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APPLICATIONS OF IMAGE PROCESSING IN BIOLOGY AND AGRICULTURE

J. K. Sainis, Molecular Biology and Agriculture Division,
R. Rastogi, Computer Division, and
V. K. Chadda, Electronics Systems Division.

Introduction

Images form important data and information in biological sciences. Until recently photography was the only method to reproduce and report such data. It is difficult to quantify or treat the photographic data mathematically. Digital image processing and image analysis technology based on the advances in microelectronics and computers circumvent these problems associated with traditional photography. This new tool helps to improve the images from microscopic to telescopic range and also offers a scope for their analysis. It, therefore, has many applications in biology (Sainis, et al., 1994) However, as is the case with any new technology, imaging technology also has to be optimised for each application, since what each user is looking for in an image is quite unique.

Bhabha Atomic Research Centre (BARC) is a multidisciplinary research institute with advanced research programmes in many fields of science and technology including electronics and computer sciences on one-hand and biology and agriculture on the other. BARC is, therefore, an ideal place for developing the uses of image processing technology in many scientific disciplines including biology and agriculture.

Several applications of image processing technology for biology and agriculture have been developed in the collaborative programmes involving scientists and engineers from Electronics Systems Division, Computer Division, Molecular Biology & Agriculture Division, Nuclear Agriculture & Biotechnology Division and Cell Biology Division. These applications involve use of the camera based hardware systems or colour scanners for inputting the images.

Contents

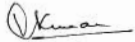
EDITORIAL

Dawn of Digital Imaging Technology in the Service of Life Sciences

Progress in basic sciences leads to technological advancements and application of new technologies in turn fuel further developments in science. A person who could use camera lucida and a graph paper to draw and measure microscopic objects was highly respected as biologist in the beginning of the 20th century. By the turn of this century the amazing developments in the dynamic field of computer applications to science have resulted in several breakthroughs. Digital imaging is a new addition to the repertoire of computer applications. This technology helps us to improve the quality of images as well as to quantify them and reduces the tedium involved in repetitive manual works.

Two feature articles in the present issue discuss about the application of this cutting edge technology to the field of biology. The first article deals with the use of imaging systems to various problems related to measurement of number of objects, their areas and also colour intensity. It also describes use of multispectral monitoring, feature extractions and digital image data bases for various problems in agriculture. The second article deals with the application of digital imaging system to cytogenetic analysis such as karyotyping, micronuclei tests as well as single cell gel electrophoresis.

These imaging systems and software programes have been developed in collaborative interdisciplinary projects in BARC and will be of immense help to all dealing with life sciences.



The software packages developed for biology include the BIAS software based on DOS and a Windows compatible ColorPro software developed in Electronics Systems Division and Comprehensive Image Processing Software (CIPS) developed in Computer Division. The salient features of these applications are described in the following.

1. Colour image analysis for estimation of leaf area, infected leaf area and chlorophyll

Leaf area estimation is very important in plant breeding. Earlier, leaf area meters were employed for this purpose. Now, image analysis can be used to measure the leaf area. Images of the leaves, captured by a camera or a scanner are analysed by the ColorPro software package developed by Electronics Systems Division. The area is obtained in pixels, which can be converted to cm^2 or $inches^2$ with the appropriate calibration of the system.

Many times a viral or a fungal attack on plants results in degradation of chlorophyll pigments in leaves. Such infected leaves have patches of green and yellow colour. In plant breeding, it is important to quantify the leaf infection, which needs area measurement of green and yellow sectors. This had been a very difficult task earlier which is now made easy by the ColorPro software. The software can perform area measurements on green and non-green sectors of the leaf, after colour thresholding the leaf image. Thus the extent of infection can be quantified without much efforts.

Plant leaf colour is also commonly used as an indication of health status of plants. The loss of chlorophyll content of leaves occurs due to nutrient imbalance, excessive use of pesticides, environmental changes and ageing. A variety of colour plates and chlorophyll meters were previously used to monitor chlorophyll content of leaves in situ. The ColorPro software can also be used for quantitative estimation of chlorophyll in situ.

The ColorPro software measures the intensity of colour for each leaf disk (Fig. 1A) in an arbitrary unit of intensity called inverse integrated gray value per pixel which is

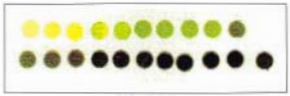


Figure 1 A

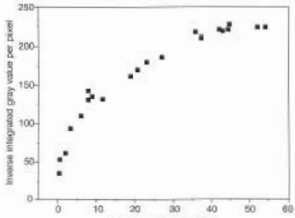


Figure 1 B

Figure 1: In situ estimation of chlorophyll in leaf disks. A: Leaf disks with different amounts of chlorophyll used for in situ estimation of chlorophyll. B: Relation of the inverse integrated gray value per pixel to the actual amounts of chlorophyll per pixel

proportional to the actual concentration of chlorophyll per pixel (Fig. 1 B). Thus the concentration of chlorophyll per pixel can be measured from the colour image of any leaf taken under standard conditions of illumination. The concentration of chlorophyll per pixel multiplied by the area of leaf in pixels will yield the total concentration of chlorophyll per leaf.

2. Protein Estimation by ColorPro

Protein estimation is an important technique in many biochemical experiments including those associated with plant biochemistry. Most routinely used methods of protein estimation are based on spectrophotometric measurements, which are cumbersome, laborious and may require large quantities of protein. A new method has been developed for protein estimation using the ColorPro software (Bannur, et al. 1999). The procedure involves spotting a constant volume of protein solutions (standard and unknown) on nitrocellulose paper and staining with Ponceau S and destaining. The digital colour scanner is used to grab the image of the pink spots. The intensity of the colour for each spot is digitised and measured in arbitrary units termed as inverse integrated gray value. This value shows a discernible increase with increase in protein concentration from 0.1 μg to 30 μg of protein per spot. The method is simple and allows the analysis of several samples simultaneously.

3. Colony counting by image analysis

The advent of biotechnology has resulted in a need for adaptation of microbial techniques in many fields of applied biology. The ColorPro software has routines, which can be used for counting bacterial colonies. Different parameters of the colony like intensity, size, form factor, connectivity and colour in the image can be taken into consideration while counting the colonies. Enhancing the contrast during acquisition and optimisation of thresholding yield better images for more accurate and faster counting.

4. Use of image analysis in study of electrophoregraphs

Electrophoretic separation of the proteins on polyacrylamide gels is a commonly used technique in biology. The protein gels often show large number of bands, which have to be compared among different samples. The storage, retrieval and analysis of electrophoregrams with the help of computer based image analysis system is useful where large number of samples are to be studied and compared with a standard pattern of bands. The ColorPro and BIAS software packages can be used for comparative analysis of protein gels, as well as DNA gels after silver staining.

5. Software for automated reading of DNA sequencing autoradiographs

DNA sequencing, the method of determining the order of occurrence of nucleotides in a DNA molecule is commonly performed either by the chain termination method or by the chemical degradation method. Figure 2 shows an example of a DNA sequencing autoradiograph and the sequence as determined from it. The sequence is read

from the bottom to the top by representing each band in the respective lane (labelled G, A, T and C) by that particular base and the order of occurrence of these bases in all the four lanes together represents the DNA sequence of the template used in the sequencing reaction (in the 5' to 3' direction).

Reading the sequence from the auto-radiographs is one of the tedious steps in large-scale manual DNA sequencing.

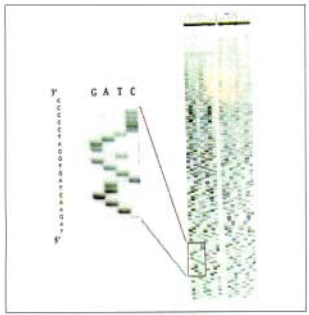


Figure 2: DNA sequencing autoradiograph

This step may also contribute to some errors in the sequence output. Windows based software has been developed in Electronics Systems Division as well as in Computer Division, that enables the user to generate the DNA sequence from digitised autoradiographs. These programs accept input images in a TIFF format (Tagged Image File Format) which in turn are generated by scanning the autoradiographs on a flatbed scanner. The user has to select the lanes and label them as G, A, T or C and detect the peaks in each lane. The program then generates the DNA sequence from the four of the selected lanes in ASCII text format and stores in a file. The program achieves 80% accuracy in automatic mode. An interface has been provided to edit missed/wrongly detected peaks in both the programs, which can increase accuracy of the sequence to 100%. The errors are mainly due to the inability of the programs to resolve the multiple peaks (doublets, triplets, etc.) as individual peaks, particularly when they occur very close to one another and form diffused bands in the lower portion of the autoradiographs.

6. Multispectral monitoring, a useful technique for agriculture

Multispectral monitoring is used for analysis of the physiological status of crops in remote sensing. These operations can be simulated with the use of CCD camera and band pass filters. The digital images of the leaves are grabbed with various bandpass filters and the reflectance is measured using the BIAS software (Fig. 3).

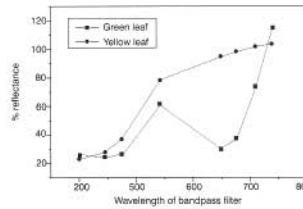
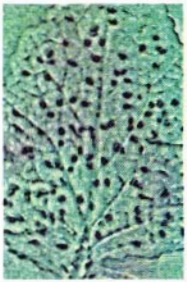


Figure : 3 Reflectance percentage of green and yellow leaves with respect to the background at different wavelengths

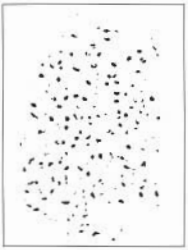
If the leaves are healthy they absorb more light in red region and also reflect more in near infra red region. BIAS software offers a facility for pixel to pixel subtraction of the images. If the image taken with red filter is subtracted, from the image taken with near infra red filter, the healthy leaves will appear brighter due to large differences in reflectance. Since the unhealthy leaves reflect considerable light in both red and near infra red regions, their image will be darker after subtraction. Thus, multispectral imaging can help to monitor the physiological status of crops in fields and will, therefore, be very useful in agriculture.

A: Image of Aphid infected Leaf

B: Hue Component extracted

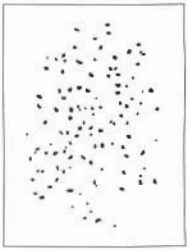


C: Adaptive Histogram Equalisation and Median Filtered Image



D: Thresholded Image (thresholded at 213)

E: Eroded Image



Count = 115
F: Dilated Image

Figure 4: Image processing and analysis for quantification of A: image of aphid-infected leaf, B-F: various image processing operation as indicated in the captions done before obtaining the final count.

7. Colour image processing and analysis to count the insects on a leaf

Insect pathogens are a menace to the plants. Plant breeders aim to develop insect resistant varieties. In these efforts a plant breeder has to quantify the extent of infection on a variety of plant parts. Colour can be an important descriptor in quantifying the insect infection. A software has been developed by Computer Division using a combination of image processing routines and true colour enhancement to count insects on a leaf. The image enhancement techniques viz. Red-Green-Blue to Hue-Saturation-Intensity conversion, adaptive histogram equalisation, median filtering, thresholding and morphological operations are used before obtaining the final count of insects on the leaf as shown in figure 4.

8. Image analysis for morphometry of wheat grains

Morphology of grains is important in determining its acceptance and identification. Morphometry can be used

to identify or characterise a particular type or a variety. Before the advent of image analysis it was difficult to quantify the morphological parameters of food grains. Manual measurements of size of grains are cumbersome, and also there is a limitation on numbers that can be measured. The CIPS software developed in Computer Division facilitates the measurement of several morphological parameters such as area, perimeter, eccentricity, compactness, major and minor axis length, average axis length, axis ratio, spread and slenderness from the gray images of the wheat seeds arranged with their crease-down. It was observed that use of scanners in transparency mode with higher resolution during image capture yielded better results. The moment analysis is also done on the gray image of each grain. The standard

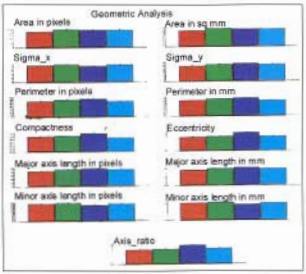


Figure 5A

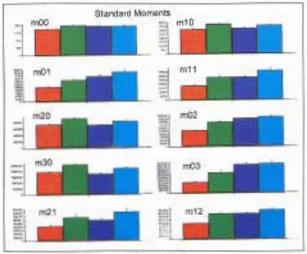


Figure 5B

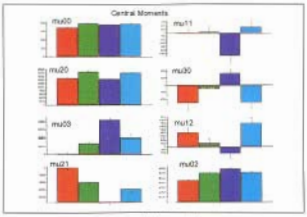


Figure 5C

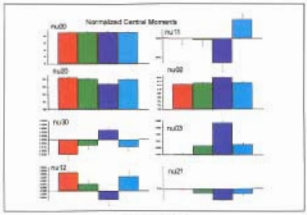


Figure 5D

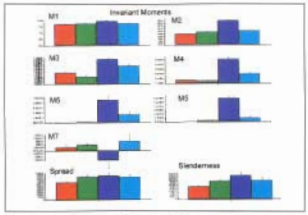


Figure 5E

Figure 5 Morphometry on seeds of four different selections of wheat. A-Geometric parameters. B-Standard Moments. C- Central Moments. D- Normalised Central Moments and E- Invariant Moments. The Y axis represents mean of 12 seeds for each parameter. The bars represent standard error. Selection 1 - red, Selection 2 - green, Selection 3 - dark blue and Selection 4 - light blue

moments are calculated for each grain, which are then used to compute the central, normalised central and invariant moments.

Figure 5 shows geometric analysis (A), standard moments (B), central moments (C), normalised central moments (D) and invariant moments (E) of the seeds of four wheat selections. In these wheat selections the geometric parameters of seeds are more or less similar, however some of the moments are distinctly different. As is evident from figure 5 A-E, the program calculates numerical values (mean and standard error) for over 40 different shape-related parameters. Comparison of all these parameters among different varieties can give useful information for variety identification.

9. Software for digital database for plant taxonomy

Taxonomy provides important information for identification and classification of plants. Conventionally, the information on the flora is stored in herbarium. The plants and plant parts are collected from the wild, identified correctly, dried among blotting papers, treated with anti-fungal, anti-bacterial chemicals, insecticides etc and stitched on a standard size herbarium sheet for long-time storage and use. All the information collected about the plant like locality of collection, date, morphological characters of plant like leaf and flower morphology, branching, venation, inflore scence, fruit and seed character are written on a separate label and pasted on the herbarium sheet. Students, taxonomists and researchers use such herbarium sheets to identify the plant/s of their collection. A considerable effort, time and money has to be spent on this method of preservation. Additionally, the maintenance costs are enormous. The most important drawback of this system is the space for the herbarium as the sheets are to be stored either in metallic or wooden cupboards. In spite of good maintenance and care, the dried, brittle plant materials are gradually damaged and valuable materials are lost due to continuous handling, weather changes, etc. Taxonomy is an important area in biology; however, the interest in this discipline has dwindled over the years with the result that there are very few people who can correctly identify plants.

A program has been developed with the help of Computer Division for storing the images of plants and plant parts along with the relevant taxonomic information. A provision has been made in this program where the database can be searched using few key words and information about any plant can be retrieved along with its image. This is a novel method for storing the taxonomic information compared to the conventional cumbersome procedure of preparing the herbarium sheets. Fresh plants can be directly imaged and their natural leaf and flower colours can be preserved in an image. This program can be used to create a digital database for taxonomy and when ready, this database can be made available on internet or CD.

Such image databases are also useful for many other purposes in biology. Using similar program breeders can store the images and other relevant information about all their selections. The images of entire plant, leaves, flowers, seeds including images of the protein gel patterns can be stored and these databases can be subsequently used/searched any time.

Conclusion

We have attempted to extend image processing and analysis technology to a broad spectrum of problems in biology. A software CytoPro has also been developed for chromosome analysis, which can quantify the microscopic images of cells and chromosomes with the help of a CCD camera mounted on the camera port of a trinocular microscope. All the applications mentioned in this article are described in details in Lecture notes for DAE-BRNS Workshop, 1998. Some of the software programs for image processing and analysis described above are now available in the market and a question is generally asked as why should one try to develop indigenous systems. The computer based image processing is undergoing rapid evolution in parallel with computing systems. The dedicated imaging systems available in the market, where user can press a few keys and get the results, are

not very versatile and more importantly, they have a very high price tag on them. Additionally, it is hard to discern as to how the results are being produced. The open ended imaging systems in BARC are mainly meant for those scientists who i) care to follow how their image data is processed before producing the final results, ii) want to upgrade and innovate their systems with changing times, and also iii) want to search new frontiers in their fields for applications of this technology.

Note :

- A FiveDay DAE-BRNS Workshop on Applications of Image Processing in Plant Sciences and Agriculture (WIPSA) was organised at MB&AD, BARC during October 26-30, 1998 to introduce plant biologists to the basic principles and applications of image processing technology in biology. This workshop was organised jointly by Molecular Biology and Agriculture Division, Electronics Systems Division and Computer Division. Over 35 senior scientists from different national laboratories and universities in India attended this workshop and expressed their desire to collaborate with BARC in the area of image processing and analysis (Nuclear India, 1998).

- The programs for applications from 1 to 6 above have been developed in Electronics Systems Division. Mr D.G. Joshi, Mr Y. V. Rao, Mrs Valli-Kumar, Mrs L. Pious, Mr S. Kar, Mr S. V. Kulgod and Mr S. V. Bannur are actively involved in this work. Mr K.G. Krishna, Mrs M. Mathur, Mr A. V. S. S. Narayana Rao, Mrs S. S. Metkar from Molecular Biology & Agriculture Division, and Dr K. S. Reddy, Mr V. Das and Dr S. E. Pawar from Nuclear Agriculture and Biotechnology Division have collaborated in the developing these software programs.

- The programs for applications 7 to 9 were developed in Computer Division. Mrs S. P. Shouche, Mr J. Rajeshwar, Dr (Mrs) S. M. Mahajan, Mr J. J. Kulkarni from Computer Division and Dr S. G. Bhagwat and Dr V. Abraham from Nuclear Agriculture and Biotechnology Division have taken keen interest in developing these software programs.

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DEVELOPMENT OF A SEMI-AUTOMATIC DIGITAL IMAGING SYSTEM CYTOPRO FOR CYTOGENETIC ANALYSIS

R. C. Chaubey, Cell Biology Division, and
S. V. Bannur, S. V. Kulgod and V. K. Chadda, Electronics Systems Division

Introduction

First computer based imaging system for human chromosome analysis was developed in 1976 (Castleman & Melnyk, 1976). This system was developed for automatic scanning of metaphases and chromosome karyotyping. However, the system was not much used because it lacked the required sensitivity and accuracy. Due to recent developments in the field of microelectronics, imaging and computational power, there is revival of interest in the field of imaging techniques all over the world, to develop and perfect systems for karyotyping and other cytogenetic applications on human and mammalian chromosomes.

Digital imaging techniques for cytogenetic analysis have drawn considerable attention all over the world. Cytogenetic analysis of metaphase chromosomes and micronucleated cells, from mammalian and human tissues, are being increasingly used in the field of radiation and chemical mutagenesis. Chromosome aberrations, particularly dicentrics and rings are specific biomarkers of radiation exposure and are used for biological dosimetry in accidental radiation exposure cases (Salomaa, et al. 1997). More specialized techniques, e.g. G-, Q- R-, high-resolution banding and the molecular cytogenetic technique, fluorescent in situ hybridization (FISH), are increasingly used to detect precise asymmetrical and symmetrical changes in chromosomes (Dauwerse et al. 1992). Manual scoring and karyotyping of chromosomes is very tedious, time consuming and requires a great deal of expertise on the part of scorer to achieve accurate results.

An indigenous digital imaging system is being developed at BARC for cytogenetic analysis. The software (CytoPro) has been developed for numerical counting (e.g. aneuploidy and polyploidy) and karyotyping of metaphase chromosomes and size measurement of cells, nuclei, micronuclei, cell / cytoplasm ratio in rodent and human cells including erythrocytes and lymphocytes.

System description

The system consists of a Leitz Orthoplan microscope, with 0.5x camera adapter lenses, high performance color camera with 750 lines horizontal resolution (KY-F55BE 3CCD, JVC Japan), Flash Bus [1]MV Video Frame Grabber, (Integral Technologies, INC, Indianapolis, Indiana, USA), P-II computer with CD writer for image storage, UMAX Astra 1220 S Scanner with Transparency Adapter and HP DeskJet 880C printer.

Methodology

A typical set of processing operations includes image acquisition, pre-processing, and segmentation analysis of parameters and object classification. A video camera provides a video signal of the sample under microscope or from a photograph. A PC-based frame grabber board digitizes this signal and stores the images into the computer. A high-resolution video camera and a frame grabber ensure acquisition of images without loss of useful information from the samples.

The system includes several processing functions to sequentially process the images for analysis. These operations can either be performed automatically one-after-another without any human operator intervention or can be carried out interactively by observing the processing results at each stage and modifying the processing parameters.

The acquired images have to be pre-processed to remove acquisition artifacts for improving the picture quality. The signal to noise ratio is improved by frame averaging technique. The fluctuations in supply voltage of a microscope lamp or other illumination sources cause the

gray level/color variations in the acquired images. These shading corrections have to be carried out to compensate for non-uniform illumination across the samples.

Next step of processing is to separate the objects (cells/chromosomes/micronuclei) from the background using a thresholding technique. The system allows either interactive setting of threshold or quicker auto-thresholding method based on Gaussian bimodal histogram model. Measurements performed are in pixel units. The system has to be calibrated to find the scaling factor with respect to camera and microscope setup. Cells, nuclei, micronuclei can now be measured and also ratios of their dimensions are available. Point and click operation facility is available for measurements.

Micronucleus test

Micronucleus assay (MNA) is one of the widely used cytological techniques for assessment of cytogenetic damage of radiation and chemical mutagens (Chaubey et. al., 1978, 1993; Morita, T. et al. 1997). This technique is relatively faster and equally sensitive as metaphase analysis and is used for biological dosimetry in radiation exposed cases (Fenech, M. 1991). The test is based on the principle that chromatin fragments, which are produced by clastogenic agents or whole chromosomes which lag behind at anaphase due to spindle malfunction, are not included into the nucleus of the daughter cells and they in turn give rise to micronuclei. The frequency of these micronuclei or micronucleated cells are taken as criteria of the extent of genetic damage. A modification of this technique has been developed for in vitro studies with rodents and human cells where cytoplasmic division is blocked by using cytochalasin B and the resulting micronucleated binucleate cells are scored for assessing the cytogenetic damage (Subha Venkat et al. 1996).

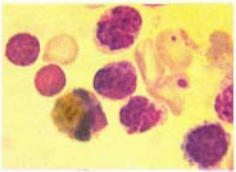
Fig.1.Top two panels shows the micronucleated erythrocytes from the bone marrow of gamma irradiated AKR mice. The bottom panel shows the image window, dialogue window for image processing and the result window showing the values of the measurements. Further examples are displayed in Fig.2, which shows the micronuclei in cytochalasin B blocked bi-, tri- and quadrinucleated lymphocytes from human peripheral blood. These lymphocytes can be measured quantitatively using CytoPro package developed at BARC. (Fig. 3)

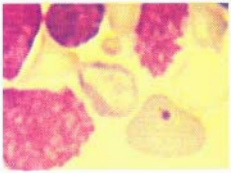
Metaphase analysis of human chromosomes

Cytogenetic analysis of metaphase chromosomes has provided valuable information about various abnormalities and their frequencies in human population. Human peripheral lymphocytes were first cultured successfully in vitro by Hungerford et al.(1959), in subsequent years phytohaemagglutinin (PHA) was discovered and was used as a mitogen by Nowell in 1960. In the same year Moorhead et al. (1960) developed the air-drying method for metaphase chromosome preparation. These landmark developments in the field of cytogenetics made it possible to use metaphase analysis and karyotyping for various applications in the area of radiation and chemical mutagenesis including clinical cytogenetics.

According to International system for Human Cytogenetic Nomenclature (ISCN), 1985, the human chromosomes are classified in to seven groups, A (1-3), B (4-5), C (6-12), plus X-chromosome, D (13-15), E (16-18), F (19-20) and G (21-22 plus the Y-chromosome).

For karyotyping of human chromosomes, various procedures such as image acquisition, pre-processing and segmentation operations have to be executed. The software provides image-editing facility to take care of touching chromosomes; and cut, copy and paste facility for taking care of overlapping problem of chromosomes. Each feature is labeled with a tag and feature specific measurements are performed on chromosomes with facilities to exclude features smaller than the desired size; load chromosome data on structure i.e. width, height, length, area; location in the image, centromere position and count; and short arm to long arm (p/q) ratio of chromosomes.





ColorPro

File Gray/Color Detect Binary Measure Edit MBAD CBD AFD FTD Windows Help

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Threshold

Pix 49.52
Units
%Roi
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R I
G H
B S
+ - ×
Result LT UT
0 206
Hist
AutoL
AutoH
OK
Clear

Result

Bone marrow smear from mice exposed to gamma rays (1.0 Gy).
Area measurement of nucleus and micronucleus.

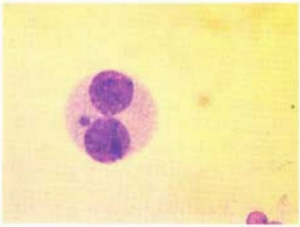
Sl. No.	File No.	Area (Sq.mic.)	LT	UT	Remarks
1	A:\AKR8.BMP	2.69	0	176	MN
2	A:\AKR8.BMP	49.52	0	206	Nucleus

Fig. 1: Bone marrow smear form gamma irradiated (1 Gy) AKR mice showing micronuclei in erythrocytes (Top two panel) and dialogue box for area measurement and the result window (Bottom panel). The cells are stained with May-Grunwald Giemsa.

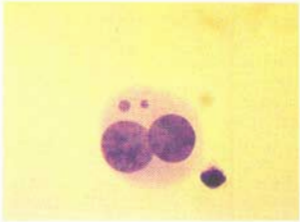
Fig. 4 shows normal human metaphase chromosomes stained with Giemsa dye. Fig. 5 shows an image display window and a dialogue window for various operations such as thresholding, editing, changing the orientation, enlargement and reduction of image; numerical values for length and p/q ratio of each chromosome, etc. Here the chromosomes are arranged on the basis of the length and p/q ratio. Fig. 6 shows a karyotype of human metaphase chromosomes into various groups as per ISCN system (1985) and the dialogue window for various operations for karyotyping.

Single-cell gel electrophoresis

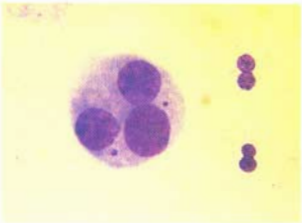
Single cell gel electrophoresis (SCGE) or the comet assay is a relatively new technique developed for monitoring DNA damage in individual cells both in vivo and in vitro (Singh et al. 1991). In this technique the cells are lysed at 4°C and treated with proteinase K at 37°C and electrophoresis is carried out in a horizontal electrophoresis unit. The cells are stained with DNA specific fluorescent dye e.g. propidium iodide or ethidium bromide and observed under fluorescent microscope with suitable filter set. Migration of DNA measurement is taken as the criteria of genetic damage. Fig. 7 shows the area



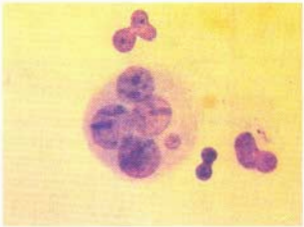
BNC from human peripheral blood with single micronucleus.



BNC from human peripheral blood with two micronuclei.



Trinucleate cell from human peripheral blood with two micronuclei



Quadrinucleate cell from human peripheral blood with single micronucleus

Fig. 2 : Cytochalasin B blocked human lymphocytes showing bi-, tri and quadrinucleate cells with one or two micronuclei stained with Giemsa dye.

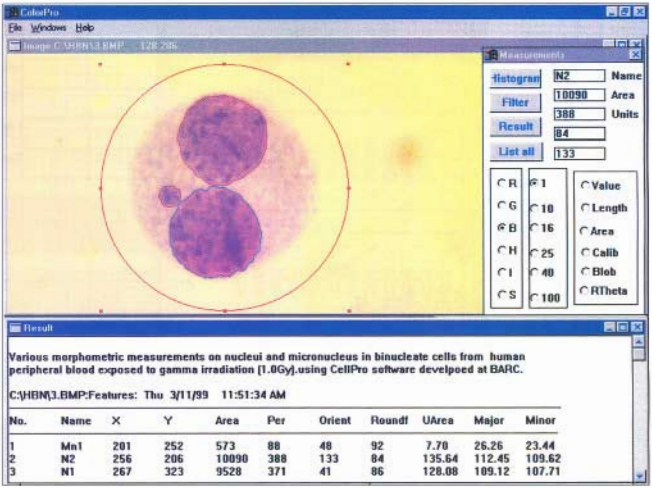


Fig. 3: Micronucleated binucleate cell from human peripheral blood showing size measurement of nuclei and micronuleus.

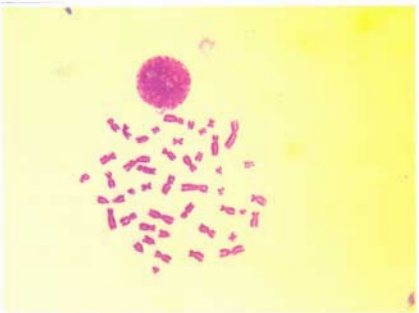


Fig. 4: Human metaphase plate, showing 46 (2n) chromosomes

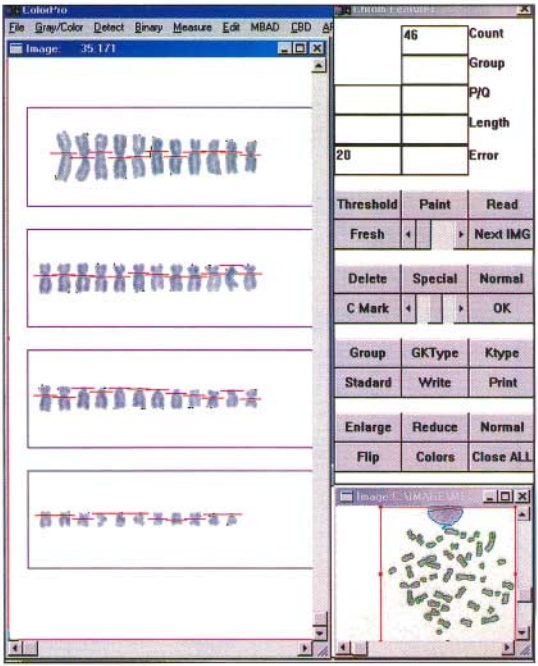


Fig.5 Human metaphase chromosomes arranged on the basis of size. This operation of the software can be used for karyotyping animal and plant chromosomes

measurement of DNA from control and treated nuclei following single cell gel electrophoresis. The figure shows the area measurements in control nucleus, damaged nucleus, dialogue box and the results windows.

Advantages of digital imaging system

The Digital imaging system for cytogenetic analysis provides several advantages. The software allows wide range of image enhancement operations; powerful image measuring tools, quantitative measurements, complete set of morphological operations, time and frequency

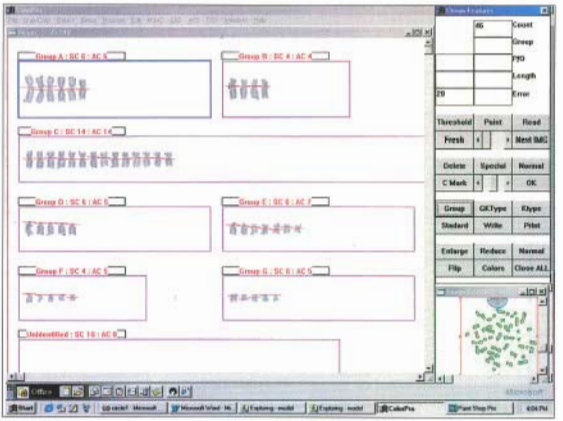


Fig. 6: Human metaphase chromosomes karyotyped by the CytoPro system

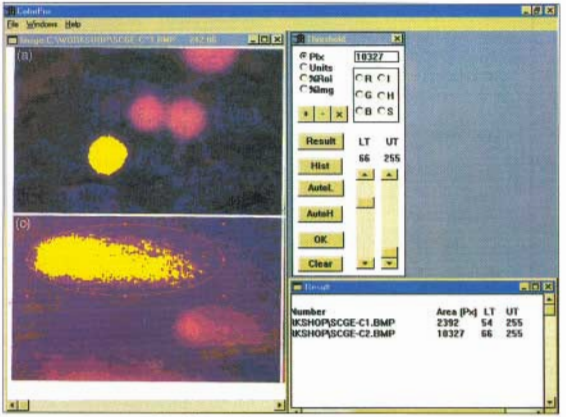


Fig. 7: Area measurement on control (panel a) and radiation (1Gy) exposed nuclei as measured by single cell gel electrophoresis. Figure also shows the dialogue box for area measurement and the result window.

domain processing; storage and retrieval of images; scanning, verification of results, statistical analysis and a user friendly graphical interface. The software has been developed and requires validation for various applications. The software has already been validated for area measurement of micronuclei and shows good reproducibility.

Acknowledgements

The work reported here forms a part of the IX'th Plan Project "Research in Frontier Areas of Biosciences" of Cell Biology Division. This project was initiated as a Collaborative program between Cell Biology Division and Electronics Systems Division, by Bhabha Atomic Research Centre, Mumbai. We wish to thank Dr. P.S.Chauhan, Head, Cell Biology Division and Mr R.K.Nigam, Head, Electronics Systems Division, for their keen interest and encouragement throughout this work. We wish to thank Mr H.N.Bhilwade, and Ms. Subha Venkat, Ph.D. Student under Dr K.S. Krishnan Fellowship, working at Cell Biology Division, for their help during this work. We also wish to thank Dr (Mrs.) J.K.Sainis, Molecular Biology and Agriculture Division for providing us an opportunity to present a part of this work at DAE-BRNS Workshop on Applications of Image Processing in Plant Sciences and Agriculture (WIPSA) at BARC, October 26-30, 1998.

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DECONTAMINATION OF FULL PRIMARY HEAT TRANSPORT SYSTEM OF MAPS II

The primary heat transport system of Madras Atomic Power Station II (MAPS II) was successfully decontaminated during Feb. 24-25, 1999. The fifth campaign in Indian PHWRs and the second attempt at MAPS-II by the indigenous decontamination process went off smoothly and brought down the radiation field in the PHT system components by a factor ranging from 2 to 14. The regenerative dilute chemical decontamination (DCD) process employed here used Ethylene Diamine Tetra Acetic Acid (EDTA) as the main formulation constituent. An organic acid, a reductant and a corrosion inhibitor were the other constituents of the formulation added to aid the dissolution of the radioactivity contaminated oxide layer without significant loss of base metal. The schematic of the DCD process is given in decontamination of the whole heat transport system with fuel in the core and continuous regeneration of formulation chemicals by cation exchange resin are the special feature of this process. Regeneration enables decontamination to be carried out with only 0.1% w/v concentration of the formulation. The formulation constituents are added to the heavy water coolant by a slurry addition pump. During the decontamination the chemical parameters, required for evaluating the progress of decontamination, were monitored jointly by water and steam chemistry laboratory and MAPS chemical laboratory.

In this campaign ten lead shielded cation exchange columns of 500 litre volume each were used. Increased availability of the cation resin helped to remove more amount of magnetite/oxide and the associated activity from the PHT system structural materials. Higher decontamination factors (DFs) were achieved in many components especially on steam generator surfaces. The DFs achieved on various components of the PHT system are given in Table-1. A total of 260 kg of iron and

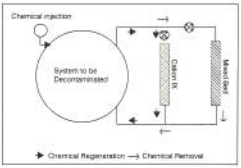

Fig. 1 Logistics of dilute chemical decontamination

82 Ci of activity that includes ^{60}Co (50 Ci), ^{137}Cs, ^{106}Ru, ^{59}Fe and ^{58}Co were removed from the system and collected on ion exchange resins.

Evaluation of coupons installed for monitoring the corrosion loss suffered by various structural materials during the DCD process gave a corrosion rate value of 0.20 μm/h for carbon steel and the base metal loss was below the detection limit of the weight loss method for monel-400, zircaloy-2 and SS-304.

Table 1

Decontamination Factors for the component/material

Component/Material	DF
Carbon steel surfaces (General)	2-5
Carbon steel feeders & Headers	4-14
Monel 400 surfaces :	
Boiler cabinets	2.6-3.5
Shutdown HXs	2-2.6
Bleed cooler	2-3.5

Subsequent to decontamination and removal of formulation constituents by the mixed bed ion exchange columns, the coolant was brought to the normal operating conditions and the system was hot conditioned at 195°C for a duration of 96 hours. The hot conditioning ensured that loose oxide particulates are removed from the PHT system, no organics are left behind in the system crevices and exposed free surfaces are covered and healed with a fresh magnetite film.

Significant reduction in radiation field by the chemical decontamination campaign is expected to result in large saving in MAN-REM during the on-going and subsequent shut down maintenance jobs of the power station.

POKHRAN -II LEADERS FELICITATED

In a glittering function organised by the Department of Atomic Energy on May 11, 1999 at the Tata Institute of Fundamental Research to mark the first anniversary of Pokhran-II, the chief architects of the Pokhran-II nuclear tests comprising Dr R. Chidambaram, Chairman, Atomic Energy Commission and Secretary, Department of Atomic Energy, Dr Anil Kakodkar, Director, BARC, Mr. M.S. Ramakumar, Director, Automation and Manufacturing Group, BARC and Dr S.K. Sikka, Director, Solid State & Spectroscopy Group, BARC were felicitated by Dr P.C. Alexander, Governor of Maharashtra.

As reported widely in the media, Dr P.C. Alexander stressed the need for India to become a super power in the next millennium, both economically and technologically, so that we become a self-reliant powerful nation.

Dr R. Chidambaram, in his address, paid glowing tributes to the scientists of DAE, DRDO and the Indian army in making both Pokhran-I and Pokhran-II successful. The tests have vindicated the wide ranging scientific and technological expertise available in the nuclear establishment. He said that there was a perfect match between the calculated yield and the measured yield in the tests. There was little doubt that the nuclear tests were a "bull's eye hit". Dr R. Chidambaram told the elite gathering that developing nuclear weapons was only a

Dr R. Chidambaram, Chairman, Atomic Energy Commission and Secretary to the Government of India, Department of Atomic Energy, being honoured by Dr P.C. Alexander, the Governor of Maharashtra with a memento

Dr Anil Kakodkar, Director, BARC, being honoured by Dr P.C. Alexander, the Governor of Maharashtra with a memento.

Mr M.S. Ramakumar, Director, Automation & Manufacturing Group being honoured by Dr P.C. Alexander, the Governor of Maharashtra with a memento.

Dr S.K. Sikka, Director, Solid State and Spectroscopy Group, BARC, being honoured by Dr P.C. Alexander, the Governor of Maharashtra with a memento.

Dr P.C. Alexander, the Governor of Maharashtra being greeted with a seedling of Lagerstroemia speciosa. (It is known as Taman in Marathi and declared as the Flower of Maharashtra. Ref. BARC Newsletter, No. 181, February 1999, p. 7-8)

fraction of the DAE's capability, and that its main role is providing abundant and cheap electricity to the masses.

Dr Anil Kakodkar said that Pokhran-II was a perfect demonstration of BARC's scientific and technological capability. He praised the team spirit of all the scientists who participated in the programme. He said that the felicitation at the function was for all the scientists who took part in the tests.

At the function, Dr Alexander presented a memento and a shawl to each of the four scientists.

BARC SIGNS AN MOU WITH MARATHWADA AGRICULTURAL UNIVERSITY, PARBHANI

Dr Anil Kakodkar, Director, BARC led a delegation of scientists consisting of Dr. M. Samuel, Director, Biomedical Group, Dr P. S. Rao, Head, NA&BTD, Dr R. Mitra, Dr S. E. Pawar and Dr S. G. Bhagwat to Marathwada Agricultural University, Parbhani, Maharashtra on March 11, 1999. Dr Kakodkar Director BARC and Dr C. D. Mayee, Vice-Chancellor, Marathwada Agricultural University, Parbhani signed an MoU for research collaboration in the areas of agriculture and food preservation. One of the major components of the collaboration is to impart training to the University staff and post graduate students at BARC in the selected areas such as use of nuclear techniques and modern biology. During the visit, the delegation visited the various departments and the facilities at the University. The staff and students of the University felicitated Dr Kakodkar for his achievements and significant contributions to the Indian Nuclear Energy Programme. He addressed a packed audience to the societal benefits of the Indian Nuclear Programme. The talk was followed by a press conference.

Dr.Anil Kakodkar, Director, BARC and Dr C. D. Mayee signing the MoU. Standing from left : Dr P. S. Rao, Dr A. M. Samuel, Dr Nayeem and Dr S. E. Pawar

Dr. Anil Kakodkar, Director, BARC tasting wheat seeds in the experimental wheat field at Parbhani. Standing from left :Dr A. M. Samuel (partly covered), Dr S. E. Pawar, Dr S. G. Bhagwat, Dr V. D. Patil, Dr Nayeem and Dr R. K. Mitra (extreme right).

HIGH MILEAGE AWARD FOR BARC BUSES

Transport Maintenance Unit, Central Workshops, BARC is carrying out maintenance of around 350 vehicles including 130 TATA heavy vehicles (100 buses + 30 other heavy vehicles).

Tata Engineering & Locomotive Company Ltd. (TELCO) have announced that five buses in our fleet have been selected for high mileage performance award. The criteria for the award is that the vehicle is run with original vehicle aggregates and with only minor repairs for 4,50,000 kms. The condemnation norms set by the government for heavy vehicles is 4,00,000 kms. And age of ten years, whichever reaches later.

BARC SCIENTIST HONOURED

Dr D.C. Biswas of Nuclear Physics Division was awarded the 1997 S.N. Seshadri Memorial Award of Indian Physics Association. He shared the award with Mr A.N. Ramaprakash of IUCAA, Pune. Dr Biswas was given the award for making important contributions in the development of gas detectors for heavy ion experimental research.

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