Implication of the Image Analysis Methods in Biotechnology*

By KIICHI FUKUI, KOH-ICHI KADOWAKI and KYUYA HARADA

Department of Molecular Biology, National Institute of Agrobiological Resources
(Tsukuba, Ibaraki, 305 Japan)

Introduction

Image information must have been the most important information when a human being makes a judgment, since more than 70% of external information taken by him are the visual information. Image information has been, however, paid a little attention and has been regarded as if it is inferior to the numerical information in the quality of information. The main reasons that are empirically believed are as follows. Firstly, photography, that is, conventionally a representative recording medium for the analog image information does not always guarantee reproducibility and perpetuity of the information. Secondly, image information has been difficult to be treated numerically. Thirdly, it had also been difficult to handle a lot of information by which human visual satisfaction could be attained, since both the hardware and the software of the system had been too primitive to manage it.

While image analysis methods have been developed recently and have begun to be applied to the biological fields by rapid development of microelectronics and image analysis theories. Image analysis methods can play an important role in order to seize reproducibly and quantitatively the construction and morpholology of an organism, in which construction and morphology have very important meaning together with quality and quantity of the constitutive materials in a tissue, in an organ and also in an organism.

We report here a chromosome image analysis system (CHIAS) developed especially for chromosome studies and present a concrete case of chromosome analysis. Several utilities of the CHIAS in biotechnology are also examplified, using case examples.

System architecture of the CHIAS

Basic design concepts of the CHIAS are that it can semiautomatically analyze the plant chromosomes directly picked up from an automated microscope. Detailed description of the system architecture of the CHIAS has been already reported[1,2].

Plate 1 shows the newest version of the CHIAS and its block diagram[3]. Images to be analyzed are taken into the system by a high resolution TV camera mounted on the microscope or attached at a copy stand with a zooming lens. They are digitally converted and are stored in each of 15 image frame memories of the CHIAS. An image frame memory consists of a 512×512 pixel matrix and each pixel has 256 steps of gray levels. Each image frame has also an overlay memory with one bit per pixel. Although an automatic operation mode is employed for the CHIAS, basically, a man-machine interactive operation mode can be adopted at every step of the image manipulation on the study demand. It means that the researcher can make a decision where his experience and knowledge are indispensable as in the case of recognition of a satellite or centromeric posi-

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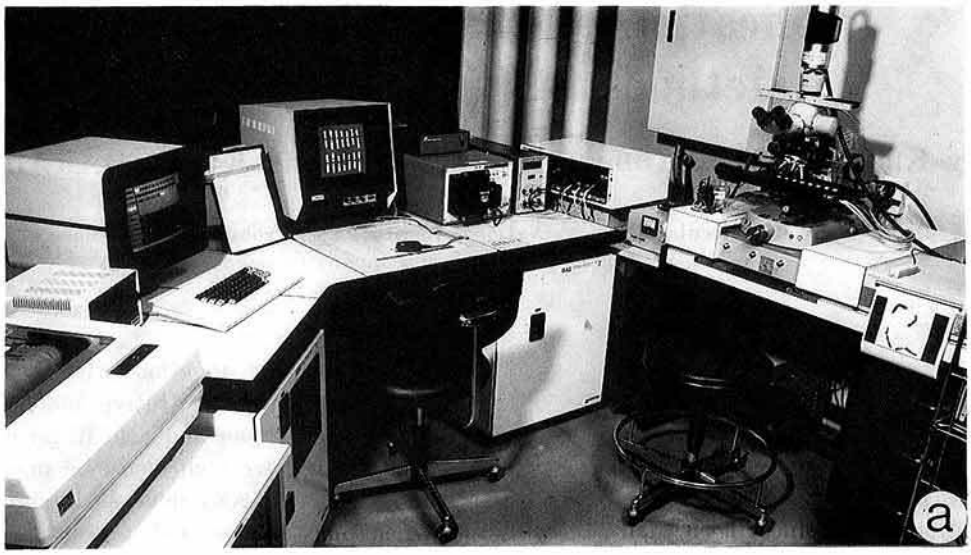

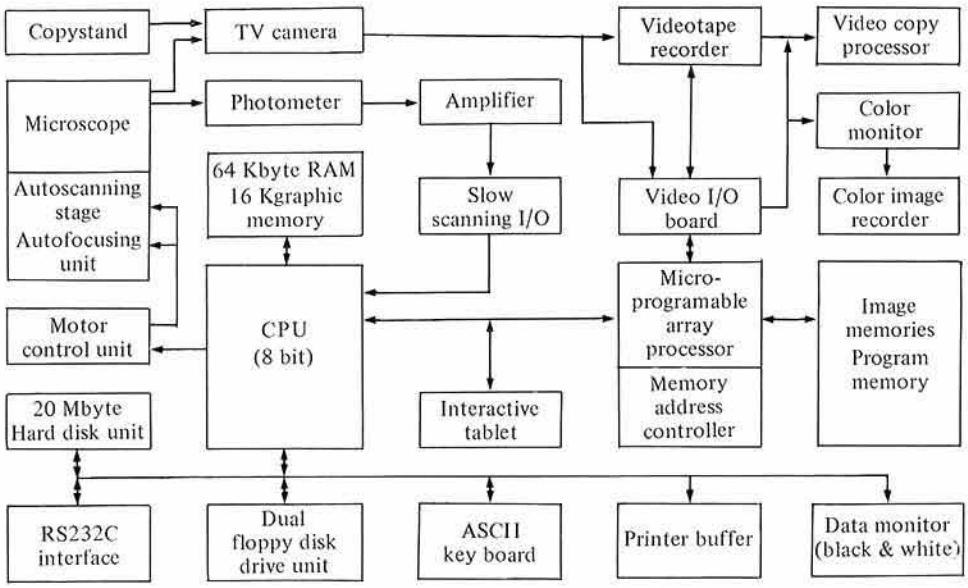

Plate 1. An overall view of the chromosome image analyzing system, CHIAS and its block diagram

tions. The CHIAS has, thus, a very flexible system architecture of a man-machine interactive mode.

Plate 2 shows an example of a command menu among more than 130 of image commands of IBAS-II (Zeiss/Kontron, Oberkochen, FRG), which is a main frame of the CHIAS. It is, thus, very easy to analyze a great variety of biological samples combining adequately these image commands, although the CHIAS has been originally developed for the chromosome analysis.

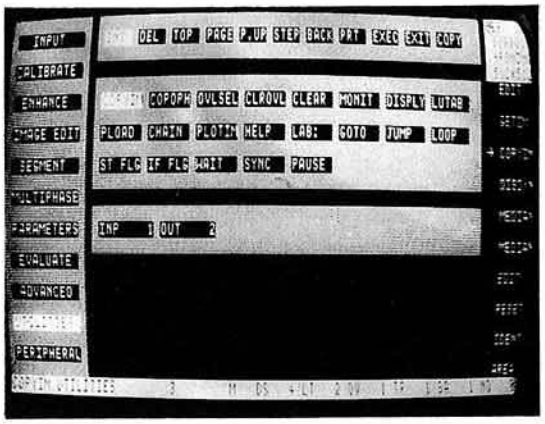

Plate 2. An example of command menu of the CHIAS displayed on a black and white monitor

Application of the imaging methods in biotechnology

1) Chromosome analysis

The software of the chromosome analysis is roughly divided into four parts. They are the program for automatic finding of the metaphase plates under the automatic microscope, the program for obtaining the numerical data of the chromosomes, and the programs for karyotyping and idiograming of the chromosomes.

Plate 3 shows the sequential representation of each manipulated chromosome image of haploid barley chromosomes. Although the more detailed description has been given elsewhere[3] already, the outline of the image manipulation is as follows. Metaphase plates were automatically searched and the overlay lines were drawn when they were found (Plate 3a). Then high magnification (4000×) was adopted for the target chromosomal plate. Twenty images of the same barley metaphase spread were taken into the system by each of 30 msec* and an averaged image of them was generated as an original image for the analysis (Plate 3b). Contrast of the image was then enhanced digitally (Plate 3c). Binarization of the gray image was proceeded and elimination of the dust particles was followed both automatically and interactively (Plate 3d). Plate 3e shows the chromosome binary image with a clean background. Centromeric and the satellite partitions were drawn interactively using the pseudo-colored chromosome images, which were colored differently based on the density level of each pixel. Pseudo-coloration could facilitate the human recognition of the centromeres and the satellite partitions much (Plate 3f). After interactive drawing of each mid-rib line on the chromosome, it was divided into the parts of the satellite, the short arm and the long arm. They were identified by the CHIAS using the different colors (Plate 3g) and the numerical data of length were obtained. Chromosome pairing of the putative homologous chromosomes was automatically carried out and each chromosome was arranged adequately in the karyotyping frame (Plate 3h). Chromosomes were rearranged in their order of length or according to the species specific order. The analysis was finalized by storing the results obtained in a floppy disk or by outputting them on a color image recorder etc.

Conventional karyotype analysis has been carried out by using photographs and a long dark room working has been inevitable. In the case of the CHIAS, however, no such operation is needed as it can pick up the chromosomal image directly from the microscope. Therefore a great deal of labor and time saving are both attained. In fact, 15 minutes are enough to complete data acquisition and karyotyping of a single metaphase plate now.

2) Pattern analyses of gel electrophoresis

Gel electrophoresis is now one of the essential techniques for the studies of macromolecules and is widely employed in the studies of proteins and nucleic acids. It sometimes happened that the gels would be broken by rough handling of it, as their physical strength is not enough. They would be broken especially when they are dropped on the floor and are not dried uniformly.

* msec: milli-second

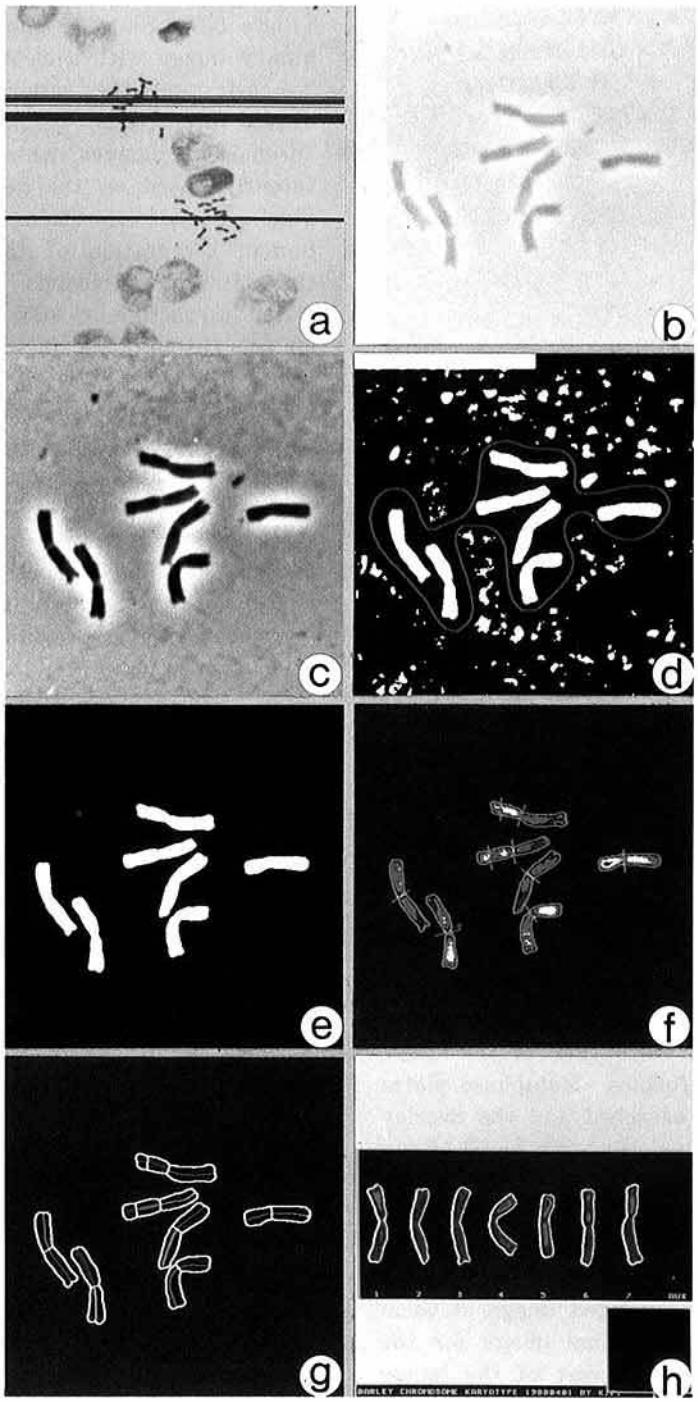

Plate 3. Sequential representation of the image analysis of a metaphase plate of haploid barley

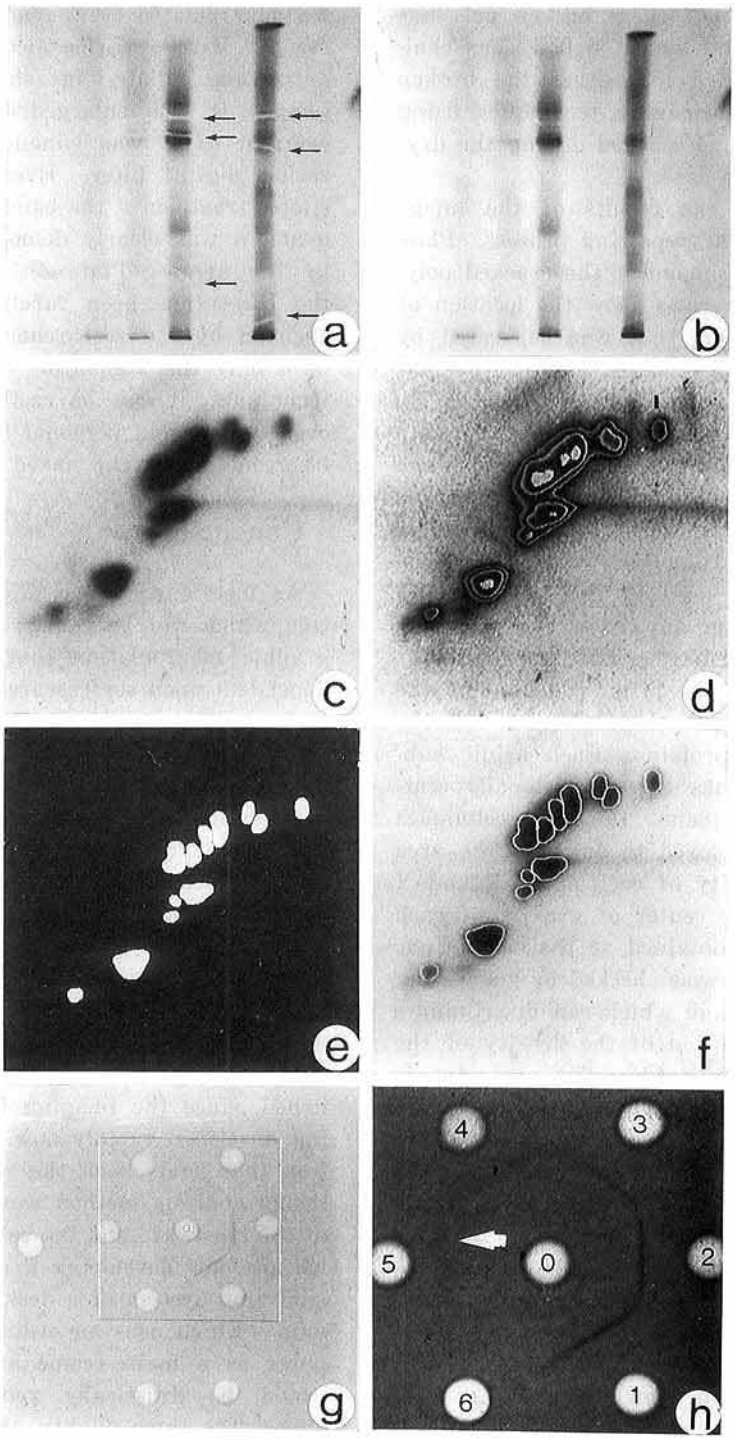

Plate 4. Several examples of image manipulation for the images of the gel electrophoreses

Physical restoring of these broken gels has been impossible and almost nobody has challenged it. We tried to recover the broken gel image by the imaging techniques using a polyacrylamide gel cracked during the drying as an example case.

Plate 4 shows the results of the image manipulation of the repairing process. Plate 4a is the original image of the cracked polyacrylamide gel. Arrows show the location of the cracks. The gel that was separated by the cracks was again sticked together by that length in order to fill the cracks. Plate 4b shows the image whose repairing had completed and the contrast of the original image was enhanced. It appeared that no disturbance by the cracks was observed and the original gel image was completely restored. The method that was used in this case can be applied for restoring any cracked gel and thus this method would have a wide application.

Plate 4c shows the original image of the two dimensional gel electrophoresis of the soybean storage proteins. Each acidic subunit of glycinin has two-dimensionally scattered on the gel plane. Imaging techniques can be easily employed to quantify the area and average density of each spot. X and Y coordinates of the center of gravity of each spot can be also obtained at the same time. At first, each spot was checked by generating a pseudo-color image which can discriminate the actual distribution of the density of the clustered spot (Plate 4d). The next step is generating a binary image of each spot (Plate 4e), following the check by the contour line generation (Plate 4f). The contour lines of the binary discriminated spots were superimposed onto the original gray image in order to check whether the discrimination was adequately done or not. Numerical parameters of each spot mentioned above were then measured.

Plates 4g and 4h show the imaging technique applied for enhancement of precipitation bands of micro-ouchterlong. Imaging techniques make it possible to quantify even the band that has been looked over by the naked eyes' observation. As a case example, we manipulated the image of precipitant reached with anti-rice alcohol dehydrogenase antiserum. Plate 4g shows the original image. It was enlarged two hold and the contrast of it was enhanced by a normalization digital filter. Even by these fairly simple treatments, the band between the wells 0 and 5 was clearly demonstrated as shown by the arrow (Plate 4h). Quantitation of the bands has been rarely carried out and decision by the researchers' naked eyes has been only the standard. Using the imaging techniques, it can be easily solved that the over-looking and personal fluctuation can not be avoidable by the naked eyes' judgment.

Conclusion

As mentioned above, the personal fluctuation which can be caused during the photographic manipulation that has been totally dependent upon the researchers' personal skill and experience, would be avoidable by imaging techniques. Moreover, the drastic time saving can be attained and not only the numerical information but also the image information can possess complete reproducibility and accuracy since they are digitized. Application of the imaging techniques to the biotechnological fields would allow to obtain the information which has been impossible to obtain, objectively.

It can not, however, be said that the standard application method has been established, since the imaging techniques are being developed rapidly now. Another obstruction that holds back the wide utility of the image analysis method would be a cost both of the hardware and the software. Therefore, we are now developing a chromosome image analyzing system of a desk-top type, CHIAS-mini[4] which uses an ordinary personal computer as a main frame and, thus, the cost would be drastically reduced maintaining almost the same quality of the original system, CHIAS.

It is, therefore, anticipated that the standardization of imaging techniques would be attained and the image analysis method would

be widely utilized as an indispensable method in biotechnology in the near future.

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