0273-2289/93/4103—0219$03.60

High-Tech Breakthrough DNA Scanner for Reading Sequence and Detecting Gene Mutation

A Powerful 1 lb, 20 μm Resolution, 16-Bit Personal Scanner (PS) that Scans 17″ × 14″ X-Ray Film in 48 s, with Laser, UV and White Light Sources

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Received November 6, 1992; Accepted January 19, 1993

ABSTRACT

The 17″ × 14″ X-ray film, gels, and blots are widely used in DNA research. However, DNA laser scanners are costly and unaffordable for the majority of surveyed biotech scientists who need it. The high-tech breakthrough analytical personal scanner (PS) presented in this report is an inexpensive 1 lb hand-held scanner priced at 2–4% of the bulky and costly 30–95 lb conventional laser scanners. This PS scanner is affordable from an operation budget and biotechnologists, who originate most science breakthroughs, can acquire it to enhance their speed, accuracy, and productivity. Compared to conventional laser scanners that are currently available only through hard-to-get capital-equipment budgets, the new PS scanner offers improved spatial resolution of 20 μm, higher speed (scan up to 17″ × 14″ molecular X-ray film in 48 s), 1–32,768 gray levels (16-bits), student routines, versatility, and, most important, affordability. Its programs image the film, read

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DNA sequences automatically, and detect gene mutation. In parallel to the wide laboratory use of PC computers instead of mainframes, this PS scanner might become an integral part of a PC-PS powerful and cost-effective system where the PS performs the digital imaging and the PC acts on the data.

Index Entries: DNA; sequence; electrophoresis; fingerprinting; PCR; autoradiography; 17″×14″ X-ray film; archiving; scanner.

INTRODUCTION

At present, most biotechnologists have no choice but to manually read the DNA sequences on X-ray film. The film is laid on a white background or on a transilluminator viewbox. The users' eye, with visual acuity of 400 μm power of discrimination, reads the sequence. The user points to the base A, C, G, or T with an electronic pen and the computer reads that position and records the entry. Thus, computer-assisted sonic or nonsonic digitizers (pen-readers) lack the needed resolution of 60 μm or better because the human eye remains the sequence reader.

Additional inaccuracy comes from sound sensors. The linear arrays of 256 sound sensors use one vertical array on the left side of the computer screen to read Y position of the pen, and another horizontal array on the upper side of the screen to read the X position. Some pen readers have the linear array of sensors at four borders of the transilluminator itself. Again, the human eye is the reader contributing to inaccuracy. Furthermore, the resolution of reading the X-Y position of the pen by sonic sensors is inadequate especially when sound disturbances and pen-based errors are taken into consideration.

An advanced version of the pen-reader, often called the DNA sequence digitizing system, was recently introduced by Hitachi. It utilizes subsurface electrodes implanted on the transilluminator beneath the X-ray film. Having 1016×1016 electrodes/sq. in. improves the resolution of reading the X-Y position, but the inaccuracy remains because the human eye determines where the pen touches the DNA base on the X-ray film.

The new generation of CCD video cameras of 512×512 pixels, which is adequate for reading 10 cm×10 cm SDS gels, has an incompatible optical resolution for reading molecular 17″×14″ X-ray film. Even these video systems are expensive and require a capital equipment budget. The advanced 1024×1024 or 2048×2048 CCD cameras are drastically more expensive. The improved resolution these video systems provide remains unutilized because the human eye determines where the mouse points on the displayed image.

Even though all these conveniences did not add significant improvement in reading the sequence, the net effect was slightly negative. Such apparent improvements were cosmetic in nature and have pushed the

prices of pen-readers and of video imaging farther up into highly priced capital equipment.

On the other hand, dedicated instruments to accurately read sequences were developed, but the $100,000+ price tag is prohibitive. The laser system from DuPont (Boston, MA) can read sequences on 17″ × 14″ X-ray film. The DNA sequence reader from Applied Biosystems (Foster City, CA) uses molecular sieve columns and selectively reads the UV-fluorescence of A, C, G, and T DNA fragments present in the flowing eluate. Both systems are research quality but very hard to afford.

Adequate resolution of scanners is as important as their affordability. The resolution of scanners or video imagers needs to be compatible with that of the electrophoretic separation of DNA fragments on SDS polyacrylamide gels. The SDS high resolution (*1,2*) of about 60 μm cannot be faithfully scanned with white light scanners of 300–800 μm resolution. The well-separated bands on the gel appeared overlapping on the densitometric tracing because the resolution obtained by the gel was lost during scanning. The advent of laser densitometry with 5 μm resolution came to the rescue and the laser scanner faithfully quantitated the separated bands on the SDS gels (*3–5*). Applying laser densitometry to various fields became a widely used technique (*6,7*). However, laser densitometers became a costly capital equipment item needing a special budget. Furthermore, the standard gel size of 10 cm × 10 cm commonly used for electrophoresis was expanded to include 42 cm × 35 cm (17″ × 14″) in DNA research. Also, clinical imaging of a full chest X-ray film 17″ × 14″ (*8–13*) commonly used for archiving became another feature that was incorporated in DNA work. These advancements in resolution, expanded gel size, and clinical imaging drove the price of laser scanners way up and availability became more difficult.

Currently we have the two extremes of unaffordable capital equipment: (1) inaccurate and laborious pen-readers of sonic or subsurface electrode types; and (2) the prohibitively priced but accurate DNA sequence readers. Yet most of the scientists who work with DNA need a reader. Herein comes the subject matter of this report: the new affordable high-technology 1-lb PS scanner with needed speed and precision. Providing the majority of biotechnologists, where most scientific breakthroughs originate, with this equipment is critical. The powerful PS scanner assisted by the personal computer can bring a significant enhancement to their productivity.

EXPERIMENTAL

Survey on DNA Scanners

From 15,392 prospective customers who responded to advertisements in 1991, we reached 1,424 electrophoresis users in 1992. Only three had

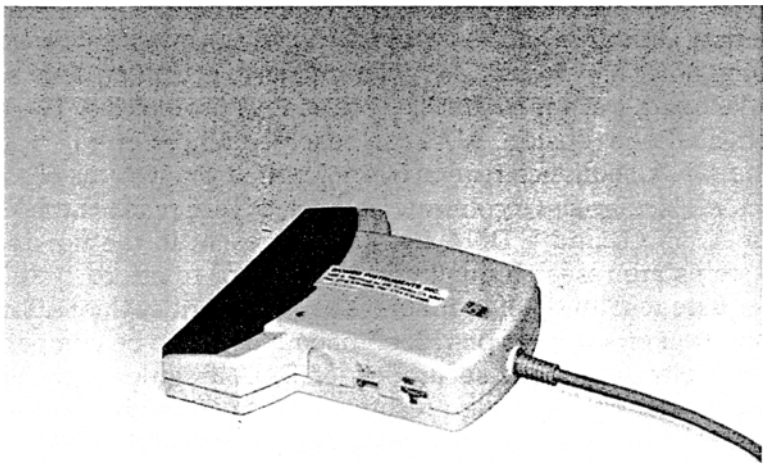

Fig. 1. High-tech analytical PS scanner, hand-held, 12 cm × 12 cm × 3 cm, 1 lb in weight, 2096 CCD linear array sensor, 16-bits, 20–60 μm resolution, scans 17″ × 14″ molecular X-ray film in 48 s, laser-UV-white light source, a densitometry station by itself to read DNA, electrophoresis, TLC, blots, mircotiter plates, and colony/plaques imaging.

shared a laser DNA reader that reads 17″ × 14″ molecular X-ray film—two from Molecular Dynamics and one from DuPont. A fourth scientist has access to an Applied Biosystems integrated sequence reading equipment shared by multiple departments. This survey indicates that only 3% of biotechnologists engaged in DNA work have advanced instrumentation. The remaining 97% have no capital-equipment budget to purchase it.

PS Scanner-PC Computer Setup

The new high-tech analytical handheld scanner, Fig. 1, weighing 1 lb at a size of 12 cm × 12 cm × 3 cm, has a built-in linear light source (laser, white, or UV) illuminating the gel (Fig. 2). The reflected light re-enters the scanner through a window to a 2048-CCD linear array sensor along the x axis. As the scanner sweeps over the sample in the y direction, the 6.7-cm roller position reader is connected with a counting system calibrated to read micrometer length units, μm. The optical signal generates a corresponding electronic one by the CCD. The electronic signal is amplified, digitized to 1,32,768 gray levels (16-bits), and selectively relayed to the computer as 1,2,3,4,8,12, or 16 bits. Assisted with a pair of 1-cm long rollers, the position reader (roller) guides scanning in a straight line. A guide for accurate scanning and gel positioning is available but not needed

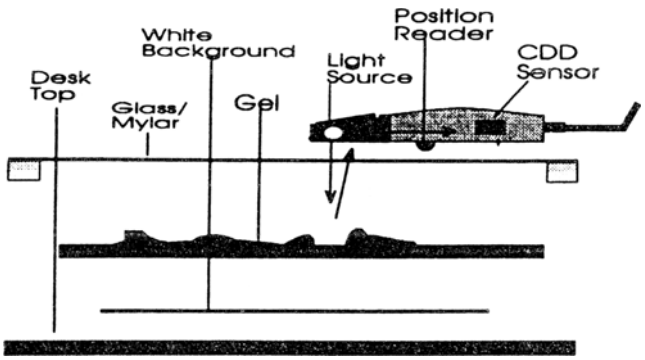

Fig. 2. Scanning with reflectance or UV-fluorescence. Linear 2046 element CCD along x and position reader along y as the scanner sweeps over a sample. There is a glass plate to prevent touching wet gels. In transmittance or absorbance modes, the gel is between the light source and the scanner.

because DNA lanes on 17″ × 14″ X-ray film are rarely straight and parallel. Most of the time the lanes are skewed, smiling, divergent, or wavy. Whether the lanes are straight or not, the computer program allows one to read the sequence.

The scanner's interface card was inserted in a 12/16-bit slot, IBM-compatible 286, 386, or 486 4 Mb RAM, 25 MHz, 40 Mb hard drive, VGA or SVGA, DOS 5.0 system. Most of the work was done on the 286 computer. The programs were automatically transferred to the hard disk C using 1.2 Mb 5.25 in. floppies in disk drive A or from 1.44 Mb 3.5 in floppies in. disk drive B. The scanner was connected to the interface card port. As the computer is turned on, the opening menu appears and the function is optioned. Then, either the student routine is selected for automatic operation or the standard function menu is run. Running the standard function allows adjustment of 48 user-defined variables. As a representative example the variables for one-dimensional gel scanning are: print report, save data 7 report, base flattening, auto zero, normalization, mapping of gel, mapping test lane, mapping reference lane, molecular weight determination from standard curve, determination of protein content from standard curve of concentration, graphic, inversion, image enhancement, global filtration, overlay of test and control, pattern recognition, scan gel after gel for unattended analyses and reporting, and single-page full report.

Imaging DNA 17″ × 14″ X-Ray Films and Other Media

Fifty-four 17″ × 14″ X-ray films were provided by various sources. Each film carries 6–12 DNA sets for sequence reading. The quality of films ranged

from good to reject. Imperfections in molecular DNA films included smiling, distorted bands, distorted lanes, skewed lanes, uneven background, over/underexposure, nonstraight lanes, radioactive splashes, and artifacts.

A wide variety of media were used to assess the versatility of the PS scanner. Such media included the following items: 7 wet gels with ethydium bromide stain, 12 Polaroid films of UV gels, 2 membrane ELISA, 3 electron microscopy photos, 17 Petri dishes with translucent and opaque colonies, 3 Petri dishes with two sorts of colonies (big opaque and tiny transparent), 2 lifted plaques, 1 DNA PCR, 1 set of G lanes (first lane control, second experimental mutation) and a few unspecified DNA X-ray films intended as unknowns to test for footprinting or fingerprinting to detect gene mutation or nicking, 7 one-dimensional mini gels (2.5 cm×2.5 cm, 8×8, and 10×10), 4 two-dimensional electrophoresis dried gels, 5 one-dimensional separation imprints on X-ray film, 2 Western blots on nitrocellulose, 8 assorted sizes of DNA on X-ray film, one chemiluminescence of X-ray film, 5 infected tree leaves, skin rash, and 1 activated phosphor imaging plate.

DNA Sequence Reading

All provided X-ray films were imaged by the PS scanner. The DNA sets were cropped, the sequence was read automatically, and then proofread and edited on zoom windows. Since we are involved in technology and methodology, the accuracy of DNA sequence reading was verified by each of four scientists only, owners of good quality molecular 17″ × 14″ X-ray films. For illustrative purposes, a representative 17″ × 14″ molecular X-ray film with multiple sets of DNA, *see* Fig. 3A. The scanning time was 48 s. The computer screen displays the image as you scan. At the end of scanning it displays a compressed mini map of the whole film. Unless optioned to rescan, the data of about 2.4 Mb is saved on the hard disk. The sequence was read from the mini map or from cropped sets. Reading the sequence from cropped individual DNA sets was the method of choice, as seen in Fig. 3. The sequence of each DNA set, as in B, was manually marked as in C, then the sequence was automatically read by the computer and bases were automatically tagged A, C, G, or T, as seen on the first zoom window in C, within 3–6 s and the galley proof editing on zoom windows was manually performed as seen in window F, within 10–35 sec. The sequence appearing in E is printed and saved on a hard disk.

For further convenience and time saving, the program contains an optional stop-scan/rescan feature that allows the user to optimize nonroutine imaging by readjusting the area to be scanned, brightness, contrast, resolution, global background correction, filtering, and reporting as optioned from the user-defined variables on the menu.

An optimal procedure for accurate reading of sequence on any molecular X-ray film regardless of good or bad quality is to stack sections of the

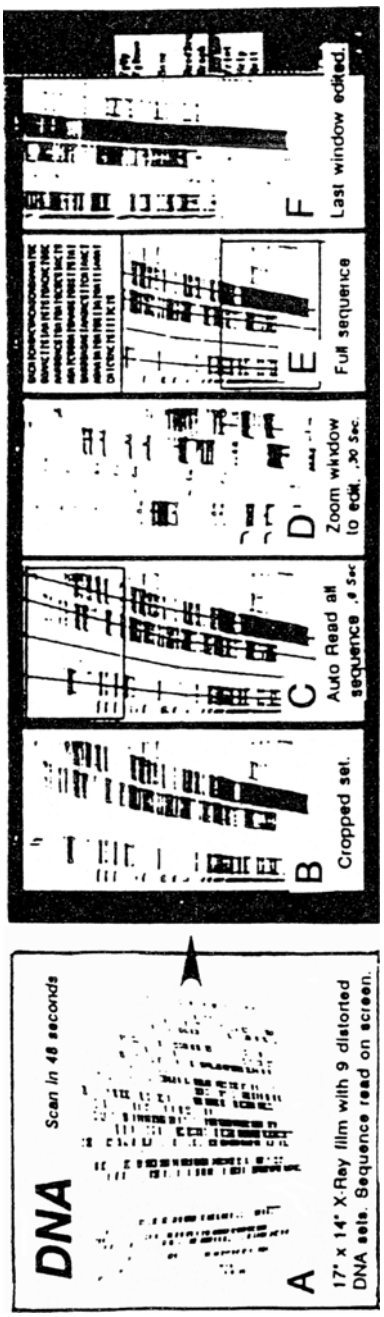

Fig. 3. DNA sequence reading: Imperfect 17″ × 14″ molecular X-ray film of 9 DNA sets, (A). Scan in 48 s. A DNA set is cropped as seen in B, the lanes are manually marked as seen in C, and full sequence is automatically read by the computer in 6 s. Bases are tagged with A, C, G, or T as seen on the first zoom window (D). The full sequence and last zoom window are seen in E. The last window in E is zoomed on as seen in F. Zoom windows allow manual proof editing of the sequence.

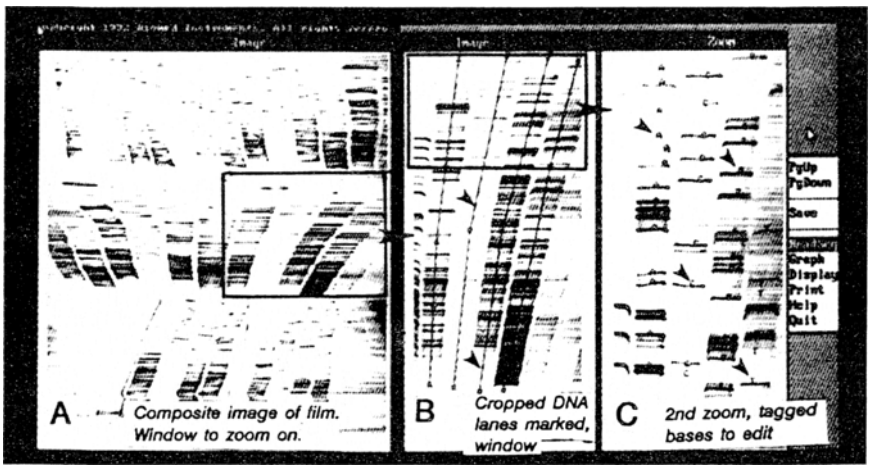

Fig. 4. Image of X-ray of Fig. 3A is stacked in segments one on top of the other to allow easy cropping as seen in (A) with lane marking of cropped and enlarged window as seen B. First, the top window of B is seen in a second enlargement zoom as seen in C. The multiple zoom windows such as seen in C, with bases tagged A, C, G, or T are utilized to proofread-edit the sequence. Each DNA set has 1–6 secondary zoom windows. A third order of zooming is available for reading closely stacked bases.

image one above the other (Fig. 4A) manually select a DNA set and frame it, as seen in Fig. 4A, and mark the lanes, as in Fig. 4B. Then, the computer reads the full sequence automatically and displays the first window with bases tagged A, C, G, or T, as seen in Fig. 4C, and then the user proofreads and edits the sequence by going over first to last enlarged windows.

For good quality films with straight and parallel multiple DNA sets, the sequence is read automatically by the computer (Fig. 5). The saved 2–4 Mb image of the film is recalled from the hard drive and its mini map is displayed on the computer monitor (Fig. 5A). Each set is then automatically marked, sequenced, printed, saved, and inaccuracies, if any, are flagged. Figure 5A shows the first DNA set marked and the frame of the fifth window. Figure 5B is the fifth window enlargement with bases of the first set marked A, C, G, or T. Manual proofreading can be done secondarily on the vertical window enlargement, if needed. Figure 5C shows the full sequence of the first set with the lower part of the last frame. Figure 5D shows the sixth or last zoom window, with bases tagged A, C, G, or T.

For molecular films with irregularities such as nonstraight, skewed, wavy, divergent, radioactive splashes, and artifacts there are two options:

1. The lanes are marked manually by pointing to multiple midpoints (Figs. 3 and 4); or

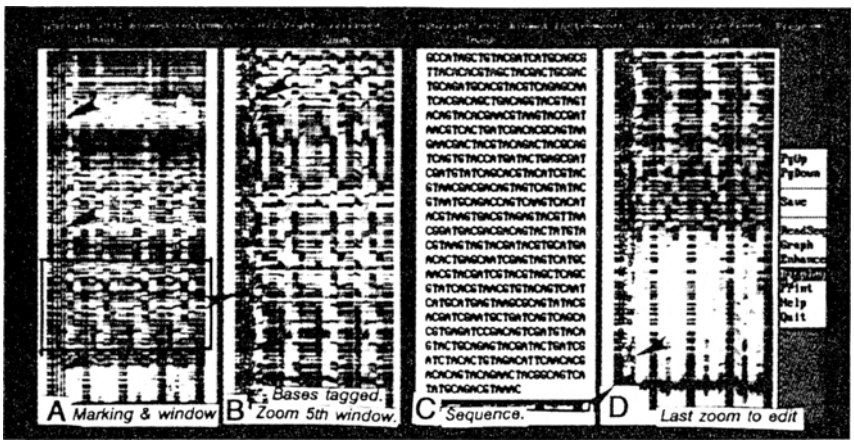

Fig. 5. Automatic sequence reading of good quality DNA 17″ × 14″ X-ray film. Image A with the leftmost DNA set is automatically marked, the enlarged fifth zoom is seen in B, and the full sequence is read, as seen in C. The last or sixth window of A is displayed in D. Editing and proofreading is not as good as in Fig. 3, but full automation is possible.

2. The artifacts are removed, the lanes are straightened vertically, the smiling bands are straightened, and the slanted or skewed bands are straightened horizontally by rotation around the centers. The image may be enhanced. Then the sequence is read automatically. This option is not recommended except as a last resort.

Most of the 54 X-ray films were imaged and then analyzed with apparent good reliability. In one film where the artifacts and round splashes are all over the film, the bases were manually tagged on zoom windows.

Image Enhancement

The radioactive imprint of A, C, G, and T DNA fragments that were electrophoresed on 17″ × 14″ polyacrylamide SDS gels and then imprinted on the X-ray film appear too dark at the side of the sample application and very faint at the other end of the film. To allow manual or automatic sequence rading, the image was qualitatively enhanced in the Y direction along the 4 DNA lanes and quantitatively in the X direction across the lanes. The quantitative enhancement across the lanes retains the ratios between the optical densities of the most dense base relative to its shadows. Quantitative enhancement is important in case of ambiguity where, for example, a dark base A, medium-dark base C, and shadows G and T appear at the same position. As seen in Fig. 6A, the original mid-segment

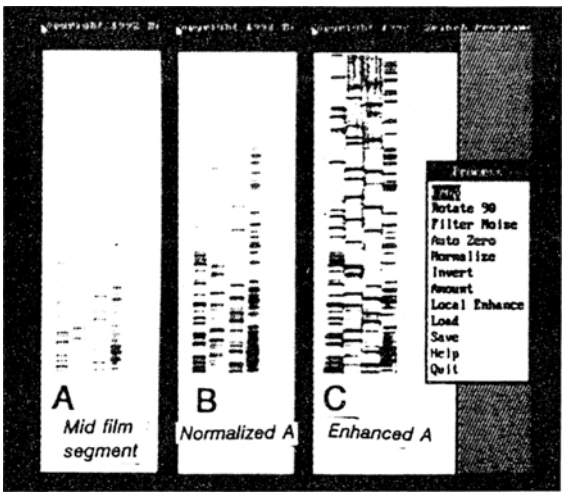

Fig. 6. Image enhancement. Original midsegment of a DNA set is seen in A, its normalized image is seen in B, and enhancement of A is seen in C. All faint bases are enhanced to be visible for proofreading and to allow automatic sequence reading. Normalization was not very helpful.

of DNA set on the X-ray film contains very faint bases that are hard to define visually. The normalized image of the segment is seen in Fig. 6B but normalization did not improve significantly the visibility of the faint bases. Enhancement of the original image of Fig. 5A resulted in all faint bases become clearly visible as seen in Fig. 6C.

Detection of Gene Mutation by Reading the Electrophoregram Overlay

Quantitative comparison of the control DNA G-lane containing 47 bases to its experimental counter G-lane revealed some differences. A segment of that region of control is seen in Fig. 7A; its counterpart of the test is seen in Fig. 7B. The densitometric tracing of A over that of B is seen in Fig. 7C. Subtracting one lane from another is seen in Fig. 7D. As seen in Figs. 7B, C, and D, a new G-base appeared in the test, indicating genetic mutation.

The presented flexible overlay of a one-dimensional electrophoretic lane over another is used for foot printing to detect the appearance or disappearance of a base and quantitates change in the protein content of each base. Reading from an enhanced image of the same lanes gave similar results. Using an image analysis program on control and test plaques and subtracting the image of control from the minor image of the test revealed that one colony is missing in the test, indicating a genetic mutation of that corresponding colony.

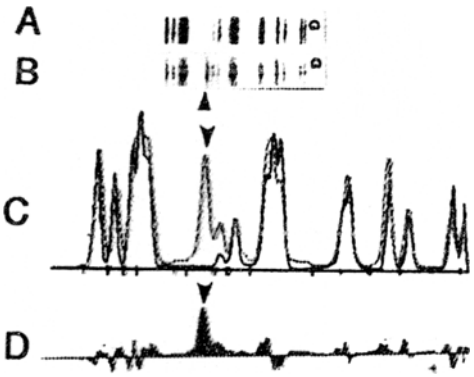

Fig. 7. Detection of gene mutation. A short segment of a long G-lane containing 47 bases of the control is seen in A, and its countersegment of test G-lane is seen in B. Lanes A and B were scanned and the graphic tracings were overlaid as seen in C. The result of subtracting one graph from the other is seen in D. As seen in B, C, and D, a new base is seen in the test but is absent in the control, indicating a genetic mutation.

Analytical PS Scanner as a Densitometry Station

Using dedicated computer programs on images that were obtained by the PS analytical scanner, the following media were successfully quantitated: one-dimensional gels, TLC, Western blots, chemilumenscence and X-ray film, 2-D gel electrophoresis and clinical two-dimensional TLC of glycoproteins in fetal maturity test, microtiter plate, membrane ELISA, electron microscope photos (image analysis), enzymatic color reactions in test tubes in their 48 tube racks, colony/plaque imaging, antibiotic sensitivity on Petri dishes, ELISA, or membrane micro ELISA, and particle size analysis.

DISCUSSION

The survey indicates that the majority of biotechnologists engaged in DNA research have no other choice except to use the human eye. The use of pen-readers, sonic, or subsurface electrodes is a convenience but has no significant advantage in terms of accuracy. Also, reading a sequence from images on the computer screen adds the advantage of screen magnification but still the human eye, with its inadequate resolution or acuity, reads the sequence. On the other hand, since the dedicated accurate systems of DuPont and Applied Biosystems are too expensive, scientists can

enhance their productivity, speed, and accuracy by the affordable analytical handheld PS scanner presented in this report.

Availing highly skilled investigators, who originate most scientific breakthroughs, with an accurate scanner is the real problem of substance. The survey indicating that most scientists who use electrophoresis or work with DNA have not obtained the needed accurate laser scanner is caused by laser scanners being a capital equipment item; bulky with prohibitive price tags. Even in an affluent economy, most researchers do not have access to the needed capital. With a closer look, the nature of the problem is the lack of advanced technology that combines power, accuracy, and affordability. The problem is parallel to that of the costly mainframe computers before the arrival of the PC.

Until the end of the 1970s, the mainframe computer was the only powerful computer system, a capital equipment item available only to departments or institutes with ample space and funds. With the advent of compact and powerful personal computers, most scientists now have the means to obtain at least one PC. The personal computer has become an affordable and needed laboratory commodity. The PC and its compatibles provide mathematical, statistical, and graphical functions with speed and accuracy that contribute to more effective scientific work. Similar development is reasonably foreseen for the PS analytical scanner—it is compact, powerful, affordable, and needed by the majority of biotechnologists and is foreseen to become an integral part of PC imaging in biotechnology laboratories.

This breakthrough in high technology introduces the PS scanner, an inexpensive compact, 1 lb scanner measuring 12×12×3 cm to replace the costly 35–85 lb conventional laser scanners that require considerable laboratory space. The PS scanner's price stands at 2–4% of equivalent conventional laser scanners. Without sacrificing performance, the new scanner has become a cost-effective affordable commodity whereas the conventional laser scanners remain high-priced capital equipment soon to be endangered.

Compared to conventional laser scanners, this PS analytical scanner has the following improved precision and performance features: 20 μm spatial resolution, high scan speed, (scan up to molecular full chest X-ray film in 48 s), selectible 8/12/16-bit (1–256/4096/32,768 gray levels) digitizing system, 0–3 OD range, UV-visible range with selectible wavelength light source, reflectance-absorbance-transmission-luminescence-fluorescence scanning mode, childproof student routines, and dedicated programs to read various media, such as electrophoresis, TLC, DNA sequence, gene mutation, and plaque imaging. However, affordability remains the breaking-in feature of this high-tech PS scanner.

The computer-assisted PS scanner appears to become a densitometry station on its own with versatile applications. The PS scanner, in addition to being able to read DNA, can quantify other media such as electrophoresis or TLC. It can read blots, microtiter plates, colonies, and plaques, and

can perform microbial identification, antibiotic sensitivity, phosphor imaging, and photometry. The needs of scientists engaged in electrophoresis, DNA, and biotechnology demand the scanner.

The PS scanner serves two major purposes. First, the PS scanner can be profitably priced at 2–10% of the conventional laser scanner weighing 30–95 lb and 120×80×40 cm in size, making the new scanner an affordable commodity. Second, the PS scanner requires virtually no laboratory space. Availability of laboratory space is as problematic as getting funds.

An experimental trial of a modified PS scanner utilizing a strong laser beam indicates a prospective use in phosphor imaging. In accomplishing this feature, the PS scanner may become a turning point for powerful techniques. Phosphor imaging is a hundred times more sensitive than X-ray film and has an 0–6 OD range instead of 0–3. The fact that the phosphor imprint is erased after a single reading is not critical because re-exposure is fast and simple. Also, this drawback is inherent to any type of scanner.

Initial field feedback looks very promising. A few departments have already expressed interest in providing a scanner for each faculty investigator who has a computer and works with DNA or electrophoresis. However, time will reveal the advantages and functions to be desired from the new PS scanner.

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