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Type P63 Digitized Color Images Performs Better Identification for Ovarian Tissue Analysis

T. M. Shahriar Sazzad[1,*], L.J.Armstrong[1], A.K.Tripathy[1, 2 1]
Edith Cowan University, WA, Australia
{t.sazzad, l.armstrong}c@ecu.edu.au
[2]Don Bosco Institute of Technology, Mumbai, India
amiya@dbit.in

Abstract. Pathology experts use microscopic biopsy slides for routine examination of different tissues especially ovarian tissues. Substantial amount of processing time is required for this type of manual identification process. Ultrasound is considered as most popular electronic scanning modalities for cancer tissue analysis rather than ovarian tissue analysis. For smaller tissue analysis a more suitable option is to use a computer based approach as it can reduce processing time and effort while increasing the accuracy rate. In this paper a complete review and analysis has been carried out on existing available approaches and a new modified approach has been presented. Comparative results indicate an acceptable accuracy and identification rate.

Keywords: histopathology; color digitized microscopic image; image artifacts; mean shift; region fusion; cluster; ovarian reproductive tissues.

1 Introduction

According to medical experts, women who do not conceive child before the age of 35 commonly face conceiving complications and expert medical consultation is often necessary. As part of the standard medical consultation process routine examination in the laboratory is required before prescribing any treatment. There are various ways to perform the routine examination process and among them ultrasound is considered as one of the most popular approach. The limitation of ultrasound is that it can only analyze large and more mature tissues and an expert interpretation is also essential for identification [1]. At present, there is no suitable approach which can accurately identify small NGFs (Non-Growing Follicles) using the ultrasound scanning device. Most appropriate approach to date is the manual microscopic identification approach for both large and small NGFs in the histopathology laboratory.

Manual microscopic biopsy slide analysis is considered as the “gold standard” in the laboratory [2]. The limitation of this “gold standard” method is that it is time consuming and has accuracy errors between experts [3]. To overcome the issues associated with microscopic approach computer based analysis approaches are more reliable [3, 4].

Generally tissues are colorless and hard to distinguish from one another [5]. Study of [5] mentioned that dyes or colored organic substances are used by experts in the laboratory during slide preparation due to the fact that dyes or colored chemicals changes tissue colors which assist experts to analyze tissues easily [5]. Different types of microscopic biopsy slides available in the pathology laboratory [6] and type P63 is one of the available slides. Type P63 has two different types which include non-counter stained (tissue stained without eosin or haematoxylin) and counter-stained (tissue stained with eosin or haematoxylin).

Among all available slides Haematoxylin and Eosin (H&E) is considered as most commonly used slide [7]. The main limitation of type H&E is it contains intensity variations [8]. Additionally, type H&E does not perform better identification for small tissues [9, 10]. In compare to H&E, PCNA (Proliferating Cell Nuclear Antigen) performs better identification [7]. Research work by [11] also suggested that type P63 could be a better choice for ovarian tissue analysis.

2 Related Work

Existing research works [1, 7, 12] related to ovarian tissue analysis are mainly semi-automated rather than an automated. Additionally most works are based on animal tissues [6]. At present, there is no research work has been carried out on human ovarian tissues using type H&E [6]. One research work carried out using type PCNA by [11], one research work by [6] using type P63 non-counter stained (100x magnification), one research work by [13] using type P63 counter stained (100x magnification) and a comparative analysis of both counter and non-counter stained was carried out by [14]. An example of type PCNA, type P63 non-counter-stained and counter-stained digitized images of 100x magnification are shown in Fig. 1. (a)

Currently, all existing research works using type P63 [6, 13, 14] are automated. Proposed work by [11] using type PCNA is not fully automated due to the fact that calibration of processing parameters are essential for a new set of images.

Conventional threshold based approach was implemented by [11] where type PCNA images were used. Type P63 images were used by [6, 13, 14] where image artifact issues were considered. Additionally, study of [13, 14] incorporated some classification approaches as well.

All other existing automated approaches [1, 15, 16] are mainly for cancer cell or tumor cell analysis.

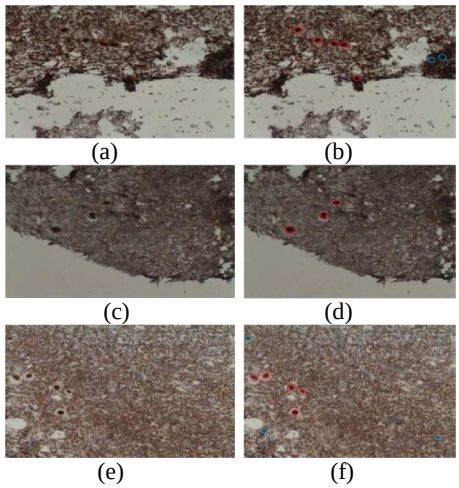


Figure 1. (a) indicates type PCNA, (b) indicates annotated image for (a), (c) indicates type P63 non-counter stained image, (d) indicates annotated image for (b), (e) indicates P63 counter stained image, (f) indicates annotated image of (e). Red marked regions are confirmed nucleus identified by 2 experts and blue marked regions are confirmed by at-least 1 expert.

3 Proposed Method

A new modified automated approach has been presented in this study. Additionally, comparison has been carried out for type PCNA, type P63 non-counter and counter stained digitized color images. A detailed flowchart of the proposed method for this study is shown in Fig.2.

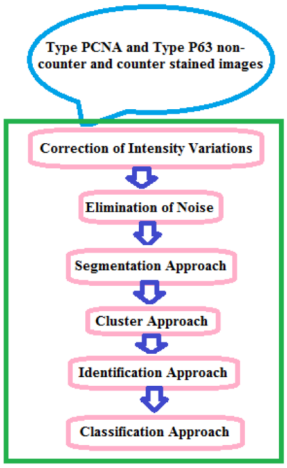


Fig. 2. Block diagram for automated identification process

3.1 Correcting Image Artifacts

Experts use color chemicals in the laboratory during biopsy slide preparation which cause intensity variations [5].

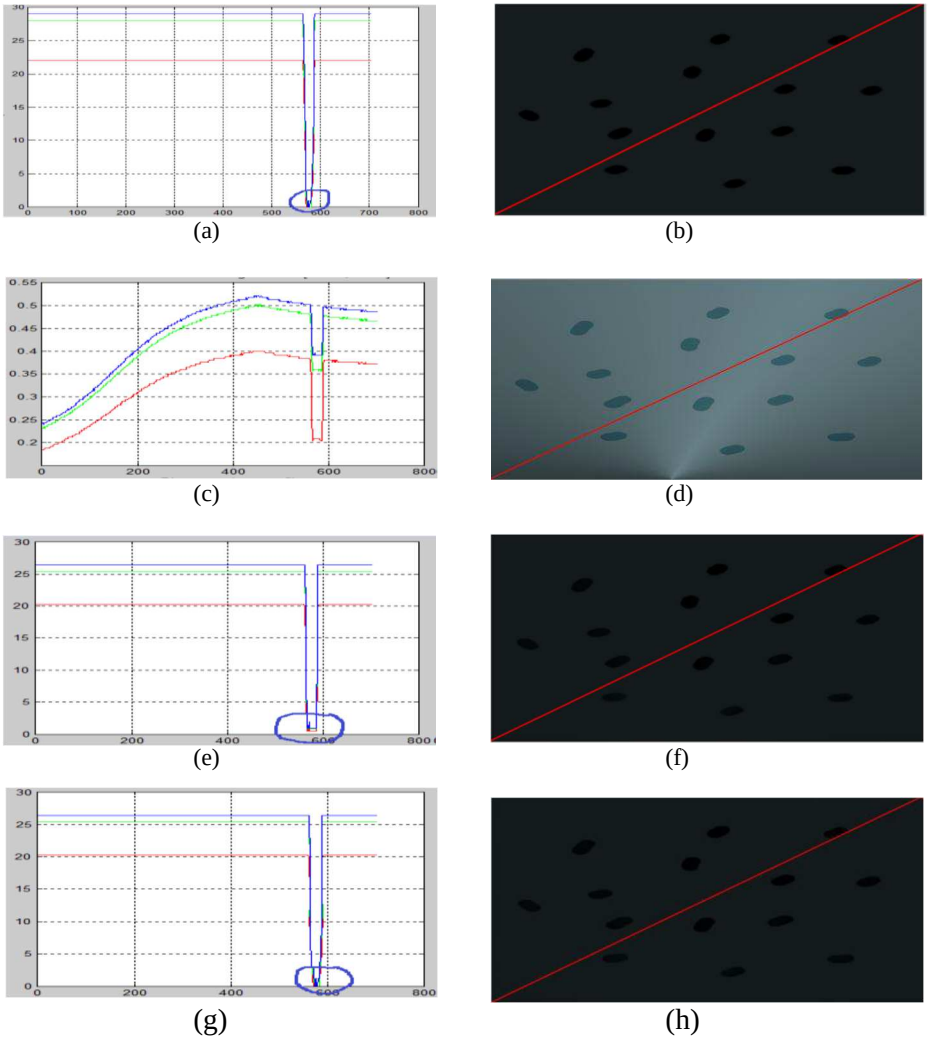
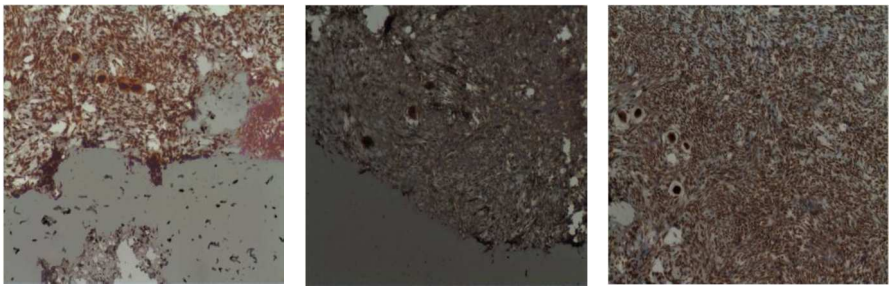


Figure 3. (a) is the original image used from [6], (b) indicates intensity graph across the red line for image (a), (c) is the image with illumination issues and (d) indicates intensity graph across the red line for image (c). (e) indicates corrected image proposed by [6] with intensity graph (f). (g) indicates corrected image of this research study modified proposed and (h) indicates the intensity graph. Intensity graph (f) indicates little dissimilarity in blue circled area but for (h) that area is almost similar to (b).

Therefore; it is necessary to correct intensity variations. Research study by [6] mentioned that morphological operation provides more suitable results among all available techniques. Work of [13, 14] also incorporated and proposed modified morphological operation of [6]. Further modification has been carried out in this research study where microscopic eye piece magnification, image magnification and cell diameter were considered to compute the disk radius for morphological operation. This new proposed modified approach was able to provide improved result shown in Fig. 3. Research study test image results are shown in Fig. 4.

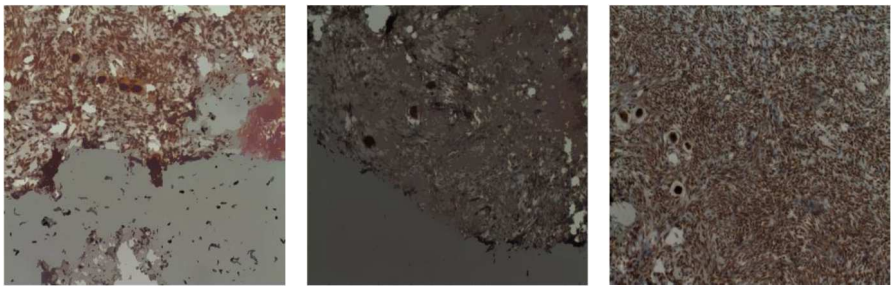


(a) (b) (c)

Figure 4. (a) indicates type PCNA, (b) indicates P63 non-counter stained and (c) indicates P63 counter-stained corrected images using this study proposed modified approach

3.2 Filter Operation

Among all existing ovarian tissue analysis approaches, median and pixel-based mean-shift filters were used where [6] proposed pixel-based mean-shift filter approach. Study of [13, 14] have incorporated the approach of [6] which was found to provide satisfactory results for type P63. Research work of [11] incorporated median filter which did not provide satisfactory result mentioned by [6]. Therefore; for this research study pixel based mean-shift filter approach was used. The results are shown in Fig. 5.



(a) (b) (c)

Figure 5. (a) indicates type PCNA, (b) indicates P63 non-counter stained and (c) indicates P63 counter-stained filtered images using [6]

3.3 Color Segmentation

For gray-scale image processing threshold and watershed based segmentation approaches are useful but not suitable for color images [11]. The modified region fusion approach proposed by [6] has shown to provide satisfactory results. Modification has been carried out by [13] to improve the results. Further modification of region fusion approach is reported in this research study.

Study of [13] has considered RGB image but in this research study RGB image was divided in 3 different channels (R,G,B). Each channel was indexed to perform the region merging operation. To minimize the standard deviation error this study followed the approach of [6]. Segmentation results are shown in Fig. 6.

(a) (b) (c)

Figure 6. (a) indicates type PCNA, (b) indicates P63 non-counter stained and (c) indicates P63 counter-stained segmented images using this study proposed approach.

3.4 Cluster Approach

Mean shift modified clustering approach proposed by [6] is a suitable approach as it does not require any predefined parameter and shown to provide satisfactory result mentioned by [13, 14]. For this research study clustering approach of [6] was incorporated.

3.5 Identify Ovarian Nucleus

For this research study same features information that were used by [6, 11, 13, 14] for the identification of ovarian nucleus regions were incorporated while eliminating border touching regions [6]. Fig. 7 indicates the identified results for this research study proposed approach

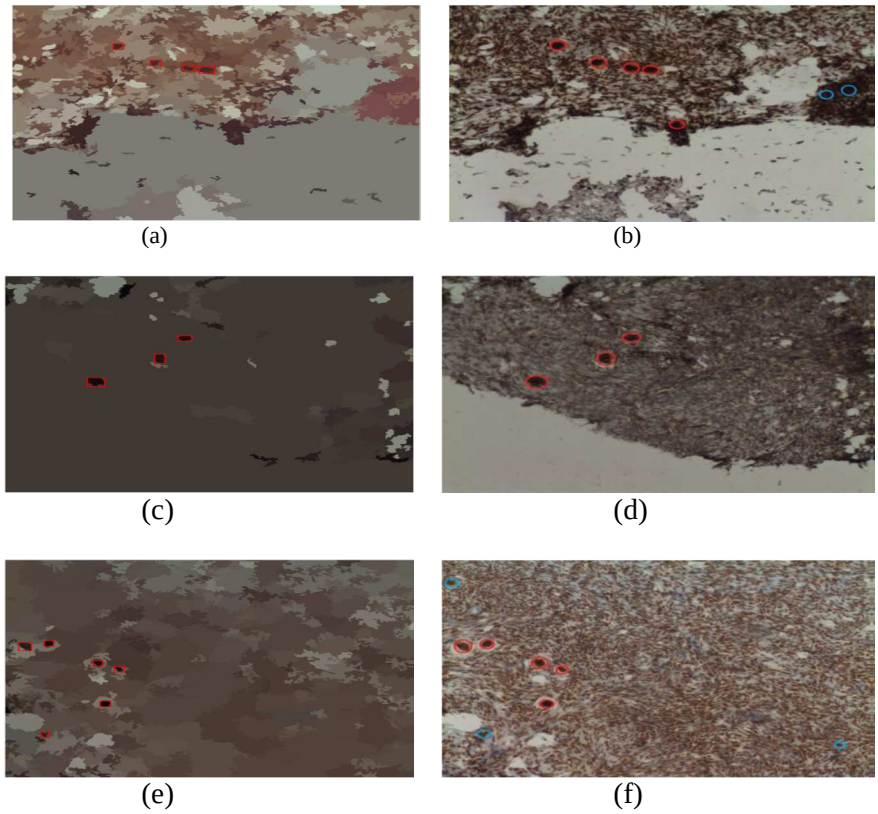


Figure 7. (a) indicates identified regions for PCNA image using this study proposed approach, (b) indicates annotated result from experts. (c) indicates identified regions for P63 non-counter stained images using this study proposed approach, (d) indicates annotated result from experts. (e) indicates identified regions for P63 counter stained images using this study proposed approach, (f) indicates annotated result from experts. One region missed in (a) for type PCNA in comparison with (b). No region missed for type P63 non-counter and counter stained images. No false region identified for any types.

3.6 Classify Identified Regions to Improve Accuracy

Although the indentified results indicates satisfactory results shown in Table II however; a suitable classifier would be a viable option to test the identified regions to improve accuracy. Study of [6, 11] did not apply any classification approach but study by [13] incorporated 3 most popular classifiers (SVM, K-NN and P-NN) from which SVM classifier was most accurate. Table 1 shows classification accuracy for this research study test images.

TABLE I. CLASSIFICATION ACCURACY TEST USING SVM CLASSIFIER

Name	Stain Type	150 images were used (50 in each group)			Accuracy %	
		Group 1	Group 2	Group 3		
SVM	PCNA	Training	Test	Test	90	90
		Test	Training	Test	89	
		Test	Test	Training	91	
	P63 (non-counter)	Training	Test	Test	96	95
		Test	Training	Test	98	
		Test	Test	Training	97	
	P63 counter	Training	Test	Test	96	97
		Test	Training	Test	98	
		Tesz	Test	Training	97	

4 Experimental Results

Table II indicates that this study proposed modified approach has an improved accuracy over 90% for type PCNA and type P63 which maintains “gold standard” criteria [11].

Study proposed method by [11] do not satisfy “gold standard” criteria as the accuracy rate was found under 80% for all types of images as shown in Table III. Other approaches [6, 13, 14] provides satisfactory results and among all approaches this study proposed approach has the most accuracy rate shown in Table III.

TABLE II. IDENTIFICATION RESULT FOR TYPE PCNA, TYPE P63 (NON-COUNTER AND COUNTER STAINED) IMAGES

Number of test images PCNA (403), P63 non-counter stain (493), P63 counter stain (475)	Image Type	Accuracy Rate (%)
Proposed method	PCNA	90.5
	P63 (non-counter stain)	95.5
	P63 (counter stain)	96.5

5 Discussion and Conclusion

Existing automated computerized approaches for ovarian tissue analysis were reviewed in this research study. All automated cancer cells identification approaches were not considered due to the fact that cancer tissues are different from ovarian reproductive tissues.

Research study test image results indicates that type P63 digitized color images performed better identification for ovarian tissue analysis in compare to type PCNA. This is a novel study due to the fact that this is the first published study where type PCNA and type P63 digitized images were compared for ovarian tissue identification.

Additionally, a significant amount of type PCNA and type P63 (non-counter and counter) stained images were used for comparison and analysis purposes. If expert observation variability can be minimized then it would be possible to increase the accuracy rate.

TABLE III. COMPARATIVE RESULT FOR ALL AUTOMATED APPROACHES

Number of test images PCNA (403), P63 non-counter stain (493), P63 counter stain (475)	Image Type	avg. processing time (sec)	Precision	Recall
Proposed method	PCNA	24.37	0.91	0.90
	P63 (non-counter stain)	21.76	0.95	0.96
	P63 (counter stain)	22.21	0.96	0.97
Automated approach [13]	PCNA	24.73	0.89	0.90
	P63 (non-counter stain)	22.30	0.95	0.955
	P63 (counter stain)	22.83	0.96	0.965
Automated approach [6]	PCNA	24.55	0.90	0.89
	P63 (non-counter stain)	22.57	0.96	0.95
	P63 (counter stain)	23,02	0.955	0.965
Automated approach [11]	PCNA	26.80	0.79	0.74
	P63 (non-counter stain)	24,10	0.75	0.73
	P63 (counter stain)	24.46	0.76	0.77
Automated approach [14]	PCNA	24.37	0.875	0.91
	P63 (non-counter stain)	22,12	0.945	0.96
	P63 (counter stain)	22.60	0.96	0.96

6 Acknowledgement and Future Work

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