Breast Cancer Histopathology Image Analysis: A Review

Mitko Veta*, Josien P. W. Pluim, Paul J. van Diest, and Max A. Viergever

Abstract—**This paper presents an overview of methods that have been proposed for the analysis of breast cancer histopathology images. This research area has become particularly relevant with the advent of whole slide imaging (WSI) scanners, which can perform cost-effective and high-throughput histopathology slide digitization, and which aim at replacing the optical microscope as the primary tool used by pathologist. Breast cancer is the most prevalent form of cancers among women, and image analysis methods that target this disease have a huge potential to reduce the workload in a typical pathology lab and to improve the quality of the interpretation. This paper is meant as an introduction for nonexperts. It starts with an overview of the tissue preparation, staining and slide digitization processes followed by a discussion of the different image processing techniques and applications, ranging from analysis of tissue staining to computer-aided diagnosis, and prognosis of breast cancer patients.**

Index Terms—**Breast cancer, computer-aided diagnosis, digital pathology, histopathology, image analysis, object detection, object segmentation.**

I. INTRODUCTION

PATHOLOGY Labs are currently undergoing a transformation toward a fully digital workflow [1]. In addition to the digital management of tissue samples, pathology orders, and reports, this includes the digitization of histopathology slides and use of computer monitors for viewing them, which aims to replace the optical microscope as the primary tool used by pathologists. This transformation has only recently been enabled by the introduction of cost and time efficient whole slide imaging (WSI) scanners, as successors to microscope-mounted digital cameras. This process of adoption of digital slide images is somewhat analogous to the digitization of radiological imaging. However, a full analogy is difficult to establish because in pathology the primary object of analysis is the tissue rather than the image [2]. Adoption of a fully digital clinical workflow in radiology benefitted from the ability to acquire radiological images digitally. By contrast, the barrier to entry is higher for histology, which is a distinctly analog modality that must always undergo an analog-to-digital conversion before computerized analysis.

A relatively large percentage of the samples that are analyzed in pathology labs are from breast cancer patients, since this disease is the most prevalent form of cancer among women [3]. Analysis methods that are routinely performed by pathologists, such as determination of the histological grade and the hormone receptor status by immunohistochemistry (IHC), can be tedious and are hampered by observer variability [4], [5]. The histological tumor grade is commonly determined according to the modified Bloom–Richardson system, which consists of semi-quantitative assessment of nuclear atypia, tubule formation, and mitotic activity [6], [7]. The analysis of immunohistochemically stained slides mainly involves the estimation of the number of cells that are positive for a particular antigen and the degree of positivity (staining intensity) [8], [9].

One of the main benefits of digital slides compared to conventional glass slides is that they enable the use of quantitative automatic image analysis methods. These methods have the potential to tackle the problems that stem from the subjective interpretation by pathologists and, at the same time, reduce their workload [10].

In this paper, we give an overview of image analysis methods that have been proposed for breast cancer histopathology images. We focus on automatic image analysis of histopathology tissue preparations imaged by brightfield microscopy, since this covers the bulk of the work that is performed by pathologists for this disease. However, some techniques developed for other tissue types or microscopy modalities that are relevant for the scope of our paper are mentioned throughout the text when appropriate. For a broader overview of digital pathology and the use of automatic methods for analysis of histopathology slides, we refer the reader to the recent reviews in [11]–[15].

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II. TISSUE PREPARATION AND IMAGING

Before we proceed to discuss the different image analysis algorithms and applications, we give an overview of the tissue preparation and staining processes and digitization of histological slides. In the typical hospital workflow, breast tumor excisions or biopsies are performed in the operating room after which the material is sent for analysis to the pathology lab. The first step of the tissue preparation process is formalin fixation and embedding in paraffin. From the paraffin blocks, sections
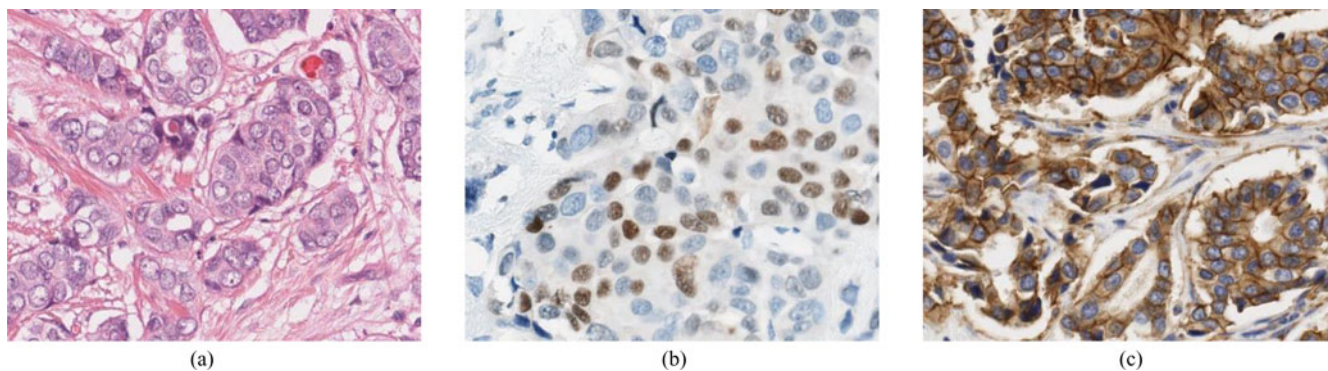
Fig. 1. Example histological stains. (a) Hematoxylin and eosin. (b) IHC staining for ER. The antibody is visualized with the DAB chromogen (3, 3'-Diaminobenzidine) and the tissue is counterstained with hematoxylin. The nuclei that are dyed with the brown stain are considered to be positive. (c) IHC staining fort HER2 with hematoxylin counterstaining. HER2 is expressed in the nuclear membranes.

with a thickness of 3–5 μm are cut using a microtome (a high precision cutting instrument) and mounted on glass slides.

The structures of interest in the tissue, in most instances the nuclei and cytoplasm, are not readily visible on the mounted sections. They therefore need to be dyed with stains that highlight them. The standard staining protocol uses hematoxylin and eosin [H&E, Fig. 1(a)]. In spite of the fact that this staining protocol has been in use for around a century, the diagnostic and prognostic procedure for all patients still almost always starts by staining the sections with H&E. Hematoxylin binds to DNA and thereby dyes the nuclei blue/purple, and eosin binds to proteins and dyes other structures (cytoplasm, stroma, etc.) pink.

IHC is a more advanced staining technique, which makes use of antibodies to highlight specific antigens in the tissue [see Fig. 1(b) and (c)]. In breast cancer, IHC is commonly used to highlight the presence of estrogen (ER), progesterone (PR), and human epidermal growth factor 2 (HER2) receptors, as well as to assess the proliferation of the tumor, for example, by highlighting the Ki-67 protein, which is associated with cell proliferation [8], [9], [16]. When performing IHC, the tissue is usually counterstained with hematoxylin to identify the nuclei and to visualize the tissue architecture (in case of nuclear antigens, visualize the nuclei in which the target antigen is absent). When quantification is of primary interest, such as with the determination of the ER, PR, and HER2 receptor status for breast cancer patients by IHC, the staining protocols need to be standardized and quality controlled in order to obtain reproducible results, which are comparable across different patients [17], [18].

Very often in breast cancer research, many different markers highlighted by IHC from hundreds or thousands of patients need to be considered. In order to achieve high-throughput analysis, researchers resort to using tissue microarrays (TMAs) [19], [20]. TMAs are constructed by punching small core biopsies (usually with a core diameter of 0.6 mm) from selected regions of the paraffin blocks containing the tissue to be analyzed, and transferring them to a recipient paraffin block in a regular pattern. The recipient paraffin block, which now contains tissue from many different subjects, is then cut and stained in a standardized manner. Because with TMAs tissues from different patients are stained under the same conditions, the resulting staining variability is significantly lower than with routinely prepared histopathology slides, which makes them more suitable for image analysis.

Currently, the typical pathology lab workflow is concluded by staining and coverslipping of the glass slides, after which they are sent to the pathologist for analysis. As digital pathology becomes more commonplace, slide digitization is added as an additional stage to this workflow [1]. The early slide digitization systems were digital cameras mounted on standard microscopes, which could capture still images. Present day WSI scanners, which enable high throughput slide digitization at relatively low cost, handle the entire scanning procedure automatically. This includes loading of the slides on the scanning platform, detection of the relevant tissue regions and focus point selection, image acquisition, compression, storing and registration on a laboratory information system. Most of the WSI scanners that are currently in use perform slide scanning at 20 $\times$ or 40 $\times$ magnification with a spatial resolution in the order of 0.50 μm/pixel and 0.25 μm/pixel, respectively. Because of the large size, the captured RGB image is compressed most commonly with JPEG or JPEG 2000. For faster navigation, the images can be stored in a pyramid structure with increasing magnification at each level of the pyramid. This also facilitates multiscale image analysis.

The tissue preparation, staining, and slide digitization processes can have a significant impact on the tissue/image appearance, and insight into them may lead to a better design of image analysis algorithms. The impact can be manifested in several ways. For example, improper fixation can lead to changes in tissue morphology and thus induce incorrect tissue morphometry by automatic image analysis. If sections are not properly stained (i.e., over- or under-stained) and mounted, this may result in out-of-focus regions and/or missing parts. Even small variations of the staining conditions can lead to variations in tissue appearance and cause problems for automatic image analysis algorithms. This issue of staining/appearance variability will be considered in more detail in the following sections.

III. STAINING ANALYSIS

When performing image analysis of histopathology images, it is of interest to separate the histological stains that dye different tissue components. For instance, if the application is nuclei
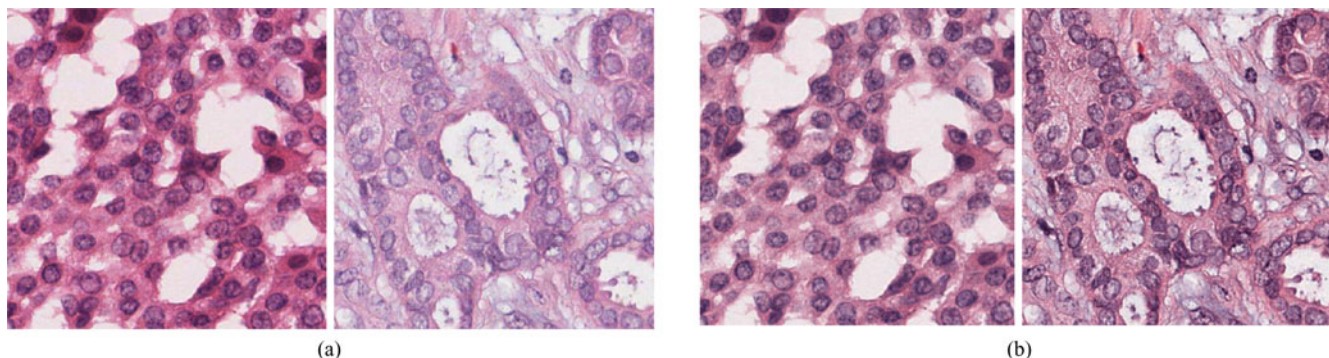
Fig. 2.   Standardization of H&E stained histopathology image appearance. (a) Two raw images with notable difference in the staining appearance. (b) Same two images with standardized appearance by a normalization method presented in [24].

detection in H&E stained sections, it can be beneficial to obtain a hematoxylin only image since the eosin-only stained components can contribute toward false positives. One approach is to perform clustering or supervised classification of the RGB pixel values in order to obtain binary or probability maps for the different stains. These methods require labeled data or identification of the cluster that corresponds to the stain of interest. Another approach for staining separations is based on the fact that the image formation process in brightfield microscopy can be modeled according to the Lambert–Beer law for light absorption. According to this law, the optical density (the logarithm of the intensity) is proportional to the concentration of stain in the tissue. Given that with RGB image sensors there are three detection channels, linear decomposition can be performed to determine the concentration of up to three stains for each pixel location [21]. Once the stain concentrations are determined, single-stain images can be derived by an inverse approach. These techniques require definition of characteristic absorption spectra for the stains that need to be separated. However, there are blind techniques that do not have this limitation [22].

It should be noted here that the commonly used DAB chromogen (3, 3'-Diaminobenzidine) is not a true light absorber, but it exhibits light scattering behavior. Thus, the behavior of DAB cannot be perfectly modeled by the Lambert–Beer law. However, in practice, good unmixing results can still be achieved [18].

One of the major difficulties in breast cancer histopathology image analysis, particularly of H&E stained sections, is appearance variability. In part, this can be explained by the heterogeneity of the disease, but a large portion is a result of the tissue preparation and staining processes. The color appearance can significantly vary between different labs owing to differences in fixation and in staining protocols and reagents. Color appearance may also vary between samples produced in the same lab as a result of preanalytic features as fixation delays and inconsistencies in the staining conditions. Finally, variability in appearance can be due to slide digitization conditions, including notably differences in optics, light detectors, or light sources used in the scanners.

Automatic image analysis methods can be significantly hampered by the variability of the tissue appearance. In addition, methods that are developed and tested on data from a single

center often must be reevaluated and adjusted when used with slides from external labs. It is desirable that histopathology image analysis methods are designed in a way that is robust to appearance variability, such as in [23]. Alternatively, the image appearance can be standardized prior to further processing. One simple approach is to determine the concentrations of the individual stains for each pixel with some of the staining separation techniques mentioned before, normalize the staining concentrations and then digitally mix the stains with common characteristic absorption coefficients to obtain a standardized image [24], [25]. This approach is illustrated in Fig. 2. In [26], a method for color standardization was proposed based on unsupervised segmentation into tissue components. The tissue was divided into four components: nuclei, stroma, epithelium, and background and the RGB histograms for each component were aligned to a template image.

IV. QUANTIFICATION OF IHC

In H&E stained slides, the features of the nuclei that are of interest to pathologists are relatively complex—their size, shape and texture, their spatial arrangement and organization into tubules, interaction with the stroma, etc. In contrast with this, most of the information that is of interest in IHC-stained sections is contained in the color and the intensity of the staining, which makes IHC-stained samples more open to design and implementation of image processing algorithms. For example, a useful and readily obtained characteristic of IHC digital slides is the determination of the percentage of pixels that are positively stained for a particular antigen.

In breast cancer patients, the ER, PR, and HER2 receptor statuses can have a major influence on the planning of adjuvant systemic treatment. Currently, the standard method of scoring IHC stained slides is by visual examination under a microscope. This procedure is prone to variability among pathologists even when strict guidelines are followed. Recent recommendations issued by the American Society of Clinical Oncology and the College of American Pathologists for testing of the ER, PR, and HER2 receptor status include encouragement of the use of quantitative image analysis techniques with the goal of improving the consistency of the interpretation [8], [9].

The ER and PR receptor statuses are customarily determined by counting the percentage of positively stained nuclei. If this percentage is above a predefined threshold (10% in Europe and 1% in the USA) the tissue is defined positive. The automatic quantification of the ER and PR status thus usually involves the use of an automated nuclei detection or segmentation algorithm [27] (this topic will be covered in detail in the following section). As an alternative to this, the percentage of positively stained nuclear area can be determined [28]. In [29], a method which computes features that reflect the area of positively stained nuclei and the nuclear intensity was proposed. These features were then mapped to an ordinal scale that is used by pathologists.

In comparison with the ER and PR receptors, which are expressed in the cell nuclei, the HER2 receptor is expressed on the cell membranes. The tumors are scored positive when more than 30% of the cell membranes show complete, uniform, and intensive staining. Cases with complete but nonuniform or weak staining in more than 10% of the cells are equivocal, and cases with no staining or incomplete staining are defined to be negative. Equivocal cases are further evaluated by other methods [30].

The largest challenge in HER2 staining quantification lies in correct membrane segmentation, which can be particularly challenging in negative cases and cases with incomplete and faint staining. A method for automated assessment of HER2 IHC is presented in [31]. In the first stage of this approach, all image pixels are classified as belonging to epithelial nuclei or cell membranes. The nuclear regions are further segmented into individual nuclei by watershed segmentation and the cell membranes are determined by adaptive ellipse fitting. Slides are then classified into one of the three scoring groups based on features describing the membrane staining intensity and completeness. In [32], following nuclei segmentation, approximate membrane contours were determined by Voronoi tessellation. The approximate contours were then refined based on the membrane staining intensity. Hall *et al.* [33] proposed a method where features for HER2 quantification were extracted based on positive controls, thus eliminating the variability in the staining between different slides.

Most of the commercially available image analysis tools include algorithms for positive nuclei counting and membrane staining quantification. A review of imaging solutions for quantitative IHC can be found in [34]. Two publicly available web applications for ER/PR and HER2 quantifications are described in [28] and [35]. In various recent studies, automatic scoring has shown high agreement with expert scoring and other methods (such as FISH for HER2 scoring) [36]–[44].

V. OBJECT DETECTION AND SEGMENTATION

A. Tissue and Tissue Components Segmentation

The typical histopathology slide contains a tissue area of approximately 15 mm × 15 mm. At the resolutions at which digital slides are captured, this will result in images with a size of up to several gigapixels. Because processing of these very large images might result in computational problems, it is common practice to identify the regions of the slides that are of interest prior to performing more detailed image analysis.

Generally, large portions of the slides are empty, i.e., they do not contain tissue. Most WSI scanners have the ability to identify empty tiles in the slide during the scanning process and avoid scanning them, which results in reduction of scanning time. One such approach for supervised tissue localization was proposed in [45].

When analysis of TMAs is performed, the individual TMA cores need to be identified and segmented and their coordinates on the TMA grid need to be assigned so they can be matched to the donor paraffin block [46]–[48].

In general, large areas of the tissue are not relevant for the problem at hand. For example, for computer-aided diagnosis of breast cancer (classification into the classes benign or malignant) only the epithelial regions of the tissue are relevant. When quantification of IHC or histological grading needs to be performed, only the tumor tissue is of interest and nontumor regions need to be excluded from the analysis. Furthermore, epithelial and stromal regions of the tumor have different significance for prognosis. Segmentation of the tumor into these components is often used as a preprocessing step when performing automatic morphometry and histological grading, or for guidance when constructing TMAs [49]. In the majority of the published studies on tissue segmentation, this kind of compartmentalization is achieved by supervised pixel-wise classification of small rectangular image regions based on color and texture features [50]–[54], although unsupervised methods have been proposed [55].

B. Nuclei Detection and Segmentation

The segmentation of nuclei in breast cancer histopathology images can be considered a basic functional block in many different applications. Quantification of IHC nuclear staining has already been mentioned in the previous section. Other applications include extraction of prognostically relevant morphometric features (size, shape, chromatin texture), automatic nuclear pleomorphism grading as part of a computer-aided prognosis system, detection of lymphocytic infiltration, and detection of malignancy and tubule formation in a bottom-up manner.

Nuclei segmentation remains a very challenging problem, particularly for routinely stained H&E sections, despite the numerous attempts to solve it. Many of the challenges arise from the variability of the tissue appearance, which is in part due to imperfections in the staining process. Furthermore, there is an inherent diversity of the appearance of epithelial cancerous nuclei, which may vary from almost normal-like round to highly irregularly shaped and enlarged nuclei with coarse and marginalized chromatin and prominent nucleoli (small round structures inside the nuclei). This is illustrated in Fig. 3. Different nucleus types, such as elongated fibroblasts and lymphocyte nuclei, often appear together with epithelial nuclei, which can decrease the specificity when only epithelial nuclei need to be detected or segmented. Additionally, nuclei may be overlapping, clustered or tightly clumped, which makes them difficult to separate. Lastly, hematoxylin-stained "junk" particles, which
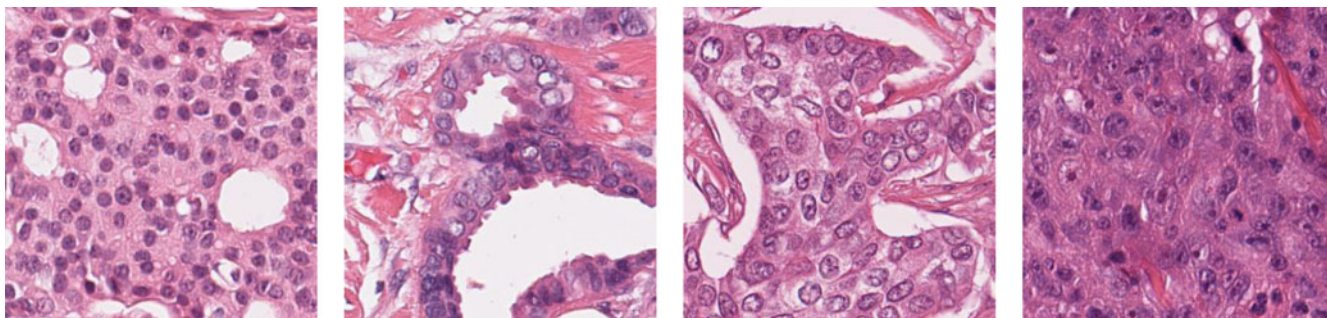
Fig. 3.   Different nuclei appearances in breast cancer histopathology images (from left to right): small and round with relatively uniform chromatin, organized into tubules, marginalized chromatin, and prominent nucleoli.

tend to appear in high-grade tumors, can hamper the nuclei segmentation.

A large variety of approaches for segmentation of nuclei in breast cancer histopathology images have been proposed. They vary not only as concerns the segmentation method proper, but also in the pre- and postprocessing steps that aim to improve segmentation performance.

The most difficult aspect of nuclei segmentation in breast cancer histopathology images is the detection of individual nuclei, especially when they are clustered closely together and overlap. Some proposed methods try and identify the individual nuclei prior to performing the segmentation procedure (marker extraction), whereas others first segment clumps of nuclei from the rest of the tissue and then proceed to separate those into individual nuclei. Methods such as the Hough transform [56] and voting along the direction of the image gradient to infer the center of the object [57], [58] have been used for both identification of nucleus markers and separation of segmented clumps. Another popular approach to separate clumps of nuclei is to split them along points of high concavity [59], [60]. In [61], separation of clustered nuclei is achieved by unsupervised Bayesian classification. Supervised methods for nuclei detection have also been proposed, with good performance [62].

With regards to the core segmentation technique, active contours are among the more popular methods [56], [57], [63]. Their objective is to find a minimum energy fit of moving contours to the image, the energy being defined in such a way that the contours are attracted to the boundaries of the objects of interest. The contours need to be seeded close to the target nuclei locations, which is why the approach is usually paired with a nuclei detection method. In [57], the level set active contours segmentation includes a repulsion term to prevent the contours of adjacent cells from overlapping. The active contour model for nuclei and lymphocyte nuclei segmentation proposed in [63] incorporates boundary, region and shape prior terms, and performs simultaneous segmentation of multiple objects in the image.

Several methods have been proposed that work by initially defining a large number of candidate regions and then selecting the ones that are likely to represent a correctly segmented object. In [64], candidate regions are identified by the maximally stable extremal regions (MSER) detector. This detector produces a large number of potentially overlapping regions. Each candidate region is evaluated with a statistical model, and dynamic programming is used to select a set of nonoverlapping regions that best fit the model. The authors of [59] proposed generating a very large number of candidate objects by identifying all possible closed contours within the image by contour tracing. A set of nonoverlapping contours is obtained by defining the objects that are most "fit" based on the contour gradient strength. In [58], candidate regions are initially identified by a marker-controlled watershed approach at multiple scales and using multiple marker types, which yields a large number of overlapping contours. Regions unlikely to represent valid nuclei are removed based on size, shape, boundary, and chromatin distribution features. Local concurrences are resolved by greedy selection of the contours most likely to represent nuclei, using the solidity of the object as a fitness value.

Other recently proposed and promising methods for nuclei segmentation in breast cancer histopathology are based on dictionaries of discriminative image patches [65] and marked point processes [66], [67].

Detection and segmentation of lymphocyte nuclei and detection of lymphocytic infiltrations can be considered specials case of nuclei detection/segmentation. In [68], region growing with high sensitivity and low specificity is used to initially segment lymphocyte nuclei and other objects. Then, maximum *a posteriori* estimation that incorporates size, luminance, and spatial proximity information is used to improve the specificity of the detector. Finally, the results from the lymphocyte nuclei detection are input to a classifier that discriminates between the lymphocyte infiltration phenomenon and the baseline level of lymphocytes. In [60], output from a Gaussian mixture clustering algorithm is used to initialize geodesic active contour segmentation. The overlapping objects are resolved by splitting them along high concavity points. Lymphocyte nuclei are distinguished from other objects by texture-based clustering.

The choice of the segmentation method is closely related to the intended application and the available time and computational resources. For example, active contours may yield superior nuclear segmentation, but at the cost of increased computational complexity, which limits their use for high throughput applications. When such high throughput processing is needed, a simple thresholding of the hematoxylin channel followed by morphological operations and splitting of clusters along high concavity points might be used, but at the cost of less accurate segmentation results.
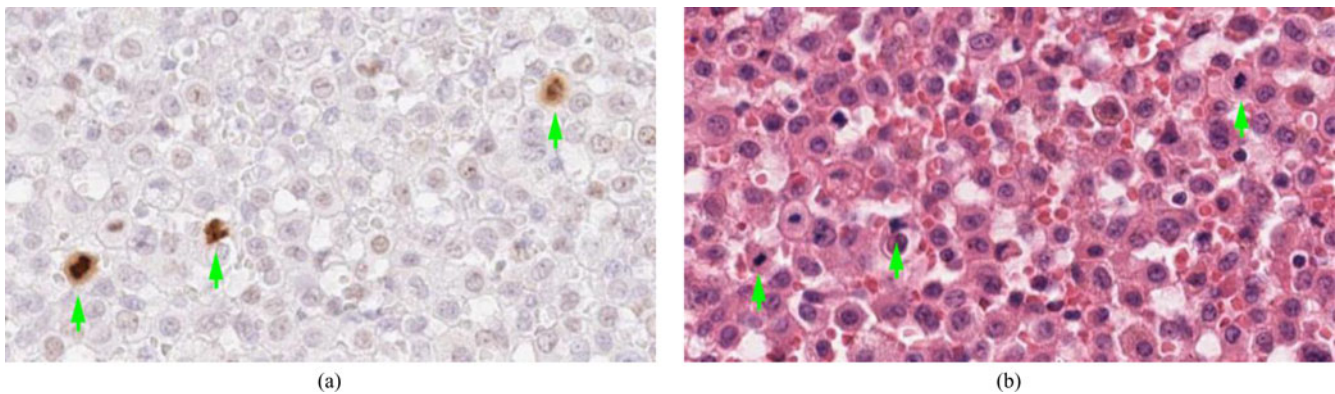
Fig. 4. Mitotic figures in breast cancer histopathology images. a) Region from a PPH3 labeled section. This staining method is specific to cells in the M phase which are clearly visible as dark brown objects (marked with green arrows). b) The same section stained with H&E. The corresponding four mitotic figures are visible as hyperchromatic objects. However, many other similar objects appear which complicates mitosis counting.

So far we have only listed methods that specifically aim at dealing with nuclei segmentation in breast cancer histopathology sections imaged with brightfield microscopy as an application. However, methods developed for other tissue types (e.g., prostate) or microscopy modalities (e.g., fluorescence microscopy) can also be applied to this problem [69]–[77]. In order to use these methods, some adaptation of some of the image processing steps will likely be needed, for example, modification of the way a nuclear staining image is obtained.

C. Tubules Segmentation

Along with nuclear pleomorphism, the degree of structural differentiation of the tissue is one of the earliest prognostic factors for breast cancer patients that have been identified by pathologists. Cancer disrupts the ability of the nuclei to communicate with each other and organize themselves into structures such as tubules, making the lack of tubule formation an indicator for advanced malignancy. The tubules are generally round or oval structures consisting of lumen surrounded by a layer of epithelial cells. The major challenge in tubule segmentation is the similar appearance of other structures, such as adipose tissue or tears formed during the tissue preparation process, which only lack the outside layer of well-arranged epithelial nuclei.

A color gradient-based geodesic active contour model for segmentation of the tubular lumen areas was proposed in [78]. The segmentation is initialized by weighted mean shift clustering and normalized cuts, and performs more favorably than the Chan-Vese region-based active contour model. This study was extended in [79] by incorporating domain knowledge to distinguish between tubules and other lumen-like areas. The authors show that the segmentation result can be used to infer the degree of tubule formation as defined in the Bloom–Richardson grading system. Although the literature for tubule segmentation in breast cancer is not extensive, methods developed for segmentation of other related structures are also applicable to this problem. A very closely related application is segmentation of glands in prostate cancer histopathology images [70], [80]–[82]. The methods developed for this application might be used for tubule segmentation in breast cancer with little adaptation. It should also be mentioned that some of the features that can

be derived by performing segmentation of tubules, can also be captured by computing features that describe the overall architecture of the tissue [83], [84].

D. Mitotic Figures Detection and Assessment of Proliferation

Of the three components that are part of histological grading of breast cancer, the assessment of tumor proliferation is probably the most important and prognostically significant one [85]. The oldest and still most widely used form of assessment of tumor proliferation is counting of mitotic figures in a predefined tissue area (usually 2 mm^2). The nuclei of the cells that are in the M phase of the cell cycle have distinctive morphological appearance in the H&E sections. Most commonly, mitotic figures manifest themselves as hyperchromatic objects without a clear nuclear membrane, with "hairy" protrusions around the edges and basophilia instead of eosinophilia in the surrounding cytoplasm. However, these are more instructive than defining features, and the bulk of the training of pathologists consists of looking at specific examples of mitotic figures designated as such by experts. The task of identifying mitotic figures is notoriously time-consuming and difficult, due to the fact that many other objects such as apoptotic and necrotic nuclei may have similar appearance, which renders it difficult even for trained experts to make a distinction. Lymphocyte nuclei, compressed nuclei, "junk" particles, and other artifacts from the tissue preparation process can also have a hyperchromatic appearance.

In addition to mitosis counting in H&E sections, there are also IHC techniques that can be used for assessment of proliferation of breast cancer tumors. However, they are not routinely used owing to increased cost and additional time delay. The most widely used IHC technique is labeling of the Ki-67 antigen that is associated with cell proliferation and expressed in all cell cycle phases except G0 [86]. Because Ki-67 labeling is unspecific to the M phase, the assessment of the number of mitotic figures is usually higher than by counting in H&E sections and it might not have the same significance. A proposed alternative to Ki-67 is phosphohistone H3 (PPH3) that has the advantage of targeting only nuclei in the M phase, and has been shown to have prognostic significance [87]. An example region labeled for PPH3 is shown in Fig. 4(a). In both of these staining

methods, the techniques for quantification of positively stained nuclei discussed in Section III can be used.

The standard approach of assessing tumor proliferation in pathology labs, however, still remains mitosis counting. Given that this is the most tedious part of the Bloom–Richardson grading system, there is a large incentive to develop an automatic mitosis detection algorithm that works with the routinely prepared H&E sections.

The earliest proposed approaches were unavoidably hampered by the limited image acquisition quality and computational power [88] and the need of specialized staining such as Feulgen to better highlight the chromatin [89], [90]. Recently, two publicly available datasets of H&E stained breast cancer histopathology images with annotated mitotic figures were made available [91], [92], which sparked further development of different mitosis detection approaches [93]–[102].

The majority of the proposed approaches work by first identifying candidate objects or locations that are then classified as mitotic figures or other objects. By far, the most distinctive feature of the mitotic figures is their hyperchromicity. In most cases, the intensity of the staining of the mitotic figures is noticeably darker than normal epithelial nuclei and only comparable to apoptotic, necrotic or compressed nuclei (artifact from the tissue preparation) and lymphocyte nuclei. This is illustrated in Fig. 4(b). The candidate extraction phase often may make use of this distinctiveness by performing thresholding, local intensity minima detection or pixel-wise classification followed by refining of the detected regions by morphological operations and/or active contours segmentation. In the second stage, more specialized features designed to capture the specific morphology of mitotic figures are used to train a classification model. However, convolutional neural networks that operate on raw RGB image patches appear to exhibit the best performance for this detection task [93], [97], [98]. The approach that was proposed in [93] is unique in the sense that it uses deep convolutional neural networks and does not perform candidate detection as an initial stage. Instead, it performs classification at every pixel location, which achieves excellent results.

A limiting factor of automatic mitosis detection is that whole slide images are typically scanned at a single focal plane. During mitosis counting, pathologists rely on "fine tuning" of the focus—something that is missing in whole slide images at a single focal plane. Digital slide scanners that perform image acquisition at multiple focal planes are now becoming more common; however, this feature is not widely used because of the increased storage demands. We anticipate that in the future, as storage costs go down and new image compression techniques become available, this limitation will be removed.

VI. COMPUTER-AIDED DIAGNOSIS AND PROGNOSIS

The objective of image analysis of digitized histopathology slides is to facilitate, and preferably automate, computer-aided diagnosis and prognosis (CAD and CAP) in pathology labs. CAD is defined as the detection of cancer within the examined tissue, whereas CAP addresses the more complex problem of predicting the outcome for the patient based on the available data. There are numerous challenges in achieving this objective, including the large image sizes and the lack of representative datasets with high quality annotations by multiple observers, and with patient follow-up. On the other hand, the incentives for developing CAD and CAP systems are overwhelming. Patients that are suspected of having breast cancer undergo a biopsy that is examined by a pathologist. Large numbers of cases are found to be benign [103], often easily distinguishable from cancer, which implies that a CAD system operating at high sensitivity can significantly reduce the workload of the pathologist, even if the specificity is moderate. This kind of system can also be used for quality control and assurance, for example, to identify positive cases that have been missed during the routine examination. Furthermore, the extraction of quantitative parameters from tumor regions can go a long way toward reducing the inter- and intraobserver variability of breast cancer grading, which has been well documented in the literature.

Some of the proposed breast cancer detection techniques operate by examining nuclear features, as cancer nuclei have a distinct morphology—large size, coarse chromatin texture, and irregular shape. In [56], ROIs from breast cancer histopathology slides are classified as benign or malignant based on two features related to the nuclear size: the median nuclear area and the number of large well-formed nuclei in the region. Nuclear morphometric features are also used in [104] in combination with texture and topology features for malignancy detection in breast cancer histopathology. The extraction of nuclear morphometric features, in most cases, relies on a nuclei segmentation procedure. The influence of the nuclei segmentation accuracy on the subsequent feature extraction for classification into the classes benign and malignant is examined in [105]. The conclusion of the authors was that perfect segmentation accuracy is not needed as it does not necessarily guarantee optimal performance. In [84], cancer tissue is distinguished from noncancerous tissue based on Gabor texture features, without relying on a nuclei segmentation algorithm.

In order to arrive at computer-aided prognosis of breast cancer patients, researchers have both developed image analysis systems in the context of the currently employed grading systems, and aspired to discover novel imaging biomarkers from histopathology images. In some cases, the output of the staining quantification and object detection and segmentation techniques discussed in the previous section can be directly used as part of established clinical practice, provided their output is comparable to the performance of human experts. This, for example, is the case with the quantification of positively stained nuclei and the detection of mitotic figures. In most cases, however, these techniques are used only as building blocks of more complex systems. Some proposed methods aim at predicting the cancer grade assigned by pathologists, most commonly according to the Bloom and Richardson grading system, or one of its constituent components such as the nuclear pleomorphism score [106]. Classifiers trained with features describing the tissue architecture in combination with nuclear features have been shown to be successful in distinguishing between different breast cancer grades [84], [107]. In [108], two features were identified that can successfully discriminate between the three breast cancer

grades—the density of nuclei with irregular chromatin and the density of tubules in the tissue.

It should be noted here that breast cancer grades can be seen as estimates of patient outcome based on an expert opinion. In that sense, CAP systems that aim at predicting the histological tumor grade use this as an intermediate end-point for the prediction of survival. The use of reliable intermediate end-points is crucial in a situation where the actual patient survival is not known or it is difficult to obtain. Besides the histological grade, other intermediate end-points can be used, for example, the risk scores from gene expression assays. Such work was presented in [109] and [110], where tissue architecture features were used to predict the Oncotype DX recurrence score with high accuracy, which suggests that image analysis methods have the potential to be used as a cheap alternative or supplement to gene expression profiling.

When patient cohorts with known survival outcome are available, systems can be built that directly predict patient survival based on the available data, both from imaging and from other sources, thus avoiding the use of possibly unreliable intermediate end points. One such example can be found in [111], where the mean nuclear area calculated by automatic nuclei segmentation was shown to be prognostically relevant for male breast cancer patients in addition to other histological and clinical features. The fractal dimension of the tissue, extracted from invasive breast cancer TMAs stained with pan-cytokeratin (specific to epithelial components), which was previously associated with tumor grade [112], was also shown to have prognostic value [113]. In [114], the authors proposed a system that successfully predicts patient outcome based on automatically extracted quantitative features from histopathology images. In addition, they identified three novel and previously unrecognized stromal features that are significantly associated with patient survival.

An exciting and promising area of research is the integration of imaging biomarkers from histopathology images with genomic data. Structural information about the tissue is lost when preparing the molecular assays, hence imaging biomarkers may be complementary to genomic data. In a recent study [115], the authors used an image analysis approach to derive knowledge about the tumor cellular composition (percentage of cancer, stromal and lymphocytic nuclei), which they used to correct copy number data and more accurately estimate the HER2 amplification. In addition, they combined image features with genomic information to train a predictor of survival of ER-negative breast cancer patients. This predictor had a better performance in comparison with using image features or genomic information only. In another study [116], the authors developed a workflow for image analysis of histopathology images and integration of morphological features with genomic data for biomarker discovery. Four of the morphological features were identified as biomarkers that can separate patients into groups with different outcomes.

Many of the proposed methods for automatic breast cancer detection, grading, and prognosis have been trained and evaluated only on relatively small regions from the image slides, either digitized TMA slides or manually selected regions from whole-slide images. Although these methods are useful in a semiautomatic setting, it is necessary for a high throughput and automatic application to either produce an output for the entire slide (or even, from a set of slides originating from one specimen), as in [107], where this is achieved by using a multifield-of-view framework, or to perform extraction of relevant ROIs for the problem at hand and limit the analysis solely to those regions [50], [54], [117].

VII. DISCUSSION

Over the past few years the interest in analysis of histopathology images has been steadily increasing, prompted by the introduction of WSI scanners into pathology labs and the imminent acceptance of digital slides as a primary diagnostic modality.

The use of quantitative techniques is viewed as a solution to the problem of observer variability of the interpretation of histopathology slides, both by pathology professionals and image analysis experts. In the case of breast cancer, several applications for quantification of immunohistochemically stained tissue have already gained approval from the United States Food and Drug Administration (FDA). Methods that work with routinely prepared H&E stained slides have great potential to make an impact on the pathology workflow. However, ensuring robustness is challenging owing to the complexity of the tissue characteristics that need to be analyzed. Nevertheless, progress has been made both in the development of basic image analysis tools for H&E stained sections (i.e., object detection and segmentation methods) and in the development of systems that predict patient outcome either directly or by use of intermediate endpoints such as the histological grade as assigned by pathologists. Further improvements are needed in order to produce methods that have performance levels that are suitable for clinical application.

At multiple points throughout the text we have mentioned that the steps of tissue preparation, staining, and slide digitization that precede image analysis can influence the results. The more quantitative analysis of histopathology image data will become part of routine pathology practice, the more optimization of tissue preparation, staining, and slide digitization will be needed. However, some variation in the appearance will still be present even under closely monitored conditions, so analysis methods will have to be developed in a way that is robust to such a variation.

Perhaps the main obstacle in the development of new histopathology image analysis methods lies in the lack of large publicly available annotated datasets. While the advent of WSI scanners has produced vast quantities of image data, it is difficult to obtain ground truth annotations in a form that can readily be used for development and testing of image analysis methods, even when this data is tied to the pathology reports. For example, the tumor grade or the mitotic activity index that can be found in pathology reports are often based on the analysis of a particular region from a single slide viewed under a microscope, while the slide itself has been selected from a set of slides originating from the same sample. Information about the selected slide and the region within that slide where analysis was performed is not recorded and thus correspondence between the routine annotation and the image data is difficult to establish. In addition,

owing to the large observer variability, annotation by multiple observers is needed to produce high quality ground truths, which is both time-consuming and expensive, particularly for large datasets. Making annotated image datasets publicly available will provide a breeding ground for the development of new image analysis algorithms and will enhance the objectivity of method comparison and improve the quality of computer-aided diagnosis and prognosis.

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