IJCTA, 9(10), 2016, pp. 4611-4622

Automated Image Analysis for Breast Cancer Histopathology Grading-A Review

***Maqlin Paramanandam **Robinson Thamburaj ***Joy John Mammen**

Abstract : Over the past two decades, image analysis researchers have been able to employ derived effectual image processing techniques for histopathology images, as a result of advances in Digital Pathology. With Breast cancer being the most prevalent cancer in women, this paper presents a comprehensive review on the state-of-the-art image analysis research focusing on automated quantitative analysis of breast cancer images. It begins with a background of breast cancer histopathology which includes tissue preparation, the staining process, breast cancer grading protocol and imaging followed by a discussion on the significance of image analysis for breast cancer histopathology, a brief narrative on image acquisition and the digitization processes and lists the various image analysis methods proposed for breast cancer histology grading. This paper could also serve as an introduction to new researchers entering the field.

Keywords : Breast cancer, histopathology, digital pathology, image analysis

1. INTRODUCTION

Breast cancer is the most frequently occurring cancer among women (1.67 million new cases diagnosed in 2012 alone (25% of all cancers) as well as being the most common cause of cancer death among women (522,000 deaths in 2012) [2]. In routine breast cancer Diagnostic Algorithm the patient is referred for a biopsy when a mammogram or any diagnostic imaging reports a growth suspected to be malignant. The tissue extracted is examined by the pathologist under a microscope to determine the aggressiveness of the disease, whereby the histological grade of the tumor is assigned. As the assessment is done based on the pathologist's visual examination of the tissue, it is reported to be hampered by considerable amount of subjectivity and inter and intra-observer variability [3]. In order to mitigate this issue of observer variability and provide quantitative reproducible parameters researchers have conducted investigations and suggested the use of image analysis methods [4, 5]. Thanks to the significant advances in computational and digital Breast Cancer Histology Grading technologies over the last few decades leading to the evolution of a whole new technology called Whole Slide Image (WSI) scanners which are used to transform a biopsy slide into a digital image, hence promising significant opportunities for digitized histopathology [6-9].

This paper presents a detailed review on quantitative analysis of Breast Cancer histopathology images. The study focuses exclusively on breast cancer histopathology images acquired from Hematoxylin and Eosin (H&E) stained breast histopathology tissue examined by bright field microscopes and touches on a few works on histopathology images of other organs or other image modalities when required. Interested readers are referred to Pantonowitz L., et al [8], Gurcan M.N., et al [10], [11] and Veta M., et al [12] for a broader review on digital pathology imaging, histopathology image analysis and breast cancer histopathology image analysis respectively.

* Department of Mathematics, Madras Christian College, Chennai, India (e-mail: maqlin.soj@gmail.com

** Department of Mathematics Madras Christian College, Chennai, India (e-mail: robinson@mcc.edu.in)

** Department of Transfusion Medicine, Christian Medical College Hospital, Vellore, India (e-mail: joymammen@cmcvellore.ac.in).

2. TISSUE PREPARATION

In clinical workflow, histopathology examination of tissue begins with a surgery or biopsy. The tissue extracted is then subject to chemical processing in the pathology laboratory before being sent to the pathologist for microscopic examination. The chemical processing involves 'fixing' the tissue using a chemical fixative called formalin. This is followed by embedding the tissue in paraffin wax. The paraffin embedded tissue blocks are then cut into 3-5 μm thick sections using microtome, a tool used that cuts extremely thin slices of material, known as sections and mounted on glass slides to be viewed under microscope by pathologists. Depending on the amount of tissue removed, there could be half a dozen or more slides. The objects of interest in the tissue (nuclei and cytoplasm) are not readily visible under the microscope. Staining is done to give both contrast and to reveal the objects of interest (nuclei and cytoplasm) under microscope. The commonly used stain for bright field microscope is the Hematoxylin and Eosin staining. Hematoxylin binds itself to DNA/RNA staining nuclei blue, while eosin binds itself to the protein staining cytoplasm and the extracellular connective tissue matrix pink (Figure 1).

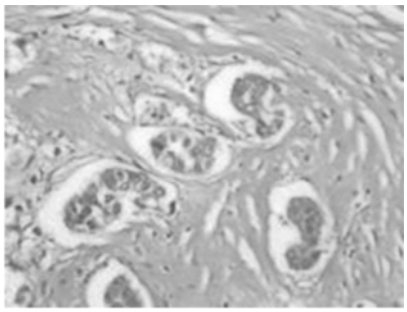

Fig. 1. Example for Hematoxylin and Eosin stain.

3. BREAST CANCER HISTOLOGY GRADING

The Nottingham modification of Scarff-Bloom–Richardson grading system (NGS) for breast cancer [1] has been the recommended breast histology grading system by various professional bodies internationally (World Health Organization [WHO] and American Joint Committee on Cancer [AJCC]. NGS grading system determines the histology grade deriving assessment from three morphological features of the tissue under examination namely tubule formation, nuclei pleomorphism and mitotic count, each of which is scored 1-3 based on how their characteristics differ from a normal tissue, as shown in Table1.

A. Tubule formation

Tubules can be characterized as rounded structures with a layer of epithelial cells surrounding a luminal region. . In cancer there is a break-down of the mechanism that the cells of normal tissue use, to form tubule or glandular structures leading to less tubular formations. An assessment of the percentage of tumor exhibiting tubular structures would depict how much the tumor differentiates from normal tissue. The part of tumor displaying tubular structures is assessed at x10 magnification and is assigned a score. The lesser the percentage of tumor forming tubules the higher the aggressiveness of cancer is found to be and it is scored higher as shown in Table 1. Fig 2 shows examples of score1 and score 3 for tubular formation.

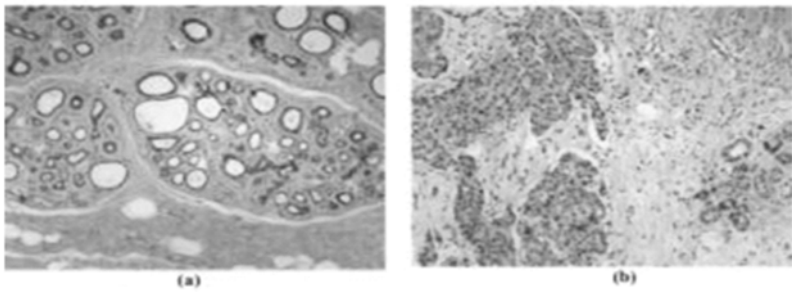

Fig 2. Example for Hematoxylin and Eosin stain captures at x40 magnification.

B. Nuclear Pleomorphism

Nuclear pleomorphism is the term used to describe the variation in size, shape and appearance of tumor cells from normal cells. The tumor is assessed at x20 magnification for nuclear pleomorphism scoring. According to NGS, a tissue's Nuclear Pleomorphism is given Score 1 is when nuclei are small, with little increase in size in comparison with normal breast epithelial cells, having regular outlines and uniform nuclear chromatin. Score 2 is given when the cells appear larger than normal, have open, vesicular nuclei with visible nucleoli, and there is moderate variability in both size and shape. Score 3 is given when there is a marked variation in size and shape, especially when very large and bizarre nuclei are present. Figure 3 shows examples of images depicting score 2 and score 3 nuclei pleomorphism.

C. Mitotic Count

The number of cells undergoing mitotic division visible under under the microscope set to a high magnification) best a fixed number of high power fields (HPF - the area of tissue depicts the proliferative activity of the tumor. Mitotic count is performed at × 40 magnification and the count is done at the tumor's periphery where the proliferation can be maximum. Scoring of mitotic count depends the on microscopic field areas under examination as shown in table 1. Figure 4 shows the mitotic figures present in a breast cancer histopathology images.

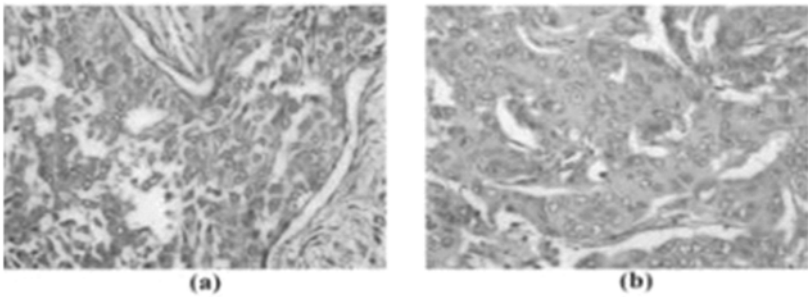

Fig. 3. Example of Breast cancer histopathology images captured at × 40 magnification. (*a*) Score 2 Nuclear Pleomorphism and (*b*) Score 3 nuclear Pleomorphism.

Table 1. Semi quantitative method for assessing histological grade in breast [1]

		Feature		*Score*
		Tubule and Gland formation		
		majority of tumours (>75%)		1
		moderate degree (10 - 75%)		2
		little or none (<10%)		3
		Nuclear Pleomorphism		
		small, regular uniform cells		1
		moderate increase in size and variability		2
		marked variation		3
		Mitotic Counts - Dependent on microscopic field areas		
		Examples of assignment of scores for mitotic counts for three different field areas		
Field diameter (mm)	0.44	0.59	0.63	
Mitotic count	0-5	0-9	0-11	1
	6-11	10-20	12-22	2
	>11	>20	>22	3

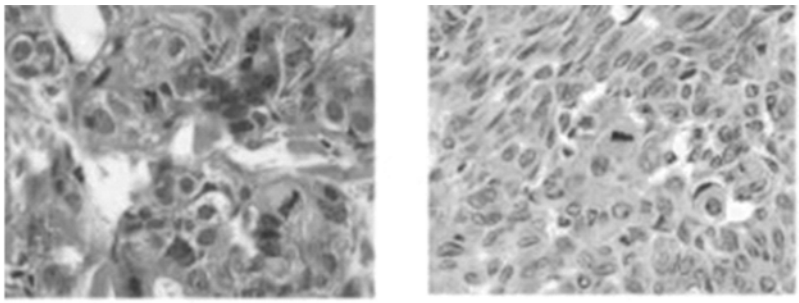

Fig. 4. Example of mitotic figures in Breast cancer Histopathology marked in green arrow heads.

The scores are added to determine the grade of the tumor (3-5 Low grade, 6-7 Moderate grade, 8-9 High grade). Lower grade tumors can be treated less aggressively, and have a better survival rate whereas higher grade tumors are treated with more aggressive treatment. Though NGS has been accepted largely, it has its share of pitfalls caused by its subjective nature and there is an immense amount of research carried on issues related to observer variability, reproducibility, relative importance of each of the three parameters in determining the overall grade, etc.

4. NEED FOR A QUANTITATIVE IMAGE ANALYSIS FOR BREAST HISTOPATHOLOGY

With breast cancer being the most prevailing [13] cancer among women, the pathology laboratories worldwide should handle relatively large number of breast histopathology slides. Histopathology analysis performed by pathologist is subjective in nature, associated with problems in consistency and reproducibility and suffer considerable amount of inter-observer variability [3] [14]. Most significantly, adjuvant chemotherapies and hormonal treatments which are used to improve patient survival are prone to have serious side effects, expensive and are therefore only administered to high risk patients. Studies prove that observer variability has significant impact on a patient's risk assessment for such therapies[15].

The subjective nature of histological grading is reflected in higher proportions on two parameters: nuclear pleomorphism and mitotic count. The estimation of variation in nuclei size and shape - clinically termed as nuclear pleomorphism (refer Table 1) is not as well defined as the other two parameters [1]. Studies have proved that it is the least reproducible feature Wolberg., et al. [16] , and there exist systematic differences between pathologists while scoring breast cancer nuclear pleomorphism, potentially affecting the overall grading of cancer Dunne and Going [17]. Elston and Ellis [1] on presenting the NGS system, had mentioned that the only way in which nuclei size and shape differences can be identified accurately is by the use of image analysis techniques. On the other hand mitotic counting hailed to be the principal prognostic factor of the NGS system [18, 19], has its share of subjectivity issues. Currently mitotic counting is done by the manual counting of mitotic figures under high power view following strict protocols. Effective care should be taken to avoid counting apoptic nuclei, pyknotic nuclei and stain artifacts which closely resemble mitoses. Apart from this it is a laborious task and takes considerable part of workload in the laboratories.

An automated breast cancer histopathology image analysis could be an efficient, error free and time saving decision support system for grading also making results obtained by different pathologists comparable. Given the need and significance of using image analysis for breast cancer grading. The following sections provide a brief description of the various image analysis methods developed in this area and the challenges involved in each aspect of automated breast cancer grading.

5. IMAGE ACQUISITION

The digitization of tissue slides can either be done using digital cameras mounted on standard microscopes which capture still images of a focused portion of the tissue slide or by using whole slide scanners which are robot-operated microscopes with specialized image acquisition software that enable high throughput imaging of the entire slide. The scanning process of a whole slide scanner is manual or entirely automatic which includes loading glass

slides onto the scanning tray, detection of relevant tissue area, focus point selection, image capture, image compression and storage. The scanner's image resolution is determined by the microscope objective used for scanning (eg, x20, x40) with a spatial resolution of about 0.25 μm/pixel. The images obtained at x40 magnification are several gigabytes large and image compression and decompression methods are used to archive the image files. Each image file is built as a multilayered pyramid and can be viewed across multiple resolutions instantaneously enabling multi-scale image analysis.

6. PRE-PROCESSING

Non-standardization in tissue preparation, staining techniques and imaging techniques lead to differences in the appearance of histopathology images. This poses a great challenge to histopathology image analysis methods. The methodology developed for datasets from one source may have to be tuned to suit images acquired at a different laboratory. There are different approaches in dealing the color variations. One approach is to separate the stains by computing the stain concentration at each pixel by color deconvolution. This is achieved by a linear decomposition of the three channels of pixel information. Once the stain concentration is determined, separate stain images can be reconstructed using an inverse approach. [20] performs stain standardization by normalizing the stain concentrations and mixing the stains with absorption coefficients and is further refined by [21]. A different approach was proposed by [22] in which color distribution of target image was matched with a reference image using a linear transformation in Lab color space. Another approach to the color standardization is robust color segmentation. A multimodal imaging technique was proposed in [23] wherein a novel sequential imaging and registration technique is presented that enables bright field and fluorescent imaging on the same tissue.

7. DETECTION AND SEGMENTATION

A most critical step is the segmentation of the objects in the breast histopathology image such as the nuclei, mitotic figures, tubule structures, etc. Each of these structures are observed by pathologists at various scale of magnification and hence they require image analysis methods developed for multiple scales. Figure 5 shows the different nuclei types of nuclei which are of interest in breast histopathology images.

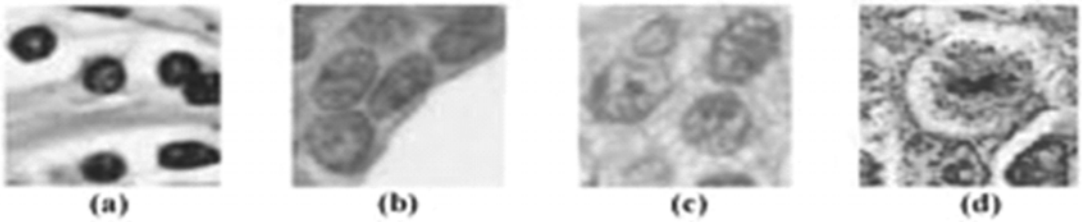

Fig 5. (*a*) Lymphocyte (LN), (*b*) Normal Epithelial nuclei (EN) , (*c*) Cancerous Epithelial Nuclei (CN) and (*d*) Mitotic nuclei (MN)

A. Region of interest (ROI) segmentation

Many image analysis methods for automated breast cancer detection and grading are developed for either small manually selected regions in the tissue slide or an entire whole slide image. In the latter case, extraction of a diagnostically relevant region of interest (ROI) is a pre-requisite. Since whole slide histopathology images are huge (several gigabytes in size) with a large portion of the image are empty tiles of non-tissue area of the slide, it is necessary to find the ROI in the image before further processing. [24] has proposed a supervised tissue localization method to locate the tissue region in a whole slide image. A few works focus on segmentation of diagnostically relevant regions in the tissue is presented. This can used as a pre-processing step before further object detection and segmentation steps. One approach is a supervised pixel classification based on color, intensity and texture features derived from candidate regions from images [25-27]. In [28], first the image is pre-processed to remove background (fat tissue regions) followed by segmentation of the tissue into Hypocellular Stroma (HypoCS) and Hypercellular Stroma (HyperCS) using gradient magnitude and phase spectra features in frequency domain.

B. Nuclei Detection and Segmentation

Numerous authors have proposed different methods for nuclei segmentation with each of them using certain image segmentation technique such as thresholding, morphological operations, watershed, active contour models, and G-cuts either separately or in combination [29]. The methodologies vary not only in their segmentation techniques but also in the approach towards nuclei detection steps.

One approach is finding a seed point within each nuclei region and then deriving the boundary of the nuclei initializing at the seed point. [30] had proposed the use of Hough transform technique for detecting nuclei seed points which were used in initializing a shape- and texture-based active contour model. A few authors have presented different voting algorithms which cast votes along gradient directions amplifying votes inside the centre of nuclei thereby locating the seed points as ones having maximum votes [31] . In [32] we presented an edge grouping method for uncovering the boundaries of nuclei detected by a gradient driven voting algorithm. An example of the resultant segmented image is shown in Figure 6. [33] applied the marker-controlled watershed approach at multiple scales, the segmentation looks into nuclei of all sizes. Two types of markers were proposed, one using radial symmetry transform (RST) and the other, the regional minima of the pre-processed image. Size, shape, boundary, chromatin distribution features and solidity of the object are all used in determining if an object is a valid nuclei or not. Another approach is to segment the nuclei regions and then resolve the overlapping or clump nuclei separation through heuristic approaches like the Concave Point Detection [34]. [35] presented an integrated region, boundary and shape based active contour to handle nuclei, lymphocytes and gland segmentation in H&E stained prostate and breast special cases, histopathology images. Figure 6 shows a result of nuclei segmentation.

Despite all the advancements in digital image analysis, the segmentation of Nuclei detection in high-grade breast cancer images is quite challenging in the case of image processing techniques due to certain heterogeneous characteristics of cancer nuclei such as enlarged and irregularly shaped nuclei, highly coarse chromatin marginalized to the nuclei periphery and visible nucleoli. The existing segmentation frameworks in literature have poor segmentation accuracy for images containing epithelial cancerous nuclei (CN) especially when CN are clustered and appear as large irregularly shaped nuclei.

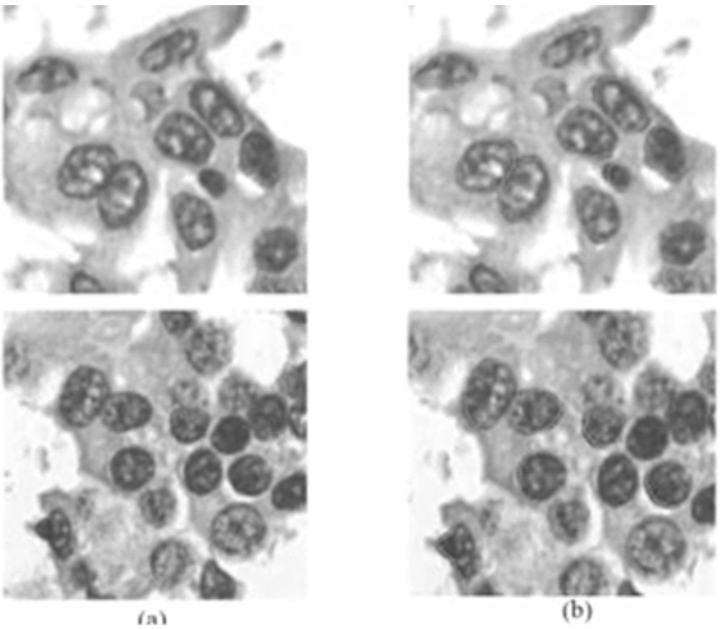

Fig. 6. (*a*) Breast cancer histopathology image patches (× 40 magnification) (*b*) Corresponding segmentation results.

C. Mitotic figure detection

The results of earliest work of image analysis in mitotic detection for breast tissue sections [36] were disappointing due to limited computational and imaging technology. Recent interests in mitotic cells detection in breast cancer

histopathology images revived when benchmark datasets of breast histopathology images annotated with mitotic figures were made publicly available at certain grand challenges [37], [38]. Automated mitotic detection has certain innate challenges due to their complex appearances. The most prominent feature of a cell undergoing mitotic division is its hyperchromaticity, effective care is taken to avoid counting other hyperchromatic elements such as lymphocytes or apoptic nuclei as mitosis. Another challenge is the variability in the shapes of mitosis in its four main phases: prophase, metaphase, anaphase and telophase. Specially, a mitotic cell in telophase, though having two separate and fully divided nuclei, should be counted as a single mitotic figure. Figure 7 shows different appearances of mitotic figures and hyperchromatic non-mitotic figures which have close resemblance to mitotic figures.

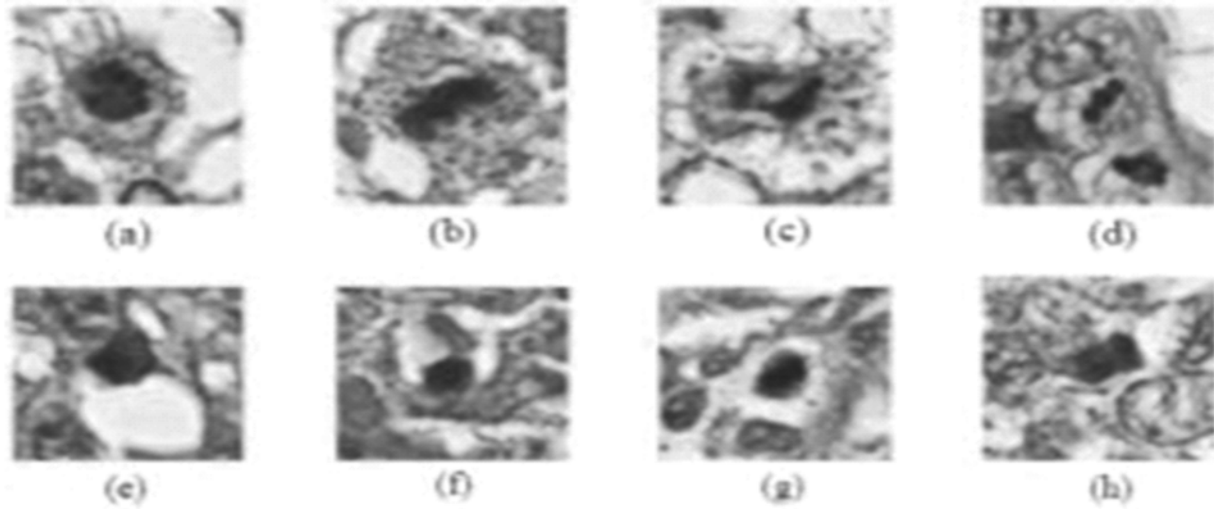

Fig. 7. Top Row: Different phases of Mitotic figures. (*a*) Prophase - chromatin pack into chromosome. (*b*) Metaphase - packed chromosomes align in the center of the cell (*c*) Anaphase - chromosomes separate and each chromatid moves to opposite poles of the cell. (*d*) Telophase - two daughter nuclei form in the cell. Bottom Row: Other Hyperchromatic nuclei in the images which closely mimic mitotic figures.

An efficient pixel classification application by supervised Deep Neural Networks [39] won the MITOS grand challenge of ICPR 2012 contest [40]. Since the DNN operates on raw pixel values it learns a set of visual features from the training data without need for human hand crafted features. However the contest was held for a relatively small dataset (5 slides in total, 10 annotated HPFs per slide) and since regions of same slide was included in both training and testing set the issues related to inter-subject variability were not taken into consideration. These issues were addressed in the next contest Assessment of algorithms for mitosis detection in breast cancer histopathology images AMIDA 2013.

The methods proposed in grand challenge AMIDA 2013 [38] can be roughly categorized into two groups: 1. Methods involving candidate detection which are classified based on certain hand crafted features into mitoses or non-mitoses classes. 2. Pixel Classifiers that when directly applied to the image pixels tend to classify them into mitotic or non mitotic class. Majority of the method belonged to the former group. The features extracted from the candidate regions were used for classification task. The winning method [39] belonged to the latter group and proved to be more powerful in mitotic detection. It presented an efficient implementation of deep convolutional neural networks to obtain a mitosis probability map for each image, from which mitoses were detected by non-maxima suppression. An extension of MIOTS grand challenge called MITOS-ATYPIA [41] was held at ICPR 2014 conference. The results of the contest have been released but the description of the winning techniques are not yet disclosed.

D. Tubule and gland Segmentation

The literature available for tubule segmentation in breast cancer histopathology images is very scant. Tubular or gland segmentation methodologies developed for prostrate histopathology images [42, 43] can be used in breast histopathology images with slight modifications. A color-gradient based geodesic active contour model was proposed to detect tubules in breast histopathology images in [43]. The contour was initialized by a mean shift

clustering method and normalized cuts. The work was extended in [44] by incorporating domain knowledge of the appearance of tubular structures in the tissue. A set of 22 graph-based image features characterizing the spatial linking between the tubular attributes is extracted from the O'Callaghan neighborhood constructed for nuclei closely surrounding every potential lumen. These features are used to discriminate between true tubules and false tubules. The results for identified tubules are shown in Figure 8. In [45], the segmentation of nuclei was achieved through level set evolution initialized by a grid method. The tubules structures are then identified based on uniformity, distance and direction criteria of nuclei surrounding the lumen. Tubule segmentation techniques face challenges when there are other objects such as fatty tissue and tissue folds produced during tissue preparation are present and which resemble tubular structures in their appearance except for the presence of a layer of epithelial cells.

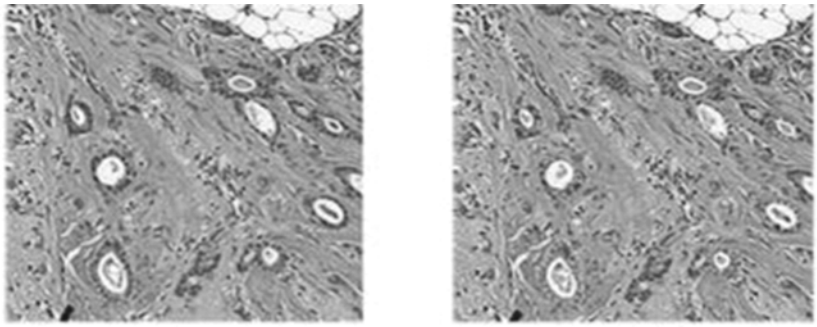

Fig. 8. Segmentation of tubules by method presented in [44]. (*a*) histopathology image patch (*b*) the centroid of only potential lumen classified as tubules (green circles) are shown along with the surrounding nuclei (blue squares) (Image Source [44])

8. COMPUTER BASED DIAGNOSIS AND PROGNOSIS

Previous sections limited discussions about image analysis techniques in pursuit of automated breast cancer prognosis that concur with the current grading system. Another approach is a comprehensive analysis of novel features extracted automatically from breast histopathology images, which are prognostically relevant and compare its performance with existing grading system. For instance [46] defines a set of features called tumor nest (TN) features extracted from the segmented breast histopathology image. The TN cell features generated in this work could quantify nuclei pleomorphism, and morphologic complexity of malignant epithelial architecture, both of which are independent prognostic predictors and were claimed to have better performance in predicting clinical outcomes than histological grade in ROC analysis. [47] presents a breast cancer grading scheme that uses two parameters to differentiate tumor grade: 1) the number density of cell nuclei with dispersed chromatin and (2) the number density of tubular cross sections. In [48, 49] , an image analysis method to compute the fractal dimensions of breast sections of grades 1, 2, and 3 tumors was developed. The results prove that breast tumor differentiation can be characterized using the architectural complexity of epithelial cells represented by the fractal dimensions. C-Path, is a prognostic model developed by [50] to measure a rich quantitative feature set of the breast cancer epithelium and stromal regions . The results of the system strongly co-related with survival outcome of patient cohorts and in addition three stromal features were recognized to be strongly associated with patient survival. Other promising endeavors in the field are studies integrating histopathology image analysis results with the cancer genomic data. In this context , [51] developed a image analysis method that predicts survival in estrogen receptor-negative breast cancer by integrating both image-based and gene expression analyses.

9. CHALLENGES, FUTURE TRENDS AND OPEN PROBLEMS

With digital pathology seeing a quantum leap in the past two decades, there has been a great deal of image analysis research on H&E stained breast cancer histopathology images. Though the studies provide a promising starting point for automated diagnosis and prognosis of breast cancers, their robustness for everyday breast pathology usage is hampered by the complexity of the tissue characteristics.

Numerous studies have proposed image analysis methods for breast cancer histopathology images to tackle the problems of object detection, automated diagnosis and prognostic scoring. However most of the methods are developed and tested on small and private datasets obtained from a particular center which enforce straightforward performance comparison of these methods. Only recently have publicly available annotated breast histopathology image datasets for mitotic detection, nuclear pleomorphism assessment and tubule detections been prepared and disclosed in certain grand challenge websites. A meaningful performance comparison of these methods can only be performed by building a unified benchmark dataset, medically validated and obtained from large cohorts of patients.

Another issue to be addressed is the non-standardization in tissue preparation, staining and imaging processes adopted at the laboratories which causes great differences in appearance of these dataset images. This requires development of image analysis techniques robust enough to withstand such differences and produce reliable results. Nevertheless with digital pathology taking its roots in pathology laboratories via the usage of WSI scanners, which have obtained pre-market clearance for research purposes in the course of waiting for FDA approval, standardization in tissue preparation and imaging technique will be of great need.

One more prospective and promising avenue in breast cancer histopathology image analysis research lies in studies related to integration of the quantitative image analysis applications in a routine clinical breast cancer management algorithms and workflow. In addition investigations can be performed on image analysis complementing other data types such as genomic data or molecular assays. As of date, most methods have been developed to work in the ROI rather than a entire slide. Working with a slide requires developing image analysis methods for multi-scale or multi-field of view framework which has been addressed in very few studies. Furthermore, improvements should be made to the image analysis methods by incorporating advanced machine vision techniques, thus increasing their performance and accuracy to levels that meet clinical standards.

10. ACKNOWLEDGEMENT

The authors would like to acknowledge the support provided by the DST-FIST computing facility at the Department of Mathematics, Madras Christian College.

11. REFERENCES

1. C. W. Elston and I. O. Ellis, "Pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: experience from a large study with long-term follow-up. ," *Histopathology,* vol. 41, pp. 151-151, 2002.
2. J. Ferlay, I. Soerjomataram, M. Ervik, R. Dikshit, S. Eser, C. Mathers, M. Rebelo, D. M. Parkin, D. Forman, and F. Bray. (2013, 16/3/2015). GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11. Available: http://globocan.iarc.fr
3. P. D. Robbins, S. Pinder, N. De Klerk, H. Dawkins, J. Harvey, G. F. Sterrett, I. Ellis, and C. W. Elston, "Histological grading of breast carcinomas: a study of interobserver agreement," *Human Pathology,* vol. 26, pp. 873-879, 1995.
4. G. A. Meijer, J. A. Beliën, P. J. van Diest, and J. P. Baak, "Origins of ... image analysis in clinical pathology," *Journal of Clinical Pathology,* vol. 50, pp. 365-370, 1997.
5. W. H. Wolberg, W. N. Street, D. M. Heisey, and O. L. Mangasarian, "Computer-derived nuclear" grade" and breast cancer prognosis," *Analytical and Quantitative Cytology and Histology,* vol. 17, pp. 257-264, 1995.
6. R. S. Weinstein, A. R. Graham, L. C. Richter, G. P. Barker, E. A. Krupinski, A. M. Lopez, K. A. Erps, A. K. Bhattacharyya, Y. Yagi, and J. R. Gilbertson, "Overview of telepathology, virtual microscopy, and whole slide imaging: prospects for the future," vol. 40, pp. 1057–1069, 2009.
7. C. V. Hedvat, "Digital Microscopy: Past, Present, and Future.," *Archives of Pathology & Laboratory Medicine,* vol. 134, pp. 1666-1670., November 2010 2010.
8. L. Pantanowitz., P. N. Valenstein., A. J. Evans., K. J. Kaplan., J. D. Pfeifer., D. C. Wilbur., L. C. Collins., and T. J. Colgan., "Review of the current state of whole slide imaging in pathology.," *J Pathol Inform* . vol. 2, 2011.
9. P. S., P. L., and P. AV., "Digital imaging in pathology.," *Clin Lab Med.,* vol. 32, pp. 557-84, Dec 2012 2012.

10. M. N. Gurcan, L. Boucheron, A. Can, A. Madabhushi, N. Rajpoot, and B. Yener, 147–171. , "Histopathological Image Analysis: A Review. ," *IEEE Reviews in Biomedical Engineering,,* vol. 2, pp. 147–171, 2009.

11. A. D. Belsare and M. M. Mushrif, "Histopathological Image Analysis Using Image Processing Techniques: An Overview," *Signal & Image Processing,* 2012.

12. M. Veta, J. P. W. Pluim, P. J. van Diest, and M. A. Viergever, "Breast Cancer Histopathology Image Analysis: A Review," *Biomedical Engineering, IEEE Transactions on,* vol. 61, pp. 1400-1411, 2014.

13. F. Bray, J.-S. Ren, E. Masuyer, and J. Ferlay, "Global estimates of cancer prevalence for 27 sites in the adult population in 2008," *International Journal of Cancer,* vol. 132, pp. 1133-1145, 2013.

14. H. F. J. Frierson, R. A. Wolber, K. W. Berean, D. W. Franquemont, M. J. Gaffey, J. C. Boyd, and D. C. Wilbur, "Interobserver reproducibility of the Nottingham modification of the Bloom and Richardson histologic grading scheme for infiltrating ductal carcinoma.," *Am J Clin Pathol.,* vol. 103, pp. 195-198, 1995.

15. J. M. Bueno-de-Mesquita, D. S. A. Nuyten, J. Wesseling, H. v. Tinteren, S. C. Linn, and M. J. v. d. Vijver, "The impact of inter-observer variation in pathological assessment of node-negative breast cancer on clinical risk assessment and patient selection for adjuvant systemic treatment," *Ann Oncol* vol. 21, pp. 40-47., 2010.

16. W. H. Wolberg., Nick, and O. L. Mangasarian., "Importance of Nuclear Morphology in Breast Cancer Prognosis. ," *Clin Cancer Res,* vol. 5, pp. 3542-3548, 1/11/1999 1999.

17. B. Dunne and J. J. Going, "Scoring nuclear pleomorphism in breast cancer," *Histopathology,* vol. 39, pp. 259-265, 2001.

18. L. Medri, A. Volpi, O. Nanni, A. M. Vecci, A. Mangia, F. Schittulli, F. Padovani, D. C. Giunchi, A. Vito, D. Amadori, A. Paradiso, and R. Silvestrini, "Prognostic Relevance of Mitotic Activity in Patients with Node-Negative Breast Cancer," *Mod Pathol,* vol. 16, pp. 1067-1075, 0000.

19. P. J. van Diest, E. van der Wall, and J. P. A. Baak, "Prognostic value of proliferation in invasive breast cancer: a review," *Journal of Clinical Pathology,* vol. 57, pp. 675-681, 2004.

20. M. Macenko, M. Niethammer, J. S. Marron, D. Borland, J. T. Woosley, G. Xiaojun, C. Schmitt, and N. E. Thomas, "A method for normalizing histology slides for quantitative analysis," in *2009 IEEE International Symposium on Biomedical Imaging: From Nano to Macro*, 2009, pp. 1107-1110.

21. M. Niethammer, D. Borland, J. S. Marron, J. Woosley, and N. E. Thomas, "Appearance Normalization of Histology Slides," in *Machine Learning in Medical Imaging: First International Workshop, MLMI 2010, Held in Conjunction with MICCAI 2010, Beijing, China, September 20, 2010. Proceedings*, F. Wang, P. Yan, K. Suzuki, and D. Shen, Eds., ed Berlin, Heidelberg: Springer Berlin Heidelberg, 2010, pp. 58-66.

22. E. Reinhard, M. Ashikhmin, B. Gooch, and P. Shirley, "Color Transfer between Images," *IEEE Comput. Graph. Appl.,* vol. 21, pp. 34-41, 2001.

23. A. Can, M. Bello, H. E. Cline, T. Xiaodong, F. Ginty, A. Sood, M. Gerdes, and M. Montalto, "Multi-modal imaging of histological tissue sections," in *2008 5th IEEE International Symposium on Biomedical Imaging: From Nano to Macro*, 2008, pp. 288-291.

24. S. Raja, Alomari, R. Allen, B. Sabata, and V. Chaudhary, "Localization of Tissues in High Resolution Digital Anatomic Pathology Images," 2009.

25. C. Bahlmann, A. Patel, J. Johnson, J. Ni, A. Chekkoury, P. Khurd, A. Kamen, L. Grady, E. Krupinski, A. Graham, and R. Weinstein, "Automated detection of diagnostically relevant regions in H&E stained digital pathology slides," 2012, pp. 831504-831504-8.

26. N. Linder, J. Konsti, R. Turkki, E. Rahtu, M. Lundin, S. Nordling, C. Haglund, T. Ahonen, M. Pietikäinen, and J. Lundin, "Identification of tumor epithelium and stroma in tissue microarrays using texture analysis," *Diagnostic Pathology,* vol. 7, pp. 1-11, 2012.

27. M. Peikari, J. Zubovits, G. Clarke, and A. Martel, "A texture based approach to automated detection of diagnostically relevant regions in breast digital pathology," in *International Conference on Medical Imaging Computing and Computer Assisted Intervention — Workhop Breast Image Anal.,* , 2013.

28. S. Khandpur, "Editorial view point," *Journal of Pathology Informatics,* vol. 4, pp. 1-1, 2013.

29. H. Irshad, A. Veillard, L. Roux, and D. Racoceanu, "Methods for Nuclei Detection, Segmentation, and Classification in Digital Histopathology: A Review—Current Status and Future Potential," *Biomedical Engineering, IEEE Reviews in,* vol. 7, pp. 97-114, 2014.

30. E. Cosatto., M. Miller., H. P. Graf., and J. S. Meyer., "Grading nuclear pleomorphism on histological micrographs.," presented at the 19th Int. Conf. Pattern Recog., 2008.

31. X. Qi., F. Xing., D. J. Foran., and L. Yang., "Robust segmentation of overlapping cells in histopathology specimens using parallel seed detection and repulsive level set.," *IEEE Trans. Biomed. Eng.,* vol. 59, pp. 754 -765, 2012.

32. M. Paramanandam, R. Thamburaj, M. Manipadam, and A. Nagar, "Boundary Extraction for Imperfectly Segmented Nuclei in Breast Histopathology Images – A Convex Edge Grouping Approach," in *Combinatorial Image Analysis*. vol. 8466, R. Barneva, V. Brimkov, and J. Šlapal, Eds., ed: Springer International Publishing, 2014, pp. 250-261.

33. M. Veta., P. J. V. Diest., R. Kornegoor., A. Huisman., M. A. Viergever., and J. P. W. Pluim, "Automatic Nuclei Segmentation in H&E Stained Breast Cancer Histopathology Images.," vol. 8, July 29, 2013.

34. H. Fatakdawala., J. Xu., A. Basavanhally., G. Bhanot., S. Ganesan., M. Feldman., J. E. Tomaszewski., and A. Madabhushi., "Expectation maximization-driven geodesic active contour with overlap resolution (EMaGACOR): Application to lymphocyte segmentation on breast cancer histopathology.," *IEEE Trans. Biomed. Eng.,* vol. 57, pp. 1676– 1689, Jul. 2010. 2010.

35. S. Ali. and A. Madabhushi., "An integrated region-, boundary-, shapebased active contour formultiple object overlap resolution in histological imagery.," *IEEE Trans. Med. Imag.,* vol. 31, pp. 1448-1460, Jul 2012 2012.

36. E. J. Kaman, A. W. M. Smeulders, P. W. Verbeek, I. T. Young, and J. P. A. Baak, "Image processing for mitoses in sections of breast cancer: A feasibility study," *Cytometry,* vol. 5, pp. 244-249, 1984.

37. L. Roux., D. Racocean., N. Lomenie., M. Kulikova., H. Irshad., J. Klossa., F. Capron., C. Genestie., G. L. Naour., and M. N. Gurcan., "Mitosis detection in breast cancer histological images an ICPR 2012 contest," *J. Pathol. Inform.,* vol. 4, pp. 8-14, 2013.

38. M. Veta, P. J. van Diest, S. M. Willems, H. Wang, A. Madabhushi, A. Cruz-Roa, F. Gonzalez, A. B. L. Larsen, J. S. Vestergaard, A. B. Dahl, D. C. Cire°an, J. Schmidhuber, A. Giusti, L. M. Gambardella, F. B. Tek, T. Walter, C.-W. Wang, S. Kondo, B. J. Matuszewski, F. Precioso, V. Snell, J. Kittler, T. E. de Campos, A. M. Khan, N. M. Rajpoot, E. Arkoumani, M. M. Lacle, M. A. Viergever, and J. P. W. Pluim, "Assessment of algorithms for mitosis detection in breast cancer histopathology images," *Medical Image Analysis,* vol. 20, pp. 237-248, 2015.

39. D. C. Ciresan., A. Giusti., L. M. Gambardella., and J. Schmidhuber. , "Mitosis detection in breast cancer histology images with deep neural networks," in *Med. Image Comput. Comput.-Assist. Interv.*, Nagoya, Japan, 2013, pp. 411-418.

40. L. Roux, D. Racoceanu, N. Loménie, M. Kulikova, H. Irshad, J. Klossa, F. Capron, C. Genestie, G. Le Naour, and M. N. Gurcan, "Mitosis detection in breast cancer histological images An ICPR 2012 contest," *Journal of Pathology Informatics,* vol. 4, p. 8, 2013.

41. "Mitos-Atypia-14. Mitos & Atypia: detection of mitosis and evaluation of nuclear atypia score in breast cancer histological images.," in *22nd International Conference on Pattern Recognition*, 2014.

42. Y. Peng, Y. Jiang, L. Eisengart, M. A. Healy, F. H. Straus, and X. J. Yang, "Segmentation of prostatic glands in histology images," in *2011 IEEE International Symposium on Biomedical Imaging: From Nano to Macro*, 2011, pp. 2091-2094.

43. J. Xu, A. Janowczyka, S. Chandranb, and A. Madabhushia, "A weighted mean shift, normalized cuts initialized color gradient based geodesic active contour model: applications to histopathology image segmentation," in *SPIE Medical Imaging*, 2010.

44. A. Basavanhally, E. Yu, J. Xu, S. Ganesan, M. Feldman, J. Tomaszewski, and A. Madabhushi, "Incorporating domain knowledge for tubule detection in breast histopathology using O'Callaghan neighborhoods," in *SPIE Medical Imaging*, 2011.

45. P. Maqlin, R. Thamburaj, J. J. Mammen, and A. K. Nagar, "Automatic Detection of Tubules in Breast Histopathological Images," in *Proceedings of Seventh International Conference on Bio-Inspired Computing: Theories and Applications (BIC-TA 2012): Volume 2*, C. J. Bansal, P. Singh, K. Deep, M. Pant, and A. Nagar, Eds., ed India: Springer India, 2013, pp. 311-321.

46. J.-M. Chen, A.-P. Qu, L.-W. Wang, J.-P. Yuan, F. Yang, Q.-M. Xiang, N. Maskey, G.-F. Yang, J. Liu, and Y. Li, "New breast cancer prognostic factors identified by computer-aided image analysis of HE stained histopathology images," *Scientific Reports,* vol. 5, p. 10690, 2015.

47. S. Petushi, F. U. Garcia, M. M. Haber, C. Katsinis, and A. Tozeren, "Large-scale computations on histology images reveal grade-differentiating parameters for breast cancer," *BMC Medical Imaging,* vol. 6, pp. 1-11, 2006.

48. M. Tambasco and A. M. Magliocco, "Relationship between tumor grade and computed architectural complexity in breast cancer specimens," *Human Pathology,* vol. 39, pp. 740-746.

49. M. Tambasco, M. Eliasziw, and A. M. Magliocco, "Morphologic complexity of epithelial architecture for predicting invasive breast cancer survival," *Journal of Translational Medicine,* vol. 8, pp. 140-140, 2010.

50. A. H. Beck, A. R. Sangoi, S. Leung, R. J. Marinelli, T. O. Nielsen, M. J. van de Vijver, R. B. West, M. van de Rijn, and D. Koller, "Systematic Analysis of Breast Cancer Morphology Uncovers Stromal Features Associated with Survival," *Science Translational Medicine,* vol. 3, pp. 108ra113-108ra113, 2011-11-09 00:00:00 2011.

51. Y. Yuan, H. Failmezger, O. M. Rueda, H. R. Ali, S. Gräf, S.-F. Chin, R. F. Schwarz, C. Curtis, M. J. Dunning, H. Bardwell, N. Johnson, S. Doyle, G. Turashvili, E. Provenzano, S. Aparicio, C. Caldas, and F. Markowetz, "Quantitative Image Analysis of Cellular Heterogeneity in Breast Tumors Complements Genomic Profiling," *Science Translational Medicine,* vol. 4, pp. 157ra143-157ra143, 2012-10-24 00:00:00 2012.