BONE MARROW CELL SCENE SEGMENTATION BY COMPUTER-AIDED COLOR CYTOPHOTOMETRY

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Computer scene segmentation of touching cell images in bone marrow, on the basis of color information, is achieved using digitized scans at three different wavelengths of light. With trivariate histograms and Euler's coordinate transformation, it is possible cytophotometrically to isolate, on the basis of chromatic differences, individual heterogeneous cells located in cell groups. The ability of the described computer methods to isolate correctly the touching cell images is determined by visual comparison of the cells as seen in the microscope and the computer-generated displays of the scanned and segmented scenes.

The customary requirement for cytophotometric measurement and analysis of cell preparations is an evenly distributed monolayer of single cells. Such a monolayer is readily achieved in peripheral blood specimens by manual or spinner techniques. In manually prepared bone marrow smears, however, many cell clusters of homogeneous and heterogeneous cell populations occur (2, 3). These clusters can be cytophotometrically analyzed if either (a) the cells in the clusters are mechanically dispersed by spinner type techniques or (b) the scanned images of the clusters are segmented by computer methods. From a hematologic standpoint, these clusters contain valuable diagnostic information which should not be neglected in the computer analysis. At present, it is not known whether spinner-prepared bone marrow smears retain this diagnostic information.2 The objective of our project, therefore, is to develop computer methods which can segment cell images such as those found in scans of cell clusters. Using cell images of different types of blood cells which are touching each other in a conventional bone marrow smear, this paper demonstrates that such images can be segmented by applying trivariate techniques to analyze polychromatic scans of the cells.

The experiment

Cytophotometric equipment: For the demonstration described below, the cells in Figure 1 are scanned in an Axiomat microscope (C. Zeiss, Oberkochen, Germany) interfaced to a PDP 11/50 (Digital Equipment Corp., Maynard, Mass.). The step size of the Axiomat scanning stage is 0.5 μm in both X and Y directions. Figures 2, 3 and 4 are photographed with a polaroid CU 5 camera from the screen of a Tektronix 4014 (Tektronix, Inc., Beaverton, Ore.) also interfaced to the PDP 11/50.

Cell preparation: The bone marrow aspirates are stained according to Pappenheim. This staining procedure is preferred to the Wright stain because the cytoplasmic granularity, especially in neutrophil granulocytes, is more pronounced. The bone marrow specimens included in this study are taken from the hematology laboratory of the Department of Internal Medicine at the University of Würzburg. They represent routine preparations as used in the diagnosis of blood diseases.

Cytophotometric analysis: To determine which wavelengths of light yield the best cell image separation results, the same group of cells (Fig. 1) is scanned from 420 nm to 680 nm in 20-nm steps. A sliding interference filter with a bandwidth of 20 nm is used to select the various wavelengths. At each wavelength, the cell group is scanned using a scanning stage step size of 0.5 μm, a 50 X oil-immersion objective, a 2 X optavar and a 0.5 μm measurement aperture located in front of the photomultiplier.

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2 Neurath PW: Personal communication.
Fig. 1. Microscopic field of a section of bone marrow smear stained according to Pappenheim photographed with Nomarski differential interference contrast optics to reveal cytoplasmic granulation. The beam combiner lever located on the objective was set to approximately 75% of maximum contrast. Shown is a cell group consisting of one monocyte, one erythroblast and one granulocyte all surrounded by several erythrocytes. The erythrocyte (arrow) overlapping the erythroblast is misstained a slight blue color. For the computer analysis, the cells were scanned in normal bright field as described in the text (original magnification × 500).

The area scanned is large enough to include the whole group of touching cells (Fig. 1). Scanning larger areas with more cells is not feasible for this study because of the extremely slow scanning stage available. Each scanned image is stored on a RK 05 disk. After completing one scan of the group, the sliding filter is set to the next wavelength and the same area is rescanned. This process is repeated until the same area has been scanned at all of the above wavelengths.

Using visual chromatic clues from the original microscopic images, various combinations of cell images from one, two and three wavelengths (Figs. 2, 3 and 4, respectively), as described below, are analyzed. No attempt is
Fig. 2. A, univariate histogram of the cell scene in Figure 1 digitized at 620 nm and smoothed to show the major maxima and minima. The peak farthest to the right (background) has been chopped to reveal the details of regions A and B. Smoothing algorithm: \[(H(i-1) + 2 \cdot H(i) + H(i+1))/4\] applied 10 times. B, computer-generated binary playback display of the pixels corresponding to region A in the histogram. C, binary playback display of the pixels corresponding to region B. D, display of a region similar to A in (A) except that the recorded values are from a scan of the cell group at 520 nm.

Fig. 3. A, bivariate histogram of the cell scene in Figure 1 digitized at 620 and 420 nm; B, binary playback display of the pixels in region B of Figure 2A corresponding to the points located below the arrows in (A).

made before this analysis manually to clean up or separate the individual cell images within the scan. For the combinations of wavelengths and angular rotations at which cluster points appear in the scatter diagrams (Fig. 3A), display thresholds are calculated which separate the selected clusters from the remainder of the scatter points. These thresholds are then used
Scene segmentation: For scene segmentation, a microscopic field of a bone marrow smear is purposely selected in which different cells are touching each other. The scanned and analyzed cell group is shown in Figure 1. This group consists of one granulocyte, one monocyte and one erythroblast, all surrounded by several erythrocytes. Figure 2A is a univariate histogram of the blood picture in Figure 1 scanned at 620 nm. The complexity of the scene segmentation problem is demonstrated by displaying the optical densities (Fig. 2, B and C) corresponding to the two regions A and B separated by the arrows in the histogram of Figure 2A. The pixels in region A correspond to the nucleus of the monocyte and the granulocyte, and to the nucleus as well as a major portion of the cytoplasm of the erythroblast. Pixels in region B correspond to the cytoplasm of the monocyte and granulocyte, and to some of the erythroblast cytoplasm as well as to all of the erythrocytes. It is worth noting that in Figure 2C there are no spatial gaps between some of the cell images which would make it possible to separate the touching cells from each other on the basis of simple spatial image analysis algorithms. Utilizing the color information scanned at 520 nm, only the nucleus of the erythroblast is properly identified and isolated (Fig. 2D).

Systematically to reduce the amounts of data involved in polychromatic scans and to demonstrate how stained cell images change with re-
spect to the wavelength of light, multivariate histograms and Euler's coordinate transformation are used. Multivariate histograms are formed by using the optical density value from each scan at the respective pixel as the coordinate for displaying a point. For two and three colors the results are a bivariate (square) and trivariate (cube) histogram, respectively. Figure 3A is the bivariate histogram using images scanned at 620 nm and 420 nm. As seen in Figure 3B, most of the surrounding erythrocytes are removed from the image. Because of its slight blue stain, however, the erythrocyte touching the erythroblast (arrow) is improperly grouped with the monocyte, granulocyte and erythroblast. To demonstrate that it is possible to improve the scene segmentation further on the basis of color and to remove the misstained erythrocyte, the trivariate histogram from images scanned at 620, 580 and 420 nm is generated. The resultant trivariate histogram, rotated by 60° and projected onto the two-dimensional storage tube terminal, is shown in Figure 4A. The small clusters of points indicated by the arrows C, D and E are not visible, for example, when the trivariate histogram is rotated by 0°. The playback displays corresponding to the three clusters C, D and E reveal that these points correspond mainly to the misstained erythrocytes (Fig. 4B), the cytoplasm of the granulocyte (Fig. 4C) and the cytoplasm of the monocyte (Fig. 4D), respectively.

COMMENTS

The present study shows that polychromatic scans of different types of bone marrow cells contain chromatic information which can be utilized by the computer to segment touching heterogeneous cell images such as those found in cluster formations. Whereas no thresholds for isolating the touching cell images are readily obvious in the univariate and bivariate histograms, the trivariate histogram of the 620-, 580- and 420-nm scans reveals cluster points in the scatter diagram which can be used to segment the cell scene. The sensitivity of the method to subtle color differences is demonstrated by the correct isolation of the misstained erythrocyte. The choice of wavelengths is determined by the color of the stained cells which are to be analyzed. Once the wavelengths necessary for an adequate scene segmentation have been determined, only images from these wavelengths need to be measured and analyzed cytophotometrically.

From a computer-aided cytophotometric standpoint, trivariate histograms and Euler's coordinate transformation are useful algorithms for systematically reducing the amounts of data in polychromatic scans and preprocessing the data for subsequent feature extraction and cell classification. Mathematically, the analysis is not limited to three colors. Depending upon the complexity of the scene to be segmented, additional scans may or may not be cost effective. The method applied in this study is an extension of the bivariate "whitening transformation" described by Bacus (1). The scene segmentation results reported by Bacus, however, are from images of spinner-prepared peripheral blood smears which contain only one leukocyte surrounded by several erythrocytes. The Boolean picture point operations applied by Young and Paskowitz (4) to localize nuclei, cytoplasmic and red cell picture points also analyze spinner-prepared peripheral blood images containing several erythrocytes and one leukocyte.

The method suggested here is directly applicable to segmenting images of histologic sections. In such tissue sections, monolayers of evenly distributed single cells are never available. Histologic sections can therefore be cytophotometrically analyzed only if the image of the section is segmented by computer methods. Potential applications of such analysis techniques include studies of deoxyribonucleic and ribonucleic acid changes occurring during tumor transformations. With a smaller aperture, the method should also be capable of localizing subcellular particles such as cytoplasmic granules.

In this pilot study, human interaction was required to determine the best cell separation. For the interactive procedures, the flicker-free image playback facility of the Tektronix 4014 storage tube, which is large enough to contain several histograms and images simultaneously, has proven invaluable. The terminal provides for fast turnaround of the scan data displayed in three to four optical densities and for visual comparison by the investigator of the computer results with the cell images as seen in the microscope. It is conceivable that these interac-
tive decisions can be replaced at a later stage in the project by automated procedures once the appropriate parameters for cell segmentation have been identified.

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