Indian Journal of Science and Technology, *Vol 8(23), DOI: 10.17485/ijst/2015/v8i23/85321, September 2015*
ISSN (Print) : 0974-6846
ISSN (Online) : 0974-5645

Analysis of Nuclei Detection with Stain Normalization in Histopathology Images

K. Sabeena Beevi* and G. R. Bindu

Department of Electrical Engineering, College of Engineering Trivandrum, Thiruvananthapuram - 695016, Kerala, India; sabeena3000@gmail.com, bgr100@gmail.com

Abstract

This paper proposes a unique detection technique to identify nuclei from microscopy images of breast histopathology slides. The method involves stain normalization, color decomposition and hysteresis thresholding. A comparative analysis of different pre-processed images on nuclei detection reveals an accuracy of 97% for Blue Ratio (BR) images. The technique uses three color channels for accurate detection of nuclei. An analysis of two stain normalization procedure is also presented.

Keywords: Blue Ratio Image, Histopathology, Stain Normalization

1 Introduction

The field of medical image computing develops computational and mathematical methods for solving problems related with medical image and their use for biomedical research and clinical care. Dramatic increase in computational power and advanced digital signal processing techniques leads the development of powerful Computer Aided Diagnostics (CAD) approaches to radiological data. The limited spatial resolutions of standard radiology modalities cause many disease process to appear similar or even in-distinguishable. This limitation can be challenging for the clinical team who seek a definite diagnosis to initiate a definite treatment. To know its underlying cause histopathological examination of permanent sections are essential. Histopathology is the study of signs of disease using microscopic examination of surgical specimen which is processed after a sequence of technical procedures like dehydration, sectioning, staining and then fixed on to glass slides[1]. Manual analysis of histology tissue still remains to be the primary way to identify cancerous tissues and usually done by visual inspection of the slide by pathologists or technologists. Such manual intervention has the disadvantages of being very time consuming for high content screening and being very difficult to grade in a reproducible manner due to variations in observations[2]. Moreover visual inspection gives only subjective results. To provide descriptive and objective information of tissue components with good reliability the process has to be automated and can be used as a diagnostic aid.

Compared with widespread use of computation process in radiology, pathology lags behind in computerized interpretations. With recent advancement of Whole Slide Imagining (WSI) an entire slide can be converted into a single digital image. Histology images are considerably different from radiology images, having large amount of objects of interest widely distributed in the image and surrounded by different neighboring tissues[3]. But in radiology, analysis focuses on one or two organs in the image which are located in similar environments. Moreover histology tissues are generally stained with different colors while radiology contains only gray intensities. Quantitative measurement of tissue properties by automatic

**Author for correspondence*

analysis of 2D histopathology images increases the objectivity and reproducibility of analysis results. The advent of computer aided techniques revolutionized the biomedical microscopy image analysis. Different stages of quantitative analysis include preprocessing, detection, segmentation, feature computation and classification.

Detection of cell nuclei is an initial step for analysis of tissue structure. The shape and size can be irregular even when they are uniformly sectioned[1]. Accuracy of segmentation depends on the reliability of the seed points. There are different approaches reported in the literature to implement automatic detection of nuclei on 2-D histopathology images. Initial works rely on the peaks of Euclidian distance map[4]. H-maxima transform detect local maxima as seed points, but highly sensitive to texture and results in over seeding[5]. In order to identify a cell as normal or malignant, shape and texture of its chromatin has to be considered. Watershed transforms[6] leads to over segmentation and consequently results in sub optimal detection. Cosatto et al.[7] proposed a method which combines Hough transform and Difference of Gaussian (DoG) filter to detect cell nuclei. But it suits only for circular nuclei and require excessive computation. A gamma-gaussian mixture model was proposed[8] for detection of nuclei by isolating tumor region from non-tumor areas. But it requires a context aware post processing step in the classification stage. This paper analyses two different stain normalization techniques in preprocessing stage. Then blue ratio image is formed from decomposed R, G and B planes of the normalized digital image. Detection accuracy with R plane, R with Wiener filter and BR with Laplacian of Gaussian (LOG) are also presented. The proposed method is described in detail in next section. Section III presents results and discussions and Section IV deals with conclusions.

2. Methodology

The technique has mainly three stages, viz., 1. Preprocessing. 2. Color Decomposition. 3. Detection of nuclei locations Figure 1 shows a block schematic of the proposed methodology. The first stage is preprocessing of digital histopathology images where stain normalization of different H&E stained images are carried out. Color decomposition of normalized histology images are next carried out. The detection of nuclear regions is a crucial step since the error resulting from this is transferred to later stages and causes incorrect results in analysis. This paper deals with HPF (High Power Field) images of breast cancer biopsy samples provided by the standard dataset[9]. The resolution of the image given in the dataset is 1376 ×1539 × 3.

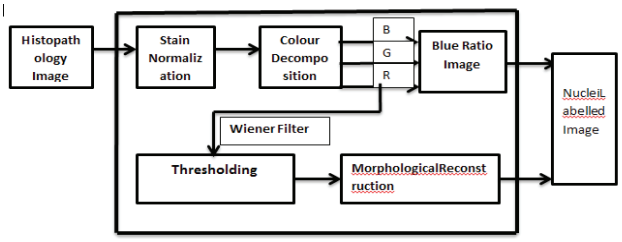
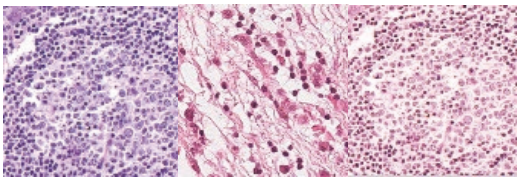


Figure 1. Block schematic of proposed method.

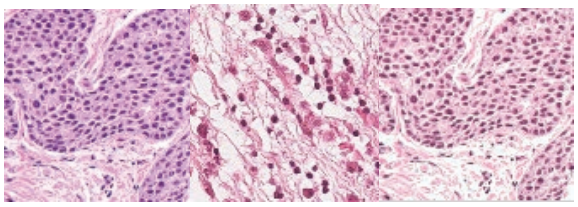
2.1 Pre Processing

One of the first steps essential for microscopy image analysis is color and illumination normalization. Hematoxyline selectively stains nuclei in to a blue-purple hue while eosin stains proteins a bright pink colour. Staining process vary widely due to different stain manufacturers, different staining practices and different storage times. Irregularities in the preparation of histology slides create complications in quantitative analysis of their results. In this paper two mechanisms for disabling many of the inconsistencies in the staining process are evaluated which reduces the differences in tissue samples due to variation in staining and scanning conditions. First one employs color normalization[10] by histogram equalization of the 3 color channels. The visual results for this method are given in Figure 2. Second method[11] is by matching the color distribution of an image to that of reference image by use of a linear transform in a perceptual color model (lab color space). Usually RGB color model is used which is perceptually not a uniform color model. Other more perpetual color models such as HSV Lab and LUV are used. Image results of this are shown in Figure 3.



(a) (b) (c)

Figure 2. Visual results of histogram normalization. (**a**) Input image. (**b**) Reference image. (**c**) Normalized input image.



(a) (b) (c)

Figure 3. Visual Results of Reinhard Normalization. (**a**) Input image. (**b**) Reference image. (**c**) Normalized input image.

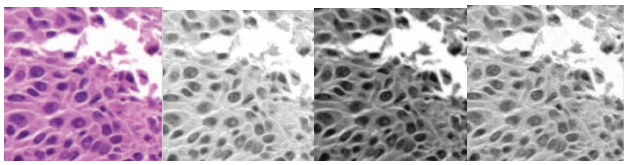
2.2 Color Decomposition

The colour image obtained after stain normalization denoted by I_{RGB} is first decomposed into R, G and B planes as the next step in the detection process.

Here nuclei detection is evaluated in three different planes as shown in Figure 4 and in Figure 5

1. R plane: Cell nuclei are darker in R plane compared to G and B planes. Hence, R image, is selected.
2. R plane filtered with Wiener filter: R image is enhanced with wiener filter for maximum nuclei detection and preserve smooth boundaries as presented by the authors in a previous paper[12].
3. Blue Ratio Image: In a blueratio image, a large pixel value is given to a high blue intensity compared to its red and green components, and a low value is given to a pixel with a low blue intensity. As nuclei appear as bluepurple areas, a blueratio image is an efficient tool to have a first clue on the position of nuclei in the image. LoG responses of BR image discriminate the nuclei region from the background.

$$BR = \left(\frac{100B}{1+R+G}\right)\left(\frac{256}{1+B+R+G}\right) \quad (1)$$

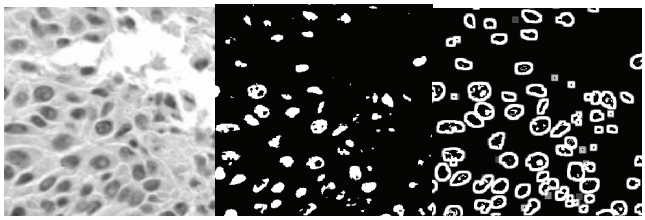


(a) (b) (c) (d)

Figure 4. (**a**) Input image. (**b**) R plane. (**c**) G plane. (**d**) B plane.

2.3 Thresholding and Morphological Reconstruction

In R and R with wiener accurate detection of nuclei is obtained by morphological reconstruction followed by hysteresis thresholding. Two thresholds T_1 and T_2 control the nuclei tracking process. I_{RB} is the binary image of I_R obtained after hysteresis thresholding and can be expressed as in Equation 2. Visual image results obtained after the detection stage are given in Figure 5. The binary image after thresholding and morphological operations is shown in Figure 5(b). The highest value of upper threshold T_1 is set to 0.9 and lowest value of lower threshold T_2 is set to 0.45. Image results are manually verified by an experienced pathologist.



(a) (b) (c)

Figure 5. (**a**) R image. (**b**) After thresholding. (**c**) BR-Log image.

$$I_{RB}(x,y) = \begin{cases} 0, & if I_R(x,y) < T_1 \\ 1, & if I_R(x,y) < T_2 \\ 0, & if T_1 \le I_R(x,y) < T_2 \text{ and } I_R(x,y) \text{ is a 4 neighbour of labelled pixels with label 0} \\ 1, & if T_1 \le I_R(x,y) < T_2 \text{ and } I_R(x,y) \text{ is a 4 neighbour of labelled pixels with label 1} \end{cases} \quad (2)$$

The upper threshold is set to high value and lower threshold to a quite low value for good results. Morphological opening by reconstruction of the binary image is performed at this stage with a disc shaped structuring element. The reconstructed image $\phi(I_{RB})$ can be expressed as follows:

$$\phi(I_{RB}) = I_{RB} - \rho_s(I_m \mid I_{RB}) \quad (3)$$

where $\rho_s(I_m \mid I_{RB})$ is the reconstructor operator with structuring element S and mask image, obtained after the opening process of I_{RB} as:

$$I_m = (I_{RB} \ominus S) \oplus S \quad (4)$$

3. Results and Discussions

Tissue slides from biopsy samples are processed and stored under different staining conditions. Hence it is required to normalise these for further analysis.This paper deals with two such normalizing techniques, so as to improve quantitative analysis of the processed results. Figure 6 shows R plane of a stained image before and after stain normalization. The detection performance is evaluated on 4 dataset of total 300 images of H&E stained HPF (High Power Field) histopathology images of breast biopsy samples. Ground truth regions are manually marked by expert pathologist. The Detected Nuclei regions (DN) are compared with the ground truth regions which are denoted as GT. Corresponding number of True Positives (TP defined as the number of true positives or correct detection) are taken to compute the detection sensitivity, Equation 5.

$$D_{SEN} = \frac{|GT \cap DN|}{|GT|} \times 100 \quad (5)$$

The actual count of nuclei in the three detection stages considered 1. R, 2. R with wiener and 3. Blue Ratio image is shown in Table 1 and detection sensitivity is compared as in Table 2. It is clear from the analysis that combining Wiener filtering with hysteresis thresholding improves detection accuracy compared with R plane. The process of nuclei detection is a complex task due to large size of the image, staining artefacts and irregular shaped nuclei. But the implementation of the proposed technique greatly reduces the complexity of the process, yet retaining high accuracy by reducing the number of colour channels. For the proposed technique all nuclear regions are correctly detected in BR image with an average accuracy of 97%.

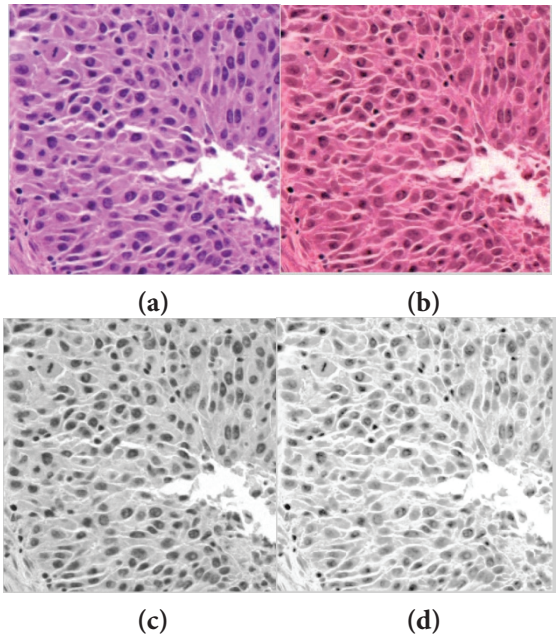


Figure 6. R plane image before and after color normalization.(**a**) Input image. (**b**) Normalized image. (**c**) R plane of original image. (**d**) R plane of normalized image.

Table 1 provides the count of nuclei on images in each data set (on an average) detected using the proposed method; as compared to manual detections. It is clear that the number of nuclei detected in blue ratio image is better than the other two planes. This clearly convinces the improved detection performance of blue ratio images. This is also evident from the table of percentage detection sensitivity shown in Table 2. Figure 7 shows a graphical representation of detected number of nuclei in 4 test set images for the three planes considered.

Table 1. Detection performance (Count of Nuclei)

Image set	Manual Detection (No)	R Plane (No)	R with Wiener (No)	Blue Ratio Image (No)
1	243	233	235	238
2	281	269	272	275
3	310	294	297	300
4	792	752	760	776

Table 2. Detection performance (Detection Sensitivity)

Dataset 1 (D_{SEN} in %)			Dataset 2 (D_{SEN} in %)			Dataset 3 (D_{SEN} in %)			Dataset 4 (D_{SEN} in %)		
R	RW	BR	R	RW	BR	R	RW	BR	R	RW	BR
95	96	97	95	96	97	94	95	96	94	95	97

4. Conclusion

An automatic detection technique for cell nuclei in breast histopathology images is described. Due to the variation in size, shape and texture along with staining artefacts, detectionof nucleibecome a challenging process. The paper focuses on analysis of twot stain normalization techniques viz. the histogram equalization technique and Rein hard normalization. The improvement in detection accuracy with blue ratio images is established with the help of quantitative measures. Reinhard normalization gives good visual results in stain normalization. With blue ratiomaximum number of nuclei can be correctly

located. The results obtained are comparable withother state-of-the-art techniques reported in the literature.

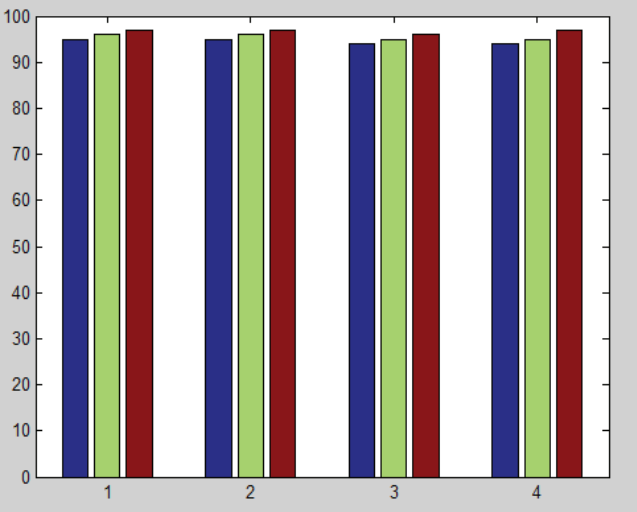


Figure 7. Detection sensitivity in R, R with Wiener and BR image.

5. Acknowledgment

The authors acknowledge the help and support rendered for this work by pathologists of KIMS Hospital, Kollam.

6. References

1. He L. Computer Assisted Diagnosis. National Institutes of Health. USA.
2. Gurcan MN, Boucheron LE. Histopathological image analysis: A review. IEEE Reviews in Biomedical Engineering. 2009; 2:147–71.
3. Andrion, Magnani C, Betta PG. Malignant Mesothelioma of the pleura: Inter-observer variability. Journal of Clinical Pathology. 1995; 48(9):856–60.
4. Al-Kofahi Y, Lassoued W. Improved automatic detection and segmentation of Cell Nuclei in Histopathology Images. IEEE Transactions on Biomedical Engineering. 2010; 57(4):630–45
5. Dalle JR, Li H, Huang CH, Leow WK, Racoceanu D, Putti TC. Nuclear pleomorphism scoring by selective cell nuclei detection. Proceedings of IEEE Workshop Applications of Computer Vision; 2009.
6. Wahlby C, Sintorn IM, Erlandsson F, Borgefors G, Bengtsson E. Combining intensity, edge and shape information for 2D and 3D segmentation of cell nuclei in tissue sections. J Microsc. 2004; 215(1):67–76.
7. Cosatto E, Miller M. Grading nuclear pleomorphism on histological micrographs. 19th International Conference on Pattern Recognition; 2008 Dec. p. 1-4
8. Khan M, Rajpooth NM. A Gamma-Gaussian mixture model for detection on mitotic cells in breast histology images. Proceedings of 21st International Conference on Pattern Recognition; 2012 Oct.
9. Roux L, Daniel R. Mitosis detection in breast histological images. An ICPR 2014 contest. 2014. Available from: http//ipal.cnfr.fr/ICPR
10. Jain A. Fundamentals of digital image processing. Prentice-Hall; 1989.
11. Reinhard E, Adhikhmin M, Gooch B, Shirley P. Color transfer between images. IEEE Computer Graphics and Applications. 2001; 21(5):34–41.
12. Beevi KS, Bindu GR, Nair SM. Automatic segmentation and classification of mitotic cell nuclei in histopathology images based on active contour model. Proceedings of IEEE International Conference on Contemporary Computing (IC3I); 2014. p. 740–744.