IPAP: IMAGE PROCESSOR FOR ANALYTICAL PATHOLOGY

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Abstract: The Image Processor for Analytical Pathology (IPAP), is a computer-based image analysis instrument which uses current technology to provide valuable information to the pathologist. Our own studies using the IPAP system are focused on the fields of tumor pathology and toxicologic pathology. The spectrum of methods for the characterization of histologic patterns in these fields consists of karyometric and histometric measurements. This paper describes the hardware configuration of the IPAP and the software system which has been developed to allow a flexible and interactive analysis of microscopical images with minimal computer training and experience. Some examples are given to illustrate the possibilities of the IPAP. (J Toxicol Pathol 7: 353-361, 1994)

Key words: Image analysis, Karyometry, Histometry, Thioacetamide, Rat, Hepatic lesions

Introduction

Computerized image analysis has become an important tool in the pathology laboratory for DNA ploidy evaluation, quantitative immunochemistry, three-dimensional reconstruction of tissue sections, and chromosomal analysis. In addition, image analysis is ideally suited to quantitative morphologic analysis, such as karyometry or histometry. Subtle histologic and cytologic features can be objectively and reproducibly evaluated to yield diagnostic and prognostic information.

The principal requirements of a modern image analysis system for quantitative morphology include flexibility, speed, accuracy of measurements, reproducibility, and easy and friendly handling. The key features necessary to fulfill these requirements lie in a powerful hardware based on the latest technology and an intelligent, versatile software implementation. Research is currently underway to develop diagnostically useful applications of morphometric image analysis. Several general-purpose image analysis workstations designed for many fields of biomedical research have been already available. However, the high cost and software inflexibility of these instruments might be prohibitive to many potential users from introducing the computer-assisted morphometry into their fields. The pathologist is usually not an expert in engineering or image analysis. To combat this problem we have developed the user-friendly, menu-driven program that allows the rapid interactive measurement of objects with minimal computer training and experience. A variety of practical applications of morphometry that we use in our laboratory are illustrated, including a morphometric analysis of rat hepatic lesions induced by thioacetamide (TAA).

Hardware configuration

The IPAP system was assembled from commercially available hardware components (Fig. 1).
This system includes a conventional light microscope, a three charge-coupled device (CCD) color video camera, an IBM PC compatible 486DX2/66-MHz microcomputer equipped with 8MB RAM, 1GB hard disk and two floppy disk drives, a specialized image analysis board Matrox Image-1280, two high-resolution monitors, a page printer, and a mouse interactive peripheral device. An automatic focusing device is a part of the microscope. The PC computer controls and coordinates the overall activities of the IPAP, including a motorized stage that moves the slides at an adjustable speed. One monitor is used to display the menu, data, and demographic information. The other monitor is used for interaction with the microscopic images.

Software systems

The software can run on MS-DOS and is written in C language for data processing and operator-computer interfaces.

We have developed a high performance image analysis software system which utilizes the Matrox Image-1280. Moreover, the power of our image analysis software system is complemented by the menu-driven user interface program. The essence of the design is to give the user flexibility in an uncomplicated framework. The program's structure leads the user through the steps of image processing and analysis. While the user experiments on the sample, using the options selected from each menu, a data file is created for repeated sample processing and data analysis. When the experiments are finished, the data are collected and analyzed, with a variety of statistical and custom-histogram displays immediately available. The measurement, statistics, and graphical data can be stored to disk in ASCII format for direct compatibility with spreadsheet programs such as Excel and Lotus 123. Images can be saved in special graphics file formats. As an example of their performance capabilities, our software systems can enhance, identify, and count 1,000 objects in less than two seconds, measure the data and perform summary statistics in less than three seconds, and provide derived histograms at the touch of button. High resolution (512×480) images can be loaded from disk in as little as 2 seconds.

Morphometric descriptors

Table 1 shows morphometric and densitometric measurement variables which can be performed routinely with the IPAP.

Our own studies using the IPAP system are focussed on the fields of toxicologic pathology including tumor pathology. The spectrum of
methods for the characterization of histologic patterns in these fields consists of karyometric and histometric measurements. Karyometric parameters comprise nuclear area, perimeter, roundness, from factor (nuclear contour index), DNA content, nucleolus/nucleus ratio, number of nucleolar organizer regions (NORs), and so on. Karyometry methods are more advanced than others; also, significant experiences have been accumulated regarding the correlation between nuclear morphometric data, grading, and prognosis of tumors\textsuperscript{10-12}. Histometric measurements are used in counting objects or estimating the extent of altered lesions. They help to improve the diagnostic performance of toxicopathologists, as it has become possible to assure the validity of conventional diagnostic criteria.

**Functional features of the system**

A maximum of user friendliness is achieved by the menu-oriented user interface (Fig. 2). The control is via the mouse and keyboard. No programming skills or experience are required.

The main subjects included are as follows.

**Study specification**

One of the attractive and powerful features of the IPAP is the provision for user-defined studies. All measurements are made on specific data sets of images, relative to a study which has certain user-defined characteristics. These characteristics are the study name, study details, experimental staining conditions, objective lens magnification, morphometric methods (karyometry or histometry), and measurement parameters. The group assignments and number of samples are also defined. Once a study is defined, it is kept as a separate set of records by the system. The end of study definition leads to the menu of operations for

### Table 1. Morphometric Parameters

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<th>Parameter</th>
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<tr>
<td>Area</td>
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<tr>
<td>Perimeter</td>
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<tr>
<td>Roundness</td>
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<tr>
<td>Diameter</td>
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<tr>
<td>Volume</td>
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<tr>
<td>Form factor</td>
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<tr>
<td>Center of gravity (Grav X, Grav V)</td>
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<tr>
<td>Feret X, Feret Y</td>
</tr>
<tr>
<td>No of holes</td>
</tr>
<tr>
<td>Area of holes</td>
</tr>
<tr>
<td>No of holes/Area of object</td>
</tr>
<tr>
<td>Intergrated optical density-object</td>
</tr>
<tr>
<td>Average optical density</td>
</tr>
<tr>
<td>Area of Voronoi pavement</td>
</tr>
<tr>
<td>Length</td>
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<tr>
<td>Individual distance</td>
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Fig. 2. Menu oriented user interface.
Panel layout is easy to pop via the mouse.
Image analysis.

Operations for image analysis

The following sequence of steps is involved in the analysis of a microscopic image.

1) Image acquisition

Using an automatic focusing device and a motorized stage which are controlled by host PC, the predefined fields can be captured automatically.

The red, green, and blue (RGB) signals received from a color camera were digitized at a resolution of 512 pixels wide by 480 pixels high with 8 bits per pixel for storage of a gray level for each color fraction.

2) Image standardization and calibration

The digitized image can be corrected for glare (reflected light) and shading (uneven illumination). The blank bright-field image are collected before the specimen on each slide is analyzed. Spatial measurements at various magnifications are calibrated with a slide micrometer. In our system, spatial calibration measurements are made in both the vertical and horizontal orientations, and the program automatically compensates for spatial anisotropy.

3) Image segmentation

Image segmentation is the process of separating objects of interest from background features in an image. Objects can be segmented by either defining object regions or object boundaries. By setting a threshold gray value, a monochrome image with 256 gray levels can be segmented into light and dark features represented by two gray levels, 0 (black) and 255 (white). The threshold can be held constant or adjusted interactively for object segmentation. We have developed an algorithm for automatic nuclear and nucleolar segmentation. The areas of immunohistochemical staining identified by diaminobenzidine (DAB) and the aniline blue stained areas can be also thresholded and segmented automatically.

4) Interactive image editing

The measurement procedure is carried out automatically as far as possible and the program is interrupted only when interactive manipulation is required. Our interactive image editing functions can be used to get the best possible results within minimal time (Fig. 3). Included are, for example, cutting and linking, fill, select region, deletion, zooming and much more.

5) Measurement and data analysis

Measurement variables are chosen from a menu of descriptors. Our laboratory uses the IPAP for basic morphometric techniques, such as nuclear or nucleolar characteristics and estimation of the extent of abnormal lesions, e.g. fibrosis. This system has also been used for an immunohistochemical analysis including measurements of neoplastic marker expression and proliferative index determination.

The in-built data analysis program gives a simple summary calculation of the mean, standard deviation, coefficient of variation, range, etc., sufficient for an initial evaluation although it is

Fig. 3. Interactive image editing.

(A) Original image. Silver colloid stain.

(B) Nuclei and nucleolar organizer regions (NORs) binarized automatically by the programmed segmentation procedure.

(C) Nuclei and NORs (red-colored parts) edited interactively by the operator.
necessary to import the numerical data into one of the standard packages for more careful statistical analysis.

The results of analysis as well as histograms are printed out on a page printer, according to the user-defined study specification.

Applications

Rat hepatic lesions induced by thioacetamide (TAA) were studied. TAA is a well known hepatotoxin which has been studied since the first report of its toxic properties by Fitzhugh and Nelson in 1948\textsuperscript{13}. A single dose of TAA produces hepatic centrilobular necrosis, while chronic administration results in liver cancers and cirrhosis in rats\textsuperscript{14}.

The experimental model used in the present work was obtained by subchronic intermittent subcutaneous injection of TAA (90 mg/kg/body weight, 2 time/week) to rats. This treatment produced livers with cirrhosis and preneoplastic nodule development by 16 weeks.

After macroscopic examination, liver fragments taken from the median and left lobe for light microscopy were fixed in 15% buffered formalin, embedded in paraffin, and cut into 5 micron sections. Consecutive sections were stained with hematoxylin and eosin (HE) and Azan for connective tissue. In addition, the proliferating cell nuclear antigen (PCNA) and the placental isozyme of glutathione S-transferase (GST-P) were examined by immunocytochemical methods. Expression of PCNA was revealed by mouse monoclonal IgG (PC10) to rat PCNA (Novocastra Laboratories Ltd., UK) and the ABC (avidin-biotin-complex: Vectastain ABC kit; Vector Laboratories, Inc., USA) method. GST-P was examined with rabbit polyclonal antibody against GST-P (a generous gift from Professor Kiyomi

![Fig. 4. Segmentation of hepatic cell nuclei and nucleoli for karyometry.](image1)

- (A) Original image. HE
- (B) Segmented nuclei and nucleoli (red-colored-parts).

![Fig. 5. Segmentation of fibrotic area for histometry.](image2)

- (A) Original image. Azan stain.
- (B) Aniline blue stained area (red-colored parts) segmented automatically.
Sato, the Second Department of Biochemistry, Hirosaki University School of Medicine) by ABC method. Diaminobenzidine was used as the chromogen, and the sections were counterstained with hematoxylin.

Karyometric analyses of all HE stained specimens were performed to determine nuclear size, the coefficient of variance of nuclear size (index of anisonucleosis), nucleolar size, and nucleolus/nucleus ratio. At least 100 hepatic cell nuclei with intact nuclear outline were randomly selected for assessment in each case (Fig. 4). The extent of fibrotic area was assessed quantitatively by measuring the aniline blue stained area in each specimen with Azan stain (Fig. 5). Counts of PCNA positive hepatocyte nuclei were made on more than 20 randomly selected fields using a 40x objective (Fig. 6). Quantitation of preneoplastic focal lesions was performed by counting the numbers and measuring the areas/cm² of GST-P positive foci > 0.2 mm in diameter (Fig. 7).

The results of analysis are shown in Figs. 8-11.

Treatment of TAA led to an increase in size of hepatocellular nucleus and nucleolus and an anisonucleosis in rats. Repeated doses of TAA caused a persistent stimulation of hepatocellular proliferation through 16 weeks of treatment.

Proliferation index was maximal at 12 weeks of treatment, while a striking increase in induction of preneoplastic lesions and fibrosis was observed after 16 weeks of treatment. These findings suggest that the number of hepatocytes positive for PCNA might be affected by the extent of the preneoplastic lesions and/or the progression of fibrosis.

It was thus concluded that the combination of computer-assisted morphometric methods with immunocytochemistry is an effective tool for the assessment of experimentally induced liver injury.
Fig. 8. Changes of hepatocellular karyometric parameters in rats treated with TAA. The bar represents the mean value in each group. In TAA treated rats, all parameters were higher than the controls.

Fig. 9. Changes of hepatocellular proliferation index in rats treated with TAA. The bar represents the mean value in each group. The highest values were noted at 12 weeks of TAA treatment.

Discussion

Recent reports mention that increasing use is being made of various types of image processing for the rapid and objective analysis of specimens for diagnostic purpose in the pathology laboratory. The advantages of quantitative morphometric analysis include its accuracy in and possibly may be more widely applicable.
Fig. 10. Histometric analysis of fibrotic area in rat treated with TAA. The bar represents the mean value in each group. Hepatic fibrosis was established at 16 weeks of TAA treatment.

Fig. 11. Quantitation of preneoplastic lesions in rat treated with TAA. Striking increase in induction of preneoplastic lesions was noted at 16 weeks of TAA treatment.

(A) Number of GST-P positive foci per total area of liver section.
(B) Area of GST-P positive foci per total area of liver section.

describing histologic morphology, and its reproducibility from examiner to examiner. In addition, image analysis allows the detection of very subtle effects. This paper has described a new image analysis system for the pathology laboratory, the IPAP. The machine can be assembled from commercially available hardware components. The software system we designed is a user-friendly, menu-driven program that allows the rapid interactive measurement of objects with minimal computer training and experience. Manual methods for contour extraction are very time-consuming and sensitive to human error. To resolve such problems, we have developed an algorithm for automatic nuclear and nucleolar segmentation, and quantitative cyto-
chemistry. These methods are applicable to standard histological material in which the assessment of two-dimensional images is required. Our experience with the IPAP system has shown that the machine is a powerful tool to improve the diagnostic performance of toxicopathologists, allowing for karyometric or histometric measurements. Future work will be directed towards developing more sophisticated algorithms for quantitative analysis of digitized images.

References


8. Erler, BS, Chein, K, and Marchevsky, AM: An image analysis workstation for the pathology labora-


