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Computerised Image Processing and Interactive Morphometry in Cytology

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ABSTRACT

Introduction of computers and image analysis systems to quantify the assessment of living cells for prognosis and diagnosis is gaining momentum. It also aims at relieving the operator from the tedium of microscopic observation and reducing operator bias and human error. This paper discusses the design and configuration of an interactive image analysis system built indigenounsly for cell analysis and classification. It also discusses the progress made in the diagnosis of cervical cancer and in the prognosis of breast cancer using computerised interactive morphometry.

1. INTRODUCTION

Microscopic examination of cells on the slides for diagnosis/prognosis of cancerous and other types of cells is a tedious process. Moreover, quantification of the assessment of cells manually is time consuming and is often error-prone. Image processing techniques offer a great promise in this regard. The current efforts are to achieve automation in cytology using image processing and pattern recognition techniques[1-7]. This paper discusses the image processing system designed and configured in the Defence Bioengineering & Electromedical Laboratory (DEBEL), for this purpose. The system works in an interactive, user-friendly mode wherein the user can select the cell of his interest. This is aided by a library of subroutines to compute various morphological and textural parameters of the cell, thus eliminating the need for a skilled operator for the assessment of cells. A ready reference of cell parameters could be made available by creating a database useful for diagnosis and prognosis. The paper also discusses the experiments carried out to classify the cervical cells and the progress made towards prognosis of breast cancer.

2. SYSTEM

The interactive image analysis system configuration is illustrated in Fig. 1. The processing system is built around an IBM PC AT 386 computer and 16-bit PC AT bus compatible frame grabber from Maharshi Electronics. The system is equipped with a microscope and a colour CCD video camera for acquisition of cell images. The system uses Carl Zeiss universal microscope that has a provision for mounting video camera on top. It carries a fast scanning stage with a motor control unit. The scanning stage can be

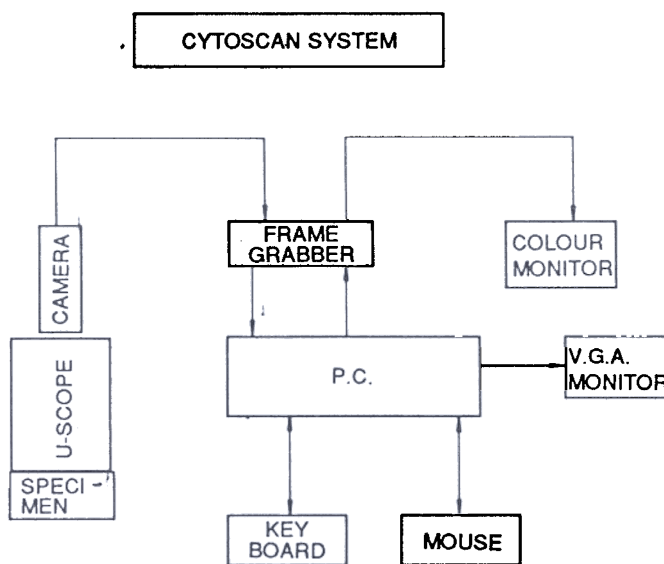

Figure 1. Block diagram of CYTOSCAN system.

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controlled by host computer through IEEE-488 interface. The stage can be moved in steps of 1 micron or higher in both X and Y directions. A solid-state CCD colour video camera from Oscar, Japan, which sends out RS-170 video output signals is used to grab the images of cells scanned below the microscope. The frame grabber accepts video data in RS-170 format sent from the camera and converts the image into 512 X 512 pixel digital image of 256 grey levels. It also converts the digital image into RS-170 signals for displaying the image on the monitor. It also stores one frame of the digitised image and the same can be accessed by the host computer for further processing. The mouse with RS-232C interface to the host computer is made use of for marking the cells of interest on the monitor and for controlling the fast scanning stage. Once the area of interest is marked, rest of the processing is done in near real-time by the various image processing software modules to extract morphological and textural parameters of the cell and to classify the cells into respective categories.

3. SOFTWARE DEVELOPMENT

Software has been developed to control the fast scanning stage of the microscope using the mouse. The stage could be moved in steps of microns or continuously at required speed. Subroutines have been developed to isolate the image portion of interest and to compute various morphological and textural parameters of the cells. Computation of morphological parameters, like nuclear area (NUA), cytoplasm area (CYA), total cell area (TCA), nucleus-to-cytoplasm ratio (NCR), nuclear optical density average nucleus intensity (ANI) and cytoplasm optical density, average cell intensity (ACI), is based on the image grey level histogram approach. The parameters, like perimeter shape, long axis and short axis are determined using geometrical approach. Texture parameters are computed by grey level run length method and Markovian method based on grey level co-occurrence matrix. Grey level run length method provides five textured parameters, namely, short run emphasis (SRE), long run emphasis (LRE), grey level nonuniformity (GNU), run length nonuniformity (RNU), and run percentage (RP). The grey level co-occurrence matrix is used to compute three textural parameters, namely, measure of homogeneity of texture (Tl), measure of contrast (T2) and measure of correlation (T3) that responds to highly ordered structures. Texture parameters provide information on chromatin distribution and clumping in the nucleus.

4. EXPERIMENTAL RESULTS

4.1 Cervical Cytology

A database of about 2000 cells of cervical smear belonging to various categories of normal (superficial, intermediate and parabasal) and dysplastic (mild, moderate and severe) cervical cells has been created and stored on the magnetic tape. The survival smear slides were acquired from Kidwai Memorial Institute of Oncology, Bangalore. The slides were picked up from general pool of cervical smears. The smears were prepared using standard Papanicolaou stain under identical controlled conditions. The abovesaid morphological and textural parameters were computed for each cell to create database after interactively isolating the cell image and the nuclear image from the background. Table I gives the comparative study (mean and standard deviation) of some important parameters of cervical cells.

Table 1. Comparison of morphological and textural parameters of cervical cells

Cell type	Cell area (sq micron)	N C R (%)	Optical density	L RE	Total run length/T1
Superficial	2321 ± 797	1.10 ± 0.4	66 ± 9	1.16 ± 0.05	1.8 ± 0.1
Intermediate	1660 ± 609	5.99 ± 1.9	113 ± 10	1.29 ± 0.05	26.7 ± 12.7
Parabasal	682 ± 249	16.0 ± 4.9	106 ± 15	1.30 ± 0.09	31.2 ± 15.6
Mild	1100 ± 500	10.2 ± 0.8	86 ± 27	1.30 ± 0.07	31.2 ± 30.0
Moderate	414 ± 178	37.4 ± 17.7	86 ± 21	1.32 ± 0.07	50.0 ± 22.0
Severe	189 ± 53	35.4 ± 17.8	77 ± 19	1.49 ± 0.26	22.0 ± 20.0

Based on the statistical information obtained from these computations, two approaches, namely, (a) Linear discriminant approach and (b) Hierarchical approach, were used for classification of cervical cells, and the required algorithms were developed.

The linear discriminant approach based on computation of Mahalanobis distance[10] gave success rate of 85 per cent for the classification of cervical cell as either normal or suspicious, whereas hierarchical approach based on decision tree gave success rate of 94 per cent. The linear discriminant approach gives good classification rate for cells of moderate and severe dysplastic type; however, the results of classification for cells of mild dysplastic type are not all that

encouraging. The hierarchical approach is better placed for correct classification of cells belonging to all types.

Table 2. Confusion matrix

	SF	IN		MID	MOD	SEV
SF	98		2			
IN	2	96	2			
PB		10	90			
MID	2	9	9	76	4	
MOD			10	2	80	8
SEV				2	18	80

SF, superficial; IN, intermediate; PB, parabasal; MID, mild dysplastic; MOD, moderate dysplastic; and SEV, severe dysplastic.

The comparison of manual classification with computer classification of the cells is shown in the confusion matrix (Table 2). The results are normalised to 100 cells per class. The best threshold level for each parameter for maximum correct classification is obtained by successively checking the results of the classification. The classifier is optimised to give minimum false negative alarms. Further attempts to decrease false negative alarms resulted in dramatic increase of false positive alarms which is also not desirable. For tagging a slide as suspicious, it is sufficient to get at least one abnormal cell correctly classified. Hence the chance of wrongly classifying a suspicious slide is almost nil. All slides that are classified as suspicious would be further examined by a cytologist to eliminate false positive cases.

The numerical composition of cells on cervical smear of normal, mild, moderate and severe cases is reported[11-12] and is also verified by experiments. Hence, the claim of correctly classifying at least one abnormal cell, even in the presence of rarely populated abnormal cells, during the scan for the purpose of tagging the slide as suspicious is very much valid.

The scanning and classification process could not be fully automated because of overlapping of cells. Monolayer dispersion of cells on the slide is one of the prerequisites for automated scanning and classification[13].

Several methods have been proposed for slide preparation that meet the requirements for automated scanning[14-15]. But these methods slightly deviate from the normal practice[16] followed in the hospitals, and implementation of the proposed methods depends on the policy matters decided by the competent medical authorities.

4.2 FNAC of Breast

It is well-known that with the advent of chemotherapy, strategies have become available for breast cancer which improve on survival on one hand but have serious side effects on the other. Hence patients with a risk for relapse should be selected for treatment. Although lymph node metastasis is widely used as a high risk indicator, it predicts prognosis accurately in only about 60 per cent of all patients with breast cancer[17]. Hence, alternative factors with additional prognostic value in breast cancer to increase the predictive accuracy of prognosis ideally to 100 per cent, are required. This is especially important as more small lymph node negative tumours are being detected in screening programmes in which the lymph node state is less important as prognostic factor. The quantitative variables, such as mitotic activity index and nuclear and nucleolar morphometric parameters have been known to enhance the predictive accuracy. It has been planned to scan around 300 FNAC (fine needle aspirated cytological) slides of breast cancer patients with equal number of lymph node positive, lymph node negative and control (benign) cases. The statistical analysis of morphometric variables and survival rate of these patients will help in determining the required prognostic indicator, which would decide therapeutic treatment.

So far, about 80 FNAC slides have been scanned. The FNAC slides are supplied by Kidwai Memorial Institute of Oncology, Bangalore. The cytological specimens are stained according to hyperchromic Papanicolaou procedure and is especially suited for quantitative analysis of nucleoli which is expected to play the deciding role in arriving at the prognostic indicator. For accurate measurement of nucleoli parameter the image is zoomed two times to give a final magnification of 4000 X. Mitotic activity is determined by observing hyper fields at 400 X magnification. The fields are chosen at regular fixed intervals that would avoid operator bias. The patient histories, like age, tumour grade, type of therapeutic treatment, lymph node status, post-operation life span, etc., are also entered into the database being created. The results of the analysis will be published as soon as the database creation work is completed.

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