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Computer video acquisition and analysis system for biological data

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Abstract

The BIAS (Biological Image Analysis System) was developed to: (i) permit accurate entry and image processing of biological data; (ii) minimize the need for specialized hardware; and (iii) aid in the human genome mapping and other projects. The first mouse/cursor key-driven module was designed to be user interactive and readily accessible to many laboratories. It contains the DRSNDS programs which automate the entering of data in a systematic format. The types of data that can be entered utilizing this module are DNA–RNA gels from either a positive or negative Polaroid™ image, autoradiograms or biotinylated images from Southern, Northern and dot or slot blot hybridization analyses. The image is acquired using a video camera and then digitized for subsequent analysis. During the analysis graphical representations of the intermediate results are provided to assure user confidence. At any point within the program the user may obtain on-line help with the current task. The output displays the mol. wt of each individual component in the appropriate context. The present version of the program produces results comparable with a human interpreter for some data. Band shifting and optical density calculations are in a prototype form to permit evaluation of various techniques. Future work is directed at expanding the system's capabilities to interpret data from other biological analyses including DNA sequencing gels.

Introduction

As a consequence of the capabilities of molecular biological techniques, the volume of information that can be produced from a single laboratory has grown exponentially. In order to process this amount of information, personal computers have become commonplace in most laboratories. Their use has generally been limited to manual entry and analysis of either restriction fragment or sequence data. Computerized data entry and analysis has improved considerably with the introduction of digitizing tablets and pens (Gingeras *et al.*, 1982; Komaromy and Govan, 1984; Staden, 1984) and has led to their widespread use. However, automated data entry systems have not gained wide acceptance due, in part, to the single task capability (Gray *et al.*, 1984; Lott *et al.*, 1985; Elder and Southern, 1987), expense (Marsman and van Resandt, 1985; Elder *et al.*, 1986; Sutherland *et al.*, 1987) or incompatibility of these systems with other hardware and software. Other obstacles to automated gel reading have been problems with positional accuracy, resolution and background noise.

The BIAS (Biological Image Analysis System) was developed to address these issues and provide an efficient user interface. It is mouse- or cursor key-driven, and provides a simple, friendly 'command sequence' that will enable even the novice user to walk through the processing of most data. An 'advanced user mode' provides a very powerful, flexible and efficient user interface to all of the capabilities of the program. The system's design is flexible enough to be applied to new kinds of analyses with no reprogramming and relatively low effort on the part of the operator.

Version 1.3 of the BIAS processes DNA—RNA gels, Southern, Northern, dot or slot blot hybridization analysis (DRSNDS) data. The capabilities and advantages of this system for entry and analysis of DRSNDS data are described in the following report.

Materials and methods

DNA–RNA gels, Northern, Southern and dot blot hybridizations were carried out as described (Krawetz and Dixon, 1984; Krawetz *et al.*, 1986). The system can process positive and negative images ranging in size from a 12 cm × 10 cm Polaroid™ film format (for DNA and RNA gels, i.e. ethidium bromide stained gels) to 25 cm × 20 cm X-ray film format (for Northerns, Southerns, slots and dot blots). Prints are illuminated with overhead spot lighting, while negative images are illuminated utilizing a standard light box.

Hardware and programming language used

The hardware requirements for the BIAS are shown schematically in Figure 1. An IBM PC/AT or compatible computer with 640 kbyte RAM memory (e.g. VT-286/10 AT, Packard Bell, CA) with EGA graphics, Logitec Bus mouse (with LOGI MOUSE driver 3.1), keyboard and Citizen 120D printer is utilized. This computer has an Intel 80286 c.p.u., operating at 6 MHz, with no wait states. A math co-processor is not required and does not appreciably improve the performance.

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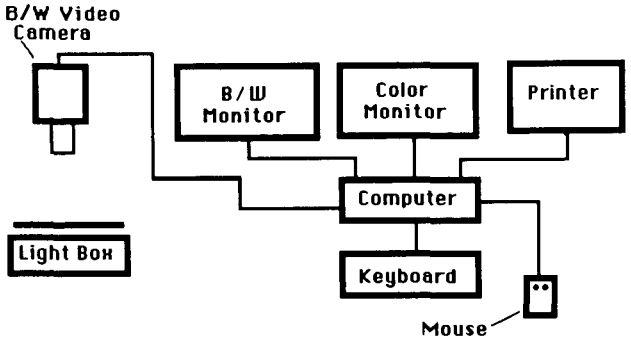

Fig. 1. Schematic representation of the BIAS (Biological Image Analysis System) hardware. The specimen is illuminated with a standard light box, and the image is then captured using a video camera and capture card, and displayed in analog form on a black and white monitor. The digitized image is subsequently processed utilizing the DRSNDS (DNA–RNA gel, Southern, Northern, Dot or Slot blot hybridization) module. Output is sent to the color monitor or printer.

The computer utilizes the MS/DOS 3.2 operating system (Packard Bell). The specimen is illuminated using a standard light box and imaged with the use of a black and white video camera (HV14, Shibaden Electric Co., Tokyo) producing a standard NTSC video signal. The video signal is then digitized utilizing this video capture card (PC-EYE 1150, Version 2.24 software, Chorus, NH). The video capture card has a resolution of 512 × 512 pixels by 6 bits, allowing 64 grey levels. The analog signal received from the video camera is displayed on a black and white video monitor (40VM-9C, General Electric Co., Owensboro, KY). The digitized signal is also displayed in 16 pseudo-colors on an EGA monitor (Packard Bell Enhanced Color Display IS/CM 1435). The monitor is driven by an EGA card with a resolution of 640 × 350 pixels, and displays 16 colors simultaneously. An EGA card and compatible monitor is required. A monochrome EGA-compatible monitor (ZVM-1470, Zenith Data Systems) produced less satisfying results, but can be used. The EGA card is driven by a custom-written assembly language program and is not compatible with GKS or other graphics packages. This entry and analysis configuration was selected in order that the major component, the computer, could also be utilized or adapted to other tasks, and to minimize the hardware cost (~$5000 Can). The programs have been written in the 'C' programming language. The Microsoft C Version 4.0 compiler was used without any modifications. Insofar as the program is written in a portable computer language, it can be run on non-IBM computers. However, this would require that some machine language subroutines be rewritten.

User interface

The acceptance and efficient use of any computer system is dependent upon the user interface and fidelity of data entry and processing. To facilitate operation, the data acquisition (i.e. image 'capture') and processing commands have been divided into two linked classes (basic and advanced) based upon user experience. A listing and brief description of the mouse- or cursor key-driven commands for both the basic and advanced user is shown in Table I. In the case of the basic user, defaults are preselected and commands are presented sequentially, to facilitate appropriate use. At each point in the command series the user may choose to execute, skip, go back to re-execute, or be given a brief description of the command. In addition, the program may be terminated or the user may enter the advanced mode. In the case of atypical data or when additional analysis is required, selection of the advanced series of user commands permits optimization of defaults, analysis parameters and command sequence to that particular data set. The user is aided with readily accessible help files for the routines. At any point while using the BIAS these may be accessed by striking

Table I. A list and description of basic and advanced user commands

Basic user commands

Command	Description
CAPT	Get an image from the video camera
DISP	Display an image on the screen
SAVE	Save image to disk
GO	Mark image minimum color on color bar
SMIN	Set minimum color on the image
GO	Mark image maximum color on color bar
SMAX	Set maximum color on the image
EXPD	Expand image to min and max colors
ORGN	Mark the origin on the image
RULE	Mark the ruler on the image
GO	Mark noise sensitivity on color bar
LANE	Mark the lanes on the image
ESTD	Mark the external standards
GRES	Graph the external standards
CLS	Clear the image window
ISTD	Mark the internal standards
GRIS	Graph the internal standards
ASHF	Automatic shift calculation
MSHF	Manual shift calculation
OD	Calculate the absolute optical densities of each band
OUT	Print out hard copy of data
QUIT	Exit program

Advanced user commands

Command	Description
LOAD	Load an image from the disk
CONF	Automatically configure the camera
SPD	Set the speed of the video capture
SETB	Set the black level of the video card
SETW	Set the white level of the video card
NEGI	Make a negative image from a positive
ROTI	Rotate the image 90°
COMP	Compensate for camera distortions
DIR	Give directory of all files
PRIM	Print image on printer
TEST	Create a test pattern
STUP	Run set up program
DOS	Run DOS commands
DBUG	Enable debugging options

Welcome to Biological Image Analysis System
DRSNDS Module Version 1.3

Name:
Type: DNA : Southern : RNA : Northern : Dot Blot : Slot Blot :

Experiment Date :
Result Book :
Result Book Page :
Probe used :
Gel Percentage :

O.D. Scale Included : yes : no :
Curve Fitting Type : logMw/D : √Mw/logD : log/log : UDefine
Lanes:
: Standards : Lane 1 : Lane 2 : Lane 3 : Lane 4 :
: Lane 5 : Lane 6 : Lane 7 : Lane 8 : Lane 9 :
: Lane 10 : Lane 11 : Lane 12 : Lane 13 : Lane 14 :
: Lane 15 : Lane 16 : Lane 17 : Lane 18 :
Notes:
:
:

<< Done >>

Fig. 2. Screen of the initial task selection menu. The user is prompted at every entry level for a response. The user may select the appropriate response or, in the case where no action is required, strike a carriage return to move to the next entry. Completion of this screen defines the type of experimental analysis and permits correlation with other experiments.

the '?' key. The help files describe the properties of the commands, their use and relationship to other commands.

Output from Southern and Northern blot hybridization analyses is correlated to the corresponding gel automatically. To accomplish this the program maintains the relationship between a Northern and an RNA gel (or a Southern and a DNA gel) by linking the names in the MS/DOS operating system. It automatically displays information about the corresponding RNA/DNA gel when the Northern/Southern is called up. Output files are maintained in standard ASCII format. Image files, which are 256 kbyte in length, are stored in a special format based upon the output of the PC-EYE video capture card.

Algorithms and routines implemented

Four algorithms were implemented to determine mol. wt (M_w). They are based on the following graphical techniques: (i) log M_w versus distance of migration; (ii) the square root of M_w versus log distance of migration (Lehrach *et al.*, 1977); (iii) the log of the M_w versus log distance; and (iv) the user-defined polynomial $M_w = k_1 + k_2 d + k_3(d^2) + k_4(d^3) + \ldots + k_{10}(d^9)$.

In method (iv) the user selects the degree of the polynomial (up to 9) and the program calculates the coefficients by a global least-squares fit. Recently Elder and Southern (1983) have

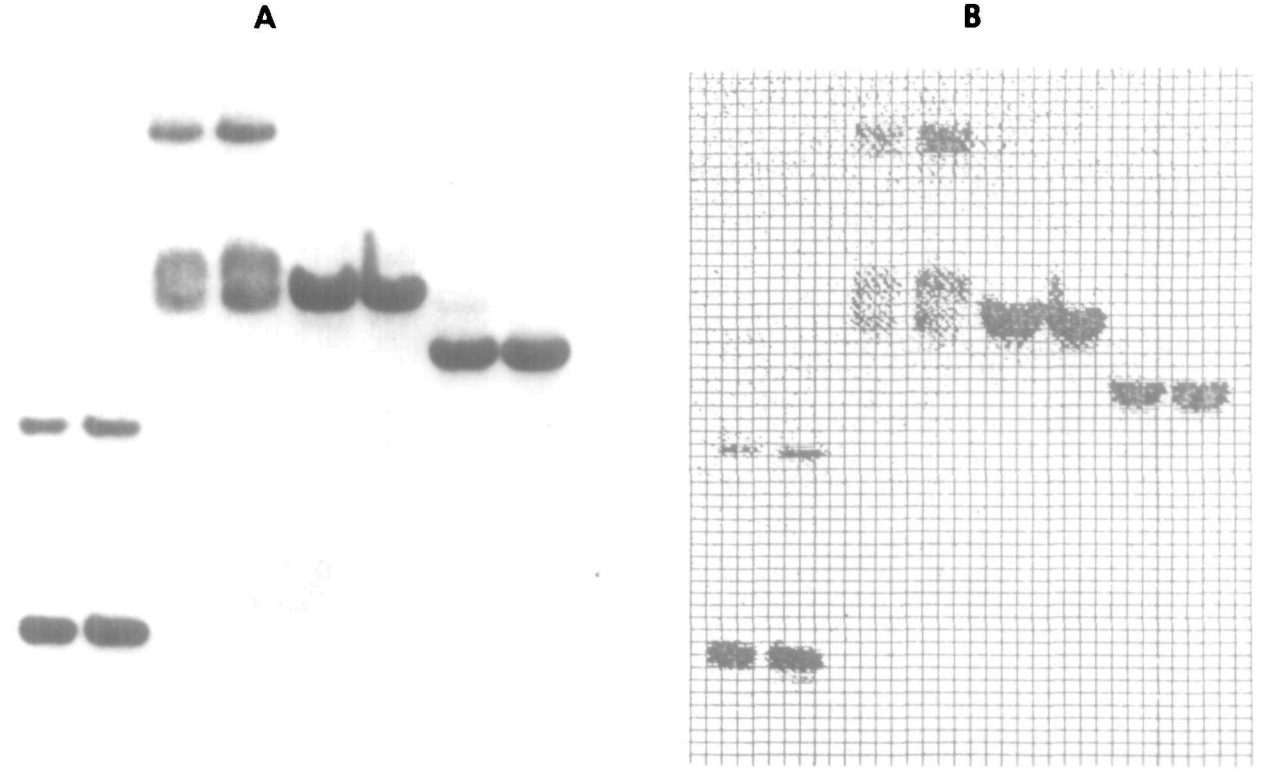

Fig. 3. Comparison of the actual image with the computer captured image. **Panel A** shows the autoradiogram of the Southern blot hybridization experiment that was analyzed with the BIAS. **Panel B** is the corresponding captured digitized image that was printed on an Epson MX-compatible printer. The grid pattern permits easy measurement of captured bands.

evaluated various sizing methods. They found 'reciprocal relations' to be an accurate representation of the relationship between mol. wt and distance migrated. We have approached the sizing problem in a similar manner through our methods (iii) and (iv), and the reciprocal relationship (Elder and Southern, 1983) would be easily added to the BIAS.

The accuracy of the user-selected curve-fitting algorithm is shown graphically during this phase of analysis as a plot of M_w versus distance of migration. This presentation was selected since it accentuates any errors in the fitting of the data.

A routine was written to correct for non-uniform lighting of the specimen and video camera sensitivity variations in different parts of the image area. It is invoked by selecting 'Comp' (for compensate) from the advanced user command menu. This routine processes the image to normalize the video levels across the image.

Data enhancement options provide automatic or manual lane correction. Lane correction is accomplished by preprocessing the video image using a convolution edge enhancement. Detection of peaks (which correspond to the center of bands), is accomplished by an algorithm that looks for local maxima subject to a user-defined sensitivity cut-off.

In any electrophoretic experiment, a problem is sometimes encountered in which the bands of known mol. wt in one or more lanes are shifted from their expected positions. This is the result of either sample or electrophoretic anomalies. In most cases within each lane at least one band has a known mol. wt. This can be utilized as an internal standard for mobility correction. In this case, the program calculates where the band(s) should appear, based upon the mol. wt/distance information derived from the external standards. It then looks for band(s) near the predicted location(s). If it finds all the bands in the predicted locations it automatically shifts the entire lane so that the bands of known mol. wt appear at the predicted location. In cases where the data is too scattered, or there are multiple bands near the predicted location, it alerts the operator to make a manual selection of the internal standard bands. All bands in the lane are shifted by a constant distance so that the internal standard bands are moved as close as possible to the predicted positions.

Optical densities are resolved into a 64-level grey scale which are represented by 16 pseudo-colors. This is the maximum resolution that can be obtained from the PC-EYE 1150 Video Capture Card. This EGA card/monitor combination can only display 16 colors at a time. These restrictions can be surmounted by substituting other hardware components and modifying the program accordingly. To determine optical densities, a density calibration strip of known values is captured along with the image. The operator then matches the calibration strip images with values in the standards file. This allows the program to find a mathematical relationship between optical densities and grey scale values. When determining the optical density of a band, the program chooses a point in the center of the band and averages points in the immediate vicinity to produce a value for band optical density. As yet, no compensation routine has been implemented to address the issue of non-linear film response that may be encountered.

Results

Hardware

The BIAS was assembled utilizing individual hardware components (Figure 1) from readily available sources. They were selected to be compatible with the existing equipment found in most laboratories. As shown in Figure 1, the image of the illuminated specimen is captured with the video camera and capture card, then displayed in pseudo-color on the EGA monitor. The image is subsequently processed with an IBM PC/AT compatible computer using the DRSNDS program module. In addition, the fidelity of the captured image can be readily verified by comparing it with the original image on the black and white video monitor.

Data entry and analysis

To evaluate the accuracy, precision and reproducibility of the system, it is first necessary to understand the sequence of operations. The first screen (Figure 2) shows the initial task selection menu. The types of data that can be entered and analyzed utilizing the DRSNDS module are DNA–RNA gels from either a positive or negative Polaroid™ image, and autoradiograms or biotinylated images from Southern, Northern, dot or slot blot hybridization analyses. Completion of the first menu (information page) permits the user to record the history of the experiment and analytical parameters (e.g. experiment type, optical density scale and standard sets). When required, the default parameters (e.g. standard sets, lane names) may be edited by using the cursor keys. These parameters vary with the type of experiment selected. They are displayed and may be altered using the results-book formatted 'fill in the blank' form which is provided by the first screen. Completion ensures subsequent data retrieval and correlation between DNA–RNA gels and the corresponding Southern–Northern hybridization analysis.

The following illustrates the operation of the program for DNA–RNA gels. The region of the specimen to be analyzed is first defined by marking the origin and setting a ruler on the captured or retrieved image with the use of the mouse or cursor keys. Subsequently, the position of each lane is marked. The user then matches the predefined mol. wts to the corresponding bands of the external standards (RNA or DNA) by matching each of the bands displayed on an inset graph to its

Fig. 4. Video and captured image of a DNA sequencing gel. (**A**) A portion of a DNA sequencing (shown on the video monitor, upper screen) was captured and the resulting image displayed on the EGA monitor (lower screen). This is an unenhanced image. The lanes are from left to right as C.A.T.G.A.C from 1 to 12. (**B**) The same image after computer enhancement.

A

B

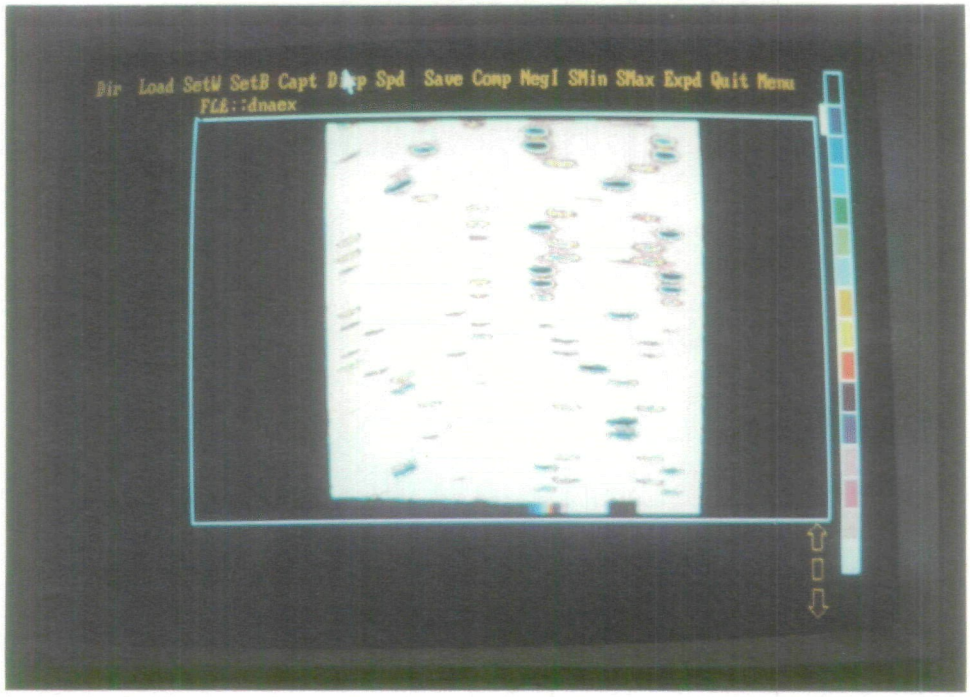
Dir Load SetW SetB Capt Disp Spd Save Comp Negl SMin SMax Expd Quit Menu
FLE:*.dnaex

Table II. The effect of sensitivity setting on band detection

(a) The effect of band detection for bands in lane 7C of Figure 4A[a]

Sensitivity level	No. of real bands detected	No. of false bands detected	No. of bands missed	Total no. of bands detected
1 (white)	4	0	5	4
2	4	0	5	4
3	4	0	5	4
4	5	0	4	5
5	5	0	4	5
6	5	0	4	5
7	6	0	3	6
8	7	0	2	7
9	7	0	2	7
10	7	0	2	7
11	8	0	1	8
12	9	0	0	9
13	9	0	0	9
14	9	0	0	9
15	9	1	0	10
16 (black)	9	8	0	17

(b) The corresponding accuracy of the detection of the bands at sensitivity level 14 in all the lanes of Figure 4A[b]

Lane	No. of bands detected	No. of bands missed	No. of false bands detected
1	9	1	0
2	3	0	0
3	6	3	0
4	4	0	0
5	4	0	0
6	10	0	0
7	9	0	0
8	9	0	0
9	5	0	1
10	5	0	2
11	9	0	0
12	9	0	0

[a]The number of real, false and missed bands was determined at the 16 sensitivity levels from level 1 (Figure 4A white, least sensitive) to level 16 (Figure 4A, black, most sensitive).
[b]The number of identified, missed or false bands as compared to the expert human interpretation of the gel is given.

appropriate mol. wt. The process is very easy and typically takes a few seconds. After the matching has established the correlation, the external standard curve is generated. The user may then select the appropriate predefined set of internal standards (e.g. vector DNA or rRNA) to compensate for inter-lane variation by band shifting. The standard set is automatically (or in atypical cases, manually) matched to the corresponding bands on the gel, enabling an internal standard curve to be generated for each lane and a corresponding band shift to be calculated and applied to the bands in each lane. This determines the mol. wts of the translated bands by ensuring that bands of identical mol. wt migrate the same distance. Optical density standards may then be utilized to calculate optical densities of the peaks of the individual bands.

Southern and Northern blot hybridization analyses are essentially processed as described above. Retrieval of the corresponding DNA–RNA gel data permits determination of the individual band's mol. wt by utilizing the previously determined external standard curve and band correction data. Slot and dot blot hybridization data are processed in a similar manner. Initially the predetermined external standards (quantity or dilution) are matched to the corresponding dot or slot with the use of the mouse. The program then automatically calculates the relative optical density of all the centers of the peaks. A standard optical density scale can be included to determine the values.

The user always knows the analytical status of the program and how the data are being processed. Graphical representations of the processed image are displayed during data acquisition and analysis. For example, as the user marks the positions of the lanes the program graphs both the unprocessed and filtered optical density data and indicates the points where bands were detected. This permits the user to confirm that the bands were correctly detected. Furthermore, when band shifts have been calculated, the internal standard curve for each lane may be simultaneously graphed on the same graph as the external standard curve. This method allows the user to verify quickly that the band shift calculations are within the expected range and that the data were processed correctly.

The program's output is available in both disk file and hard copy form. The hard copy is divided into three sections. The first is a listing similar to that shown in Figure 2, but edited to contain only the information that is pertinent to the individual analysis. The second section shows the position and optical density of each band as a scaled graph. A list of the mol. wts of the various bands and the distances in each lane that the bands were shifted concludes the final section.

Fidelity of data entry and present constraints

To evaluate the fidelity of data entry, the original image and the computer captured image were compared (Figure 3). Comparison of these images clearly shows that no visible distortion occurred during data acquisition, thus confirming accurate data entry.

The capabilities of this system to identify bands correctly was assessed. A portion of a DNA sequencing gel was captured (Figure 4A: upper screen, video image; lower screen, digitized image) and the corresponding image analyzed at various detection sensitivity levels. As shown in Table IIa, the correct identification of all bands is dependent upon the user-selected sensitivity level. The system correctly identified all bands in a selected lane, with no mis-attributed bands, at sensitivity levels 12, 13 and 14. In using the program, it has been determined that in most cases these sensitivity levels are well suited to this type of analysis task. The other sensitivity levels are provided for anomalous cases. It is anticipated that the sensitivity level could be set automatically for most autoradiograms. At the sen-

sitivity level 14, most real bands (Figure 4A, determined by an expert human interpreter) were detected (Table IIb) even though the data was not processed with an expert analysis system.

There are some limitations in the present version of the program. These are presently being addressed to enhance the usefulness and accuracy of the BIAS system.

The number of grey scale levels (64) provided by the PC-EYE 1150 video capture card limits the range of optical densities that can be resolved in a single pass. The dynamic range of some autoradiograms is wide enough to require either a higher number of bits (e.g. 256) or a multi-pass approach in which the system would 'home in' on the appropriate density levels. In addition, to calculate the optical density of a band, the present version of the software takes an average of several pixels around the center of the peak. This can underestimate the quantity of material, since in some cases the center point may be saturated. Thus, to minimize this problem, and produce more representative values for optical density, a future version of the BIAS system will possess peak integration capabilities.

Mol. wt determinations are dependent upon the correct selection of the origin point. Any variation between actual and entered positions can seriously affect the calculation. At present precise marking of the origin on the captured image is required. The improved band-shifting technique described below should address this problem.

The band-shifting algorithm currently implemented shifts all bands by the same distance. This produces acceptable results in ideal cases where the shift required is negligible. To make the program widely applicable, a scaling factor is being introduced in a future version. It will shift bands proportionally to the percentage difference in experimental versus predicted mol. wt. This will be implemented using the user-selected curve-fitting method (see Figure 1).

The program currently occupies almost the full 640 kbyte of RAM memory permitted by MS/DOS 3.2, hence it is difficult to add new features. The present version of the software addresses this problem through the use of overlays, which has a negative impact on the speed at which the program runs. This can easily be addressed through the modification of the system to utilize commercially available products that allow the user to go beyond the 640 kbyte memory limitation (e.g. 2 MB memory chips, IBM Personal System/2, Microsoft OS/2 operating system).

Discussion

As shown above, even in the case of the inexperienced or first-time user, the BIAS is simple to use. This was achieved by pre-ordering the basic user commands, providing extensive on-line help and ensuring user interaction throughout the program. It permits accurate entry and visual confirmation of data. The hardware configuration (Figure 1) ensures the system's wide accessibility to even the small laboratory. The use of the BIAS programs does not affect the use of the hardware for other purposes. The system is modular, enabling any hardware component to be easily upgraded. The advantage of this design is that future developments in either camera (e.g. higher resolution), video capture card (e.g. higher resolution) or personal computer technology (e.g. faster processing speed) can readily be incorporated into the system without rendering the remaining components obsolete. It has been our experience that alternative video cameras can easily be installed with minimal set-up time [i.e. attach the camera and run the Conf (Configure) command, 10 min or less]. Furthermore, as described in Results, more accurate optical density values could be obtained with higher resolution (256 grey scale) video capture cards. These are declining in cost and now represent a realistic, cost-effective alternative. It is interesting to note the similarity of the underlying problem in these varied input and analysis applications. The problem was clearly defined as the precise determination of position and intensity. With the use of these two parameters, the BIAS acquisition and analysis system has been assembled to input and analyze DNA−RNA gels, Southern, Northern, dot and slot blot hybridization data (DRSNDS program module). We are proceeding with the development of a second accompanying module, utilizing the same hardware to automatically enter DNA sequence data in an accurate form. As shown above (Figure 4A,B; Table IIa,b), it is clear that the portion of the DNA sequencing gel shown was captured with sufficient resolution to allow fairly accurate detection of all the bands. With the planned development of a rule-based expert system, the BIAS should be adaptable to the problem of automated entry and analysis of DNA sequence data. By applying DNA sequence interpretation principles, the rule-based system should allow the computer to make logical inferences improving the information content of the raw captured image. If full automation of DNA sequence data is desirable, and cost not a consideration, it would be necessary to construct a mechanical feeder to position the specimen for analysis, since with this video capture technology an entire DNA sequencing gel could not be adequately resolved in one capture.

The BIAS was designed as a human/machine interactive system, to be assembled from 'off the shelf' hardware. These design parameters preclude totally automated analysis. The system is designed to utilize the abilities of the operator in those areas where the human eye and brain are best (e.g. lane selection) and the capabilities of the computer in areas such as calculation, image enhancement and data storage and retrieval. This approach should yield the best overall results consistent with a reasonably economical system.

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