifier | Result |
|------------|---------|-------------------------|----------------------|--------|
| [37]       | Classification of BCa slides as score 2 or 3 based on nuclear Pleomorphism | Color deconvolution; thresholding and morphological operations; gradient in polar space | Morphology, texture; Gaussian modeling | CCR: 0.92 |
| [43]       | Classification of cells according to Nottingham histologic grade (3-Class) | Adaptive thresholding; morphological operation; Nuclei classification; Tubule detection | Texture, number of mitotic cells and tubules; linear, quadratic; ANN and DT | (The best: ANN) 3-Class CCR: 0.71 |
| [44]       | Classification of cells as benign or malignant (2-Class) and distinguishing lesion subtypes based on nuclear pleomorphism (3-Class) | Thresholding; morphological operation; multiscale representation using wavelet | Morphology, intensity, texture; k-nearest neighbor | 2-Class CCR: 0.89; 3-Class CCR: 0.80 |
| [39]       | Classification of BCa slides as low (mBR 3-5), intermediate (mBR 6-7), and high (mBR 8-9) grade classes | Color deconvolution; morphological operation; color gradient-based ACM | Architecture, texture; boosted multi-FOV classifier | (Low/high) CCR: 0.93; (Low/intermediate) CCR: 0.72 (Low/high) CCR: 0.74 |
| [45]       | Classification of cancerous and noncancerous slides | Extraction of 3400 features from manually segmented nuclei; dimension reduction by spectral clustering | (The best) Gabor-based texture; SVM (The best) architecture; SVM | CCR: 0.96; CCR: 0.93 |
| [38]       | Classification of BCa slides as benign or malignant | Adaptive thresholding; morphological operation; multi-label fast marching; watershed | Morphology, intensity, texture, architecture; k-nearest neighbor | Sensitivity: 0.97; specificity: 0.94 |
| [12]       | Classification of intraductal breast lesions as actionable (ADH and DCIS) or nonactionable (UDH) | Marker-controlled watershed | Morphology, intensity; multiple instances learning | CCR: 0.88 |
| [46]       | Classification of BCa slides as benign or malignant (2-Class) and distinguishing lesion subtypes based on overall mBR grade (3-Class) | Texton library construction by using four different filter banks; dimension reduction | Texton histogram; SVM, k-NN, DT, Bayesian, 4 boosting algorithms | (The best: Gentle AdaBoost) 2-Class CCR: 0.89; 3-Class CCR: 0.80 |
| [40]       | Classification of nuclei and ROI in breast slides as benign or malignant | Color deconvolution in CMY; difference of Gaussian; Hough transform; ACM | Morphology, texture; SVM | (Nuclei) TPR: 0.81; FPR: 0.30 (ROI) TPR: 0.92; FPR: 0.20 |
| [42]       | Classification of BCa slides based on overall mBR grade (3-Class) | Cell localization and detection of tubular formations in low-resolution global image; classifying cells as epithelial/tumor cells or candidate mitotic cells by Gaussian modeling | Tubules: Area of tubule/area of slide; rule-based Nuclei: Color; Gaussian modeling Mitotic figures: Morphology, intensity; Gaussian modeling |

mBR: Modified Scarff-Bloom-Richardson grading system, CCR: Correct classification rate, ROI: Region of interest, BCa: Breast cancer, ACM: Active contour model, UDH: Usual ductal hyperplasia; ADH: Atypical ductal hyperplasia, DCIS: Ductal carcinoma in situ, SVM: Support vector machine, TPR: True positive rate, FPR: False positive rate, ANN: Artificial neural network, k-NN: K-nearest neighbor, DT: Decision tree, FOV: Field-of-view.
from cancerous tissue has been shown. However, the
wavelet-based, Haralick, and Gabor-based features are
not easily interpretable to pathologists. Furthermore,
the high dimension of feature vector when low number
of training instances is available increases the chance
of overfitting of the classifier to hand data. Therefore,
in a study by Cosatto et al.,[9] only the median nuclear
area over an ROI and the number of large well-formed
nuclei were utilized to train a linear SVM with a labeled
dataset of 20 well-definition ductal carcinoma
classified as benign. The method has been tested on a
case was misclassified when all of the ROIs in a slide were
classified as malignant, and a malignant
ROC was calculated on a larger dataset from fifty patients
only the median nuclear
architectural
dataset of 20 well-definition ductal carcinoma
classified as benign. The method has been tested on a

In the real clinical practice, the ultimate goal is
distinguishing patients with malignancy and not the
individual ROIs. Pathologists judge each case based on
multiple ROIs and label it accordingly. In a study by
Filipezuk et al.,[18] a larger set from fifty patients (nine ROIs
per patient) were classified as either benign or malignant
using 84 features (morphological, intensity-based, and
textural) extracted from isolated nuclei in each ROI.
Sequential forward feature selection was used to reduce
number of features, and a k-nearest neighbor was used as a
classifier. The final diagnosis for each patient was obtained
by a majority voting of the classification of all nine ROIs
belonging to the same patient. A CCR of 100% was
achieved. Considering the high CCR obtained in the study,

In a study by Dundar et al.,[12] learning with multiple instances was used to train
an SVM classifier. According to the proposed classifier,
a benign case was misclassified when at least one of the
ROIs in a slide was classified as malignant, and a malignant
case was misclassified when all of the ROIs in a slide were
classified as benign. The method has been tested on a
dataset of 20 well-defined ductal carcinoma in situ (DCIS),
12 borderline DCIS, 24 atypical ductal hyperplasia (ADH),
and 39 usual ductal hyperplasia (UDH). DCIS and ADH
cases were grouped as actionable (malignant) while UDH
cases were considered nonactionable (benign). An overall
accuracy of 87.9% was obtained while the accuracy on the
borderline cases was 84.6%, comparable to that of nine
pathologists on the same set (81.2% average). Despite
encouraging result, for deploying such a system in clinical
practice as an aid to pathologists, its additive value to a
pathologist’s diagnosis should also be assessed. Moreover,
the proposed method (classification rule and features) is
not easily interpretable to pathologists.

Unlike the above-mentioned studies, pathologists do
not segment each individual nucleus within a slide; however, they analyze the scene holistically. In a study
by Yang et al.,[46] textural features based on texton-based
method were extracted without segmenting the structures
in slides. CCRs of 89% and 80% were achieved in
benign/malignant and multi-class (benign and two major
cancer subtypes) classification.

Prognosis of Breast Cancer
The advent of WSI allows extracting quantitative features
which could be helpful in predicting prognosis of BCa. In
a study by Veta et al.,[47] an automatic nuclei segmentation
algorithm[15] was utilized to extract size-related nuclear
morphometric features and their prognostic value in
male BCa was investigated. The results demonstrated
that mean nuclear area has a significant prognostic
value. In another study, Beck et al.[48] showed that
quantitative stromal features are associated with survival.
A comprehensive set of quantitative features from the
BCa epithelium and stroma was extracted by utilizing
a machine learning method called computational
pathologist. The prognostic model was based on the
extracted features and it was shown that the score from
the model was strongly associated with overall survival.
In addition, assessing significance of features revealed
that survival was strongly related to three of the stromal
features and the magnitude of association was stronger
than the association of survival with epithelial features.

The presence of lymphocytic infiltration is also a
prognostic indicator for in HER2 + BCa patients.
Currently, pathologists do not routinely report the
presence of LI as quantifying it is a tedious job. As
discussed in 1–3, recently researchers worked on automatic
detection of lymphocytes. However, a further step should
be added to grade the extent of lymphocytic infiltration.
In a study by Basavanahally et al.[13] architectural
features were extracted from IHC-stained samples, which could be helpful in predicting prognosis of BCa. In

Immunohistochemical Quantification
Currently, the standard procedure in pathology laboratories
for assessment of IHC is visual examination of samples
by a pathologist. The pathologist determines the status
of receptors by counting positively stained cells. Hence, the
procedure is tedious and prone to inter-observer variability
due to subjectiveness. Computer-assisted methods in
the field of IHC quantification aim at quantification
of information extracted from IHC-stained samples to
reduce inter-observer variability and assessment time.
HER2 receptors typically express on the cell membrane.
Therefore, membrane segmentation is one of the main
steps of automated methods for quantification of
HER2. A color-based approach,\(^{49}\) water shedding,\(^{50}\) and skeletonization\(^{51}\) were used for membrane segmentation. After the segmentation stage, a group of features was based on membrane staining intensity,\(^{49,51-53}\) membrane completeness,\(^{49,53}\) or membrane color properties.\(^{54,55}\) Instead of restricting the area for feature selection to the segmented membrane, Ali et al. utilized an algorithm which was previously used for analysis of astronomical images and extracted intensity-based features from the entire image without segmentation.\(^{11,56}\)

In contrast to HER2 receptors, ER and PR overexpression typically results in nuclear immunoreactivity, and hence, nuclei segmentation is the first step of some of the reviewed methods listed in Table 3. The extracted features from segmented nuclei in the reviewed studies were based on nuclei staining intensity,\(^{57,58}\) nuclei shape,\(^{52,57}\) or nuclei color properties.\(^{50,59}\) Rather than segmenting the nuclei, Amaral et al. proposed a method for predicting quick score values for receptor assessment based on color- and intensity-based features extracted from each pixel of test images without segmentation.\(^{60}\) As shown in Table 3, the reviewed automated methods for ER and PR assessment showed high agreement with the expert scoring.

### Table 3: Automatic and semi-automatic methods for immunohistochemical quantification

| Method [reference] | Number of samples | Reference scoring | Result |
|--------------------|-------------------|-------------------|--------|
| **ER and PR**      |                   |                   |        |
| Segmentation based |                   |                   |        |
| ImmunoRatio\(^{50}\) | 50 S VC           | \(r: 0.98\) (combined with ki-67) |
| NuclearQuant\(^{57}\) | 195 C Allred \(\kappa: 0.859; \kappa (w): 0.986\) |
| Definiens\(^*\) | 53 ROI 3-S \(a: 100\) |
| Aperio\(^{52}\) |                   |                   |        |
| Intensity analysis of segmented cell\(^{58}\) | 743 S P/N \(p: 0.74\) (ER) and 0.62 (PR) |
| Analyzing ratio of color components\(^{59}\) | 134 S P/N \(a: 85\) (ER) and 81 (PR) |
| Nonsegmentation based |                   |                   |        |
| Modified astronomical algorithms\(^{11,56,60}\) | 1769 Allred \(r: 0.82\) |
| **Her2**           |                   |                   |        |
| Segmentation based |                   |                   |        |
| MembraneQuant\(^{51}\) (based on membrane intensity) | 309 ROI 4-S \(\kappa: 0.872\) |
| Definiens\(^*\) | 23 S 4-S \(a: 100\) |
| Aperio\(^{52}\) (based on membrane intensity) | 77 S 4-S \(a: 83.0 (H^+); 73.4 (T^+); 78.0 (CS^*)\) |
| Aperio\(^*\) (based on membrane intensity) | 77 S 4-S \(a: 94.6 (H^+); 92.1 (T^+); 92.5 (CS^*)\) |
| Using normalized color Histogram\(^{53}\) | 64 S 3-S \(a: 80\) |
| Using membrane completeness and membrane intensity as features and minimum cluster distance classifier\(^{49}\) | 77 S 3-S \(\text{tb: 0.72}\) |
| Using normalized color Histogram\(^{54}\) | 77 S 3-S \(\text{a: 81-83}\) |
| Using membrane completeness and membrane intensity as features and minimum cluster distance classifier\(^{49}\) | 144 S 3-S \(\kappa (w): 0.80\) |
| ImmunoMembrane\(^{41}\) | 1648 S 4-S \(\text{a: 87; } \kappa (w): 0.57\) |
| Nonsegmentation based |                   |                   |        |
| Intensity-based thresholding\(^{61}\) | 1653 4-S \(r: 0.62\) |
| Modified astronomical algorithms\(^{11,56,60}\) | 1648 S 3-S \(r: 0.98\) (combined with ER and PR) |
| **Ki-67**          |                   |                   |        |
| Segmentation based |                   |                   |        |
| ImmunoRatio\(^{50}\) | 50 VC \(r: 0.98\) (combined with ER and PR) |
| Nonsegmentation based |                   |                   |        |
| Intensity-based thresholding\(^{61}\) | 1648 S 3-S \(\text{a: 87; } \kappa (w): 0.57\) |

\(^{1}\)Hamamatsu NanoZoomer 2.0 HT (Hamamatsu Photonics, Bridgewater NJ), \(^{2}\)Aperio ScanScope T2 (Aperio Technologies, Vista, California), \(^{3}\)Aperio Scanscope CS (Aperio Technologies, Vista, California), \(^{4}\)Commercial product; S: Slides, C: Cases, ROI: Regions of interest, VC: Visual counting, Allred: 0-8 grade system, 4-S: 4 grade system, 3-S: 3 grade system, P/N: Classification as positive or negative, \(p\): Correlation coefficient \(r: R^2\) for regression; \(a\): Agreement percentage, \(\kappa\): Cohen’s kappa, \(\kappa (w)\): Weighted Cohen’s kappa, \(\text{tb}\): Kendall’s coefficient of concordance, ER: Estrogen receptor, PR: Progesterone receptor
Similar to the methods for ER and PR assessment, quantitative assessment of Ki-67 could be done by extracting features based on either the segmented cell nuclei\cite{50} or the percentage of stained area.\cite{64} The agreement between the automated methods and the visual examination done by pathologist is reported in Table 3.

ImmunoRatio\cite{50} and ImmunoMembrane\cite{41} are two publicly available web-based applications. ImmunoRatio is a tool for quantitative assessment of ER, PR, and Ki-67 while ImmunoMembrane is an HER2 IHC analysis software. Both applications were tested and matched well with the pathologist’s visual examination.\cite{41,50}

**DISCUSSION**

The emphasis of this review was discussing the computer-based image analysis in breast pathology. In spite of encouraging results achieved by the reviewed studies, further progress is still required to make the CAD tools acceptable for clinical practice. For example, segmentation of severely overlapping and broken cells has not been fully addressed yet. Moreover, the low PPV of segmentation methods suggests that an FP reduction step should follow the initial segmentation. In addition, only a few studies focused on automatic segmentation and grading of tubule formation as well as distinguishing cancer subtypes; hence, further studies in these fields are required. Moreover, most of the studies attempted to extract features from the segmented objects. Further investigation of nonsegmentation-based methods in breast pathology is required as these methods avoid error propagation from the segmentation step and also mimic the human visual system which captures textural features.

Pathologists extract information from multiple ROIs and scales. Using multi-ROI and multi-scale approach to mimic the perception of pathologists could be a potential direction for future studies. Furthermore, clinicians prefer a CAD which provides physically interpretable features and classification rules; however, most of the current tools are “black box” systems.

One of the other major challenges of CAD is variability of breast tissue. Although standardization of slide preparation protocols, color normalization, noise reduction, and quality assurance programs will tackle the tissue variability problems to some extent, there is an inherent variability in the appearance of the objects within the breast tissue, which cannot be compensated. For example, the shape of epithelial cancerous nuclei may vary from almost normal-like round structure to highly irregularly shaped and enlarged nuclei with coarse and marginalized chromatin and prominent nucleoli. Moreover, the fact that different structures in breast histopathology slides may look similar decreases the specificity of CAD in detection of certain features. Another difficulty for segmentation-based CAD is separating clustered or overlapping cells. All these factors that affect adversely on the performance of CAD systems should be addressed to obtain a CAD which is robust enough to be used in the clinical practice of pathology.

In evaluation of CAD studies, inherent inter-pathologist variations should be considered. For example, in a study by Shaw et al.,\cite{2} it was shown that intra- and inter-pathologist agreement for detection of pleomorphism is lower than that of IHC quantification. Similarly, automatic IHC quantification tools usually achieved higher agreement with pathologists’ assessment in comparison with CADs aimed BCa grading [Tables 2 and 3].

Moreover, the additive value of CAD to pathologist’s opinion should be investigated as CAD could be potentially used as “second reader.” Finally, one of the major obstacles for researchers working on BCA digital slides is lack of publicly available data sets which enable them to evaluate the performance and robustness of their proposed algorithms. Having such reference databases whose ground truth was built based on a panel of expert pathologists would provide a unique opportunity for comparing different algorithms’ performance against each other. Recently, two publicly available databases for mitosis detection have been introduced;\cite{65} however, more databases containing virtual slides of different BCA types, different grades of BCA, and so on from different scanners are still required.

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**Conflicts of Interest**

There are no conflicts of interest.

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APPENDIX

Appendix 1
The advanced search option of the databases was used to find the articles. The search was limited to human studies. Endnote was used for reference management. After combining all references and omitting non-English references, duplicated studies were omitted by using a built-in function in Endnote. Nonoriginal studies (e.g., review paper, abstract paper or report), undetected duplicates, and nonrelevant studies were excluded based on scanning the article’s title and abstracts. Then, included papers were downloaded and fully studied and a few of them were further excluded in case they were not original or relevant to the topic of the review.

The following statement was used to search Scopus:

```
TITITLE-ABS-KEY ("breast") AND (TITITLE-ABS-KEY ["virtual slide"] OR TITITLE-ABS-KEY ["whole slide"] OR TITITLE-ABS-KEY ["digital pathology"] OR TITITLE-ABS-KEY ["digital histopathology"] OR TITITLE-ABS-KEY ["whole-slide"] OR TITITLE-ABS-KEY ["digitized histopathology"] OR TITITLE-ABS-KEY ["digital slide"] OR TITITLE-ABS-KEY ["digitized slide"] OR TITITLE-ABS-KEY ["digitized cytology"] OR TITITLE-ABS-KEY ["digital cytopathology"] OR TITITLE-ABS-KEY ["digitized cytopathology"] OR TITITLE-ABS-KEY ["cell segmentation"] OR TITITLE-ABS-KEY ["nuclei segmentation"] OR TITITLE-ABS-KEY ["nucleus segmentation"]).
```

The following statement was used to search IEEEExplore

```
("QT breast QT") AND ("QT virtual slide QT") OR ("QT whole slide") OR ("QT digital pathology") OR ("QT digital histopathology QT") OR ("QT whole-slide") OR ("digital slide") OR ("QT digital cytolgy QT") OR ("QT cell segmentation QT") OR ("QT nucleus segmentation QT") OR ("QT histology QT") OR ("QT histology image QT") OR ("QT histopathology image QT") OR ("QT mitotic QT").
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The following statement was used to search PubMed

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("Breast") AND ("virtual slide") OR ("whole slide") OR ("digital pathology") OR ("digital histopathology") OR ("whole-slide") OR ("digital slide") OR ("digital cytolgy") OR ("cell segmentation") OR ("nucleus segmentation") OR ("nuclei segmentation") OR ("histology") OR ("histology image").
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