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Cervical Smear Screening Using Cost-effective Liquid Based Cytology

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Abstract

Objective: To minimise false negativity in cervical cancer screening with Papanicolaou (Pap) test, there is a need to explore novel cytological technique and identification of unique and important cellular features.
Method: The present study explores the feasibility of low cost cervical monolayer in comparison to conventional Pap smear and available liquid based cytology techniques in extracting cytopathological features to classify normal and abnormal conditions. Comparative efficacy of newly developed technique to that of commercially prepared one was validated by automated cellular image analysis.
Result: The results show that newly developed monolayer technique for cervical smears was cost effective, capable of better cyto-pathological evaluation and also compatible for automation. The precision and sensitivity were remarkably improved for nucleus and cytoplasm using cost effective monolayer in comparison to other available techniques. ***Conclusion:*** The proposed cost-effective monolayer technology could increase the efficiency of the cervical screening to a greater extent thereby reducing the rates of faulty diagnosis.
Keywords: Cervical cytology screening, Liquid based technology, Automated image analysis, Sensitivity, Accuracy, Precision

1. INTRODUCTION

Cervical cancer is the second most common cancer among women worldwide[1]. During the last 35 years, the Conventional Pap Smear (CPS) has been recognised globally as the primary technique for screening of cervical cancer[2] as it is safe, efficient, well established and non-invasive. Many advanced technologies have been developed to reduce the false negative cases[3]. Liquid Based Cytology (LBC) is a technique employed to improve the smear preparation forming monolayer slides devoid of the blood, mucus and other debris thereby increasing the sensitivity by reducing the rate of false negative compared to CPS[4]. LBC technique successfully reduces the number of inadequate smears and provides scope to detect infectious agents[5]. However, the commercial kit based LBC is costly and inaccessible to the common people in India for cervical cancer screening[6]. In order to make the LBC technique more informative and easily accessible to the common people, an effort has been made to bring down the cost of cervical monolayer by developing an easy and low cost technique which eliminates the mucus, RBC and reduces the load of inflammatory cells and bacterial flora rather than completely eliminating them. The LBC though efficient compared to CPS, still suffers greatly from inter-observer variability especially in addressing the problem of false negativity. To reduce this shortcoming, computer assisted diagnosis supported by the image processing technique is gaining importance[5]. In this regard the proposed low cost LBC may be useful for image processing and analysis towards developing automated screening. Partial automation of screening has been achieved using image processing techniques[6]. For nucleus identification, morphological reconstruction based methods has been adopted and extended to levelsets for accurate segmentation[7].

Hence, the present study aims at characterization of cervical exfoliative cytology through development of cost-effective LBC for cervical smear and assessing its compatibility in computer assisted analysis of features.

2. MATERIALS AND METHODS

2.1 Study Sample

A total of 120 samples were collected for the study between the periods of April 2012 to January 2013 from Midnapore Medical College and Hospital, WB and National Medical College and Hospital, Kolkata. The patients were of age group 18 to 70 years. Out of 120, number of abnormal cases was 40

of which 14 were atypical squamous cells of undetermined significance (ASCUS), 10 low-grade squamous intraepithelial lesion (LSIL) and 16 high-grade squamous intraepithelial lesion (HSIL).

2.2. Sample Collection and Slide Preparation Methods

2.2.1 Conventional Pap Smear (CPS) Slides Preparation

Samples were collected using sterile Ayer's spatula covering all the zones of cervix, smeared directly on the slides and dipped immediately in 95% alcohol for fixation.

2.2.2 Commercially Kit based Monolayer (CKM) Preparation

Samples were collected from patients' cervix by means of endocervical brush provided with the kit and transferred into the supplied liquid vial. Monolayer was prepared on the charged slides following the manufacturer's protocol.

2.2.3 Newly Developed Cost-effective Cervical Monolayer (NDCM) Preparation

Samples were collected from patients covering different zones of the cervix by means of Ayer's spatula and/or endocervical brush and the cells were transferred into vials containing polysol (an isotonic aqueous solution of chloride salts of sodium, potassium, calcium and magnesium along with sodium acetate acidified with few crystals of phenol) to retain the intact morphology of the cells for future slides preparation and bio-impedance analysis.

The cervical cells in the polysol solution were vortexed for 30 seconds and then centrifuged at 2000 RPM for 5 mins. The supernatant was discarded and cell pellet was then re-suspended in 1 ml of the polysol solution by vortexing. Monolayer was prepared and dipped in 95% ethanol for 15 min immediately.

2.3. Pap Staining

Staining of the slides were performed manually following the routine protocol and using commercially prepared Harris Hematoxylin, OG 6 and EA 36 (Hi Media, India). The slides were cleaned with xylene and mounted by DPX after staining.

2.4. Microscopic Image Grabbing

The images were grabbed digitally by Axio-Cam MRc at 1388×1040 pixels by a Zeiss Observer.Z1 microscope under 20X (NA 0.8) and 40X (NA 0.55) objectives with their respective resolutions being 0.31 µm and 0.16 µm.

2.5 Validation of Compatibility of NDCM for Computer Aided Analysis

The samples prepared through LBC augments the automated Pap smear screening process. The automated screening processes are broadly classified in two steps

a) Object of interest segmentation

b) Analysis of segmented object

Analysis of segmented object is a three step process involving identification and separation of object as the first step, quantification of object as the second step and classifying features as the third step. The performance of each step is dependent on its preceding step. Performance of segmentation step directly depends on contrast between objects. Active contours are widely appreciated for image segmentation. Levelsets[8] were employed with Mumford shah energy function[9] over snakes[10] as they cope with missing and ambiguous features. The segmentation accuracies on samples prepared using CKM and NDCM are tabulated in Table 1.

2.5.1 Nucleus Extraction

The objective of this stage was to segment nucleus at high recall rate. This stage consisted of nucleus localization and nucleus boundary delineation which were sequential processes. First in nucleus localization local minima or darker regions were identified that provided locations of nucleus and subsequently passed on to second process. Based on provided location and similarity between pixels boundary all nuclei and respective cytoplasm were identified in nucleus boundary delineation process.

A. Nucleus localization

Let the digitized stained cervical smear image using RGB sensor be I . At any location $(\mathbf{x},\mathbf{y})$ on the image, the color information was represented using a vector $c_{\mathbf{x},\mathbf{y}}$ where $c_{\mathbf{x},\mathbf{y}}=\{R_{\mathbf{x},\mathbf{y}},G_{\mathbf{x},\mathbf{y}},B_{\mathbf{x},\mathbf{y}}\}$. Initially Red, Green, Blue channels were extracted from colour image I. Individual channel contrast was enhanced and uneven illumination was corrected using Adaptive Histogram Equalization (AHE). As nucleus pixels were darker to neighbouring pixels each color channel was thresholded using Otsu threshold. The thresholds for each channel were identified to be $(\mathbf{T_R},\mathbf{T_G},\mathbf{T_B})$. Masks (M_R,M_G,M_B) were obtained using these thresholds and a resultant mask (M) was generated through logical combination of M_R, M_G and M_B

$$\mathrm{M}_{\lambda}(\mathbf{x},\mathbf{y})=\begin{cases}1 & if \quad I(\mathbf{x},\mathbf{y})\le \mathbf{T}_{\lambda} \\ 0 & else\end{cases} \qquad \forall\lambda=\{R,G,B\}$$

$$M = M_{\lambda=R} \cup M_{\lambda=G} \cup M_{\lambda=B}$$

Overlaying the mask (M) on individual channels reduced the search space for nucleus. For identification of local minima in this search space a morphological reconstruction algorithm was implemented. This morphological reconstruction algorithm generated marker image (V) with valleys.

$$\delta^{(1)}(V)=(V\oplus S)\wedge G$$

where S represented disk with radius 3 pixels. G was gray representation of I .

To eliminate false valleys (False nuclei positions) introduced due to noise or imaging artefact h-minima transform was employed. Local minima within a distance of 'r' were replaced with single minima with highest peak in P. Colour values at each identified local minima were considered as a feature vector. These feature vectors were clustered to 3 regions using FCM (Fuzzy C means). Clustered pixels with minimum red, green and blue values were considered as nuclei pixels. Based on these nuclei pixels, crude nucleus boundary was identified based on gradient image[11]. All these points were combined using cubic splines to form a contour. Flood fill algorithm was then used to transform all these contours into binary objects / masks.

B. Nucleus boundary delineation

Nucleus location process provided nucleus location along with crude nucleus boundary. This contour of crude segmentation was considered as initial boundary L^0 of nucleus by nucleus boundary delineation process. This process adaptively propagated the contour to definite location L^n based on similarity index of pixels on either side of contour and contour length. As established earlier, level-sets were employed for this process. Levelsets is a iterative curve evolution technique in an Euclidian surface proposed by Osher[12]. Ravikanth extended levelsets to image segmentation application[12] given an initial contour L^0 inside that object and L^0 propagated towards L^n based on energy (similarity index of pixels on either side of contour and contour length) derived from image. Ravikanth used a finite difference scheme as a part of energy function which was error prone for discontinuous edges. Vese et al. modified gradient / energy function on the basis of Mumford Shah energy function maintaining the same paradigm[12]. The L plane of CIE L*ab conversion of I was considered as gray scale image. The level sets were implemented on this gray scale image.

C. Separation of cell from background

After nucleus extraction stage well segmented nuclei were accompanied with ill segmented nuclei and artifacts. Well segmented nuclei were filtered out on basis that nuclei were elliptical in shape; an ellipse was fitted[13] to individual segmented object. Deviation between actual object boundary and fitted ellipse boundary was considered as criteria for well segmented nucleus. Identified nuclei contours were dilated by a disk size of 20 pixels. This contour was considered as initial contour for levelsets and levelsets were reinitiated on the gray scale image. Final segmentation results of both nucleus and cytoplasm have been produced in the result section.

3. RESULTS

3.1 Comparison of CPS, CKM and NDCM

In CPS, inflammatory cells, RBC and other artefacts' were generally observed as shown in Fig 1a. These were generally absent in slides of CKM as evident from Fig 1b. The NDCM slide preparation technique provided a clear background and also retained important cyto-pathological information (inflammatory cells and microbial flora) aiding in proper diagnosis as shown in Fig 1c.

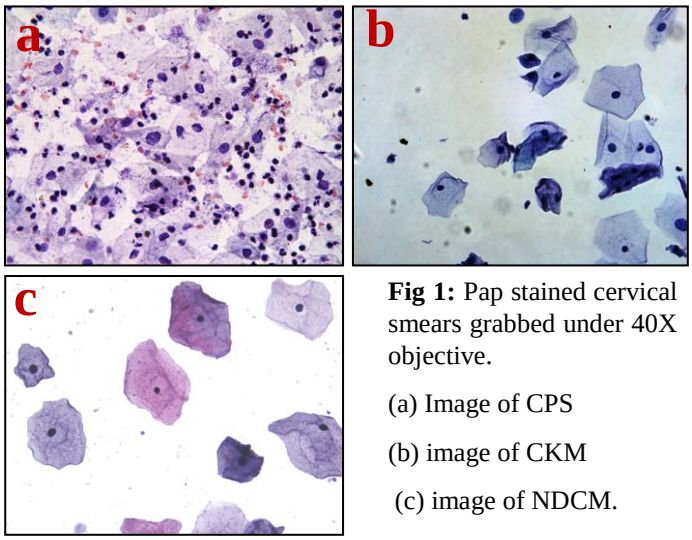

Fig 1: Pap stained cervical smears grabbed under 40X objective.

(a) Image of CPS

(b) image of CKM

(c) image of NDCM.

3.2 Compatibility Evaluation of CPS, CKM and NDCM for Computer Aided Image analysis

Fig 2,3 and 4 represented the microscopic images of CPS, CKM and NDCM slides respectively and the segmentation results of those cells and nuclei. In case of CPS, the presence of mucus and other debris reduces the efficiency of the segmentation algorithm as observed from the nuclear contour in Fig $2a_1$. The segmented nuclei were not appropriate and were of same nature as of Fig $2a_1$. The inappropriate segmented nuclei are shown by arrows in Fig $2a_2$. The same nucleus segmented algorithm was applied on both the cervical monolayer images and the results obtained in both the cases were similar and no discrepancies were observed as shown in Fig $3a_1$, $3a_2$ and $4a_1$, $4a_2$. Fig $2a_3$ showed that the automated cell and nuclear segmentation algorithm fails to run in case of CPS. But the same algorithm when applied to both types of pap stained cervical monolayer, renders appropriate and similar results shown in Fig $3a_3$ and $4a_3$.

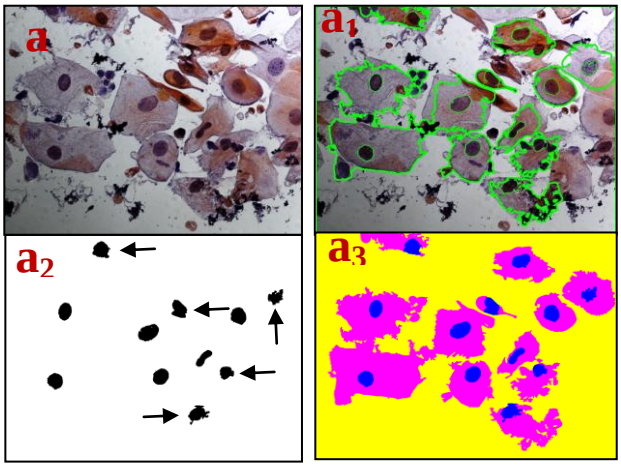

Fig 2: The automated nucleus and cell segmentation algorithm run on CPS. **(a)** Pap stained conventional cervical smear. **(a_1)** identification of both nucleus boundary and cell boundary. **(a_2)** segmentation result of nucleus on the CPS slides. The arrows in (a_2) show the in-appropriately segmented nucleus. **(a_3)** segmentation results of both nucleus and cytoplasm using flood filled algorithm.

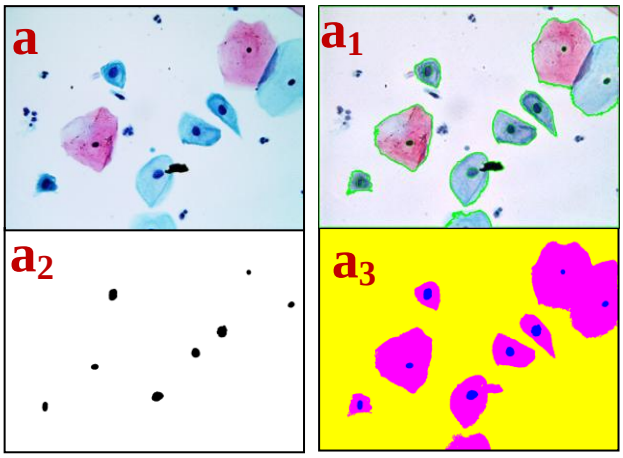

Fig 3: The automated nucleus and cell segmentation algorithm run on CKM. **(a)** Pap stained cervical smear prepared by CKM. **(a_1)** identification of both nucleus boundary and cell boundary. **(a_2)** segmentation result of nucleus **(a_3)** segmentation results of both nucleus and cytoplasm using flood filled algorithm.

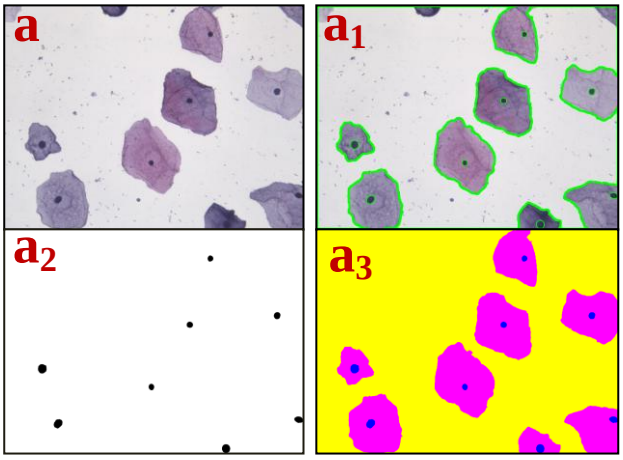

Fig 4: The automated nucleus and cell segmentation algorithm run on NDCM. **(a)** Pap stained cervical smear prepared by NDCM. **(a_1)** identification of both nucleus boundary and cell boundary. **(a_2)** segmentation result of nucleus **(a_3)** segmentation results of both nucleus and cytoplasm using flood filled algorithm.

In order to statistically validate the segmentation performance of the automation algorithm on CPS, CKM and NDCM, the parameters namely accuracy, precision, sensitivity and F-score have been computed. It was evident that the statistical values for segmentation image of the CPS slides are poorer compared to the other two monolayer preparation methods. Both CKM and NDCM gave better output on application of segmentation algorithm and their quantitative efficiency has been displayed in Table 1.

'Accuracy' reflects closeness between segmented value and actual value. The values of accuracy illustrated that NDCM was better to CKM for both nucleus and cell segmentation processes. 'Precision' reflects the closeness within the segmented values and its ability to identify positive samples. NDCM has a competitive precision in nucleus segmentation and better precision in cell segmentation in comparison to both CPS and CKM. 'Sensitivity' gives the frameworks ability to identify positive samples and NDCM shows a better sensitivity for nucleus segmentation as compared to CKM. F-score, the harmonic mean of precision and sensitivity is found to be better for NDCM compared to CKM.

Table 1: Comparative efficacy of different cervical smear preparation techniques to aid automated analysis

Methods	***Cytomorphological features***							
	Nucleus				***Cytoplasm***			
	Accuracy	***Sensitivity***	***Precision***	***F-score***	***Accuracy***	***Sensitivity***	***Precision***	***F-score***
CPS	0.978	0.711	0.673	0.692	0.801	0.729	0.662	0.694
CKM	0.998	0.867	0.884	0.875	0.986	0.940	0.988	0.963
NDCM	0.998	0.811	0.959	0.879	0.992	0.989	0.979	0.984

4. DISCUSSION

Early detection is a major step in improving survival rates in any form of malignancy and cytopathological screening plays a significant role. Routinely used CPS suffers from high level of false negativity due to cell crowding and superimposition of inflammatory cells, RBC, and other allied artefacts. Towards addressing this faulty diagnosis, CKM gained importance with better sensitivity and low false negatives. But it is not well accepted due to total absence of microbial flora and inflammatory cells' information including cost.

The presently developed NDCM could be performed as it did not employ any costly gradient solution and hence of low cost compared to CKM. Moreover, this technique was easy and the necessary manpower may be developed with short training. The NDCM successfully removes the mucus and RBC present in the sample and the cells are also well spread to form monolayer smear having very minimum cellular overlapping as depicted in Fig 1c. Moreover, it preserves microfloral information in respect to pathological status of the cervical mucosa. Hence, overall information from these slides were effective for precise diagnostic decision making with improved sensitivity[14].

In the context of cervical screening automation, CPS slides are not suitable having numerous overlapping of cells and the presence of artefacts[15]. Present study thus evaluates the utility of NDCM in automated analysis. A comparative evaluation of NDCM with CPS and CKM for automated analysis has been performed. Table 1 showed that the sensitivity of CPS were around 60% while that for CKM and NDCM were 90% and 95% respectively. Through the comparison of diagnostic performance between NDCM and CKM, the increase in accuracy from 98.2% to 99.5%, sensitivity from 88.7% to 90.05%, and precision from 95.65% to 96.9% were noted. These observations clearly demonstrated improved performance of NDCM over CKM.

4. CONCLUSION

The proposed cost-effective cervical monolayer preparation technique with improved compatibility for automation may be employed for cervical smear screening in developing countries for early detection of carcinoma cervix.

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