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FILM-ANALYSIS SYSTEMS AND APPLICATIONS

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1 INTRODUCTION

Quantitative film analysis systems are being used successfully in high technology applications, including earth's surface mapping, aerial reconnaissance, and high energy physics particle tracking. The intense interest in these types of images has led to the development of sophisticated techniques for recording and extracting high quality information from local and remote sensing systems. The transfer of this technology to medicine awaited the development of lower cost simpler systems. Such devices are now available, and being utilized for radiological image analysis.

During the past 20 years, there has been a revolution in electronic technology so that high quality image analysis systems are now widely used in hospital practice. Film analysis systems can be classified as analog or digital. Section 2 of this manuscript presents a brief review of the different systems elements that can be used, including experience obtained with a system we are using. Section 3 presents examples of medical applications to indicate the potential utility of these techniques for the development of new knowledge and for routine applications, as well.

Biomedical applications of autoradiography (ARG) have been employed since Becquerel's discovery of natural radioactivity. Heightened interest in ARG now exists in response to several recent developments. The thalidomide toxicity experience focused attention on the need to screen new drugs prior to use in humans for unsuspected focal accumulations in animals. With the rapid increase in frequency and types of nuclear medicine procedures, information on radiation dosimetry of new compounds is needed, along with dose ranges within organs. Rapid developments in positron

emission tomography (PET) technology include the synthesis of new radiolabelled compounds whose distribution, and metabolism in normal and disease states need to be evaluated. The use of in vitro ARG provides the high resolution needed to determine biodistribution in small animals, in regions which are far smaller than can be resolved by current PET technology.

For these reasons, and others that will be discussed in Section 3 of this report, we believe that these systems will be of increasing use in medical research and medical practice.

2 FILM ANALYSIS SYSTEMS

2.1 Components

2.1.1 Film. Many sizes and types of film are available commercially and the choice depends on the application. Key factors that determine the proper film are the spectral sensitivity, and resolution required. For radiographic studies, single and double-sided emulsions are available, and these are used with and without special intensifying screens depending on radiation dose and image resolution requirements. For example, when final detail is needed industrial grade film can be used without intensifying screens. Dose is of no concern in industrial radiography, and hence such small, fine grain, one-sided emulsion, high resolution films are useful for nondestructive materials testing using high energy gamma ray sources (^{60}Co). Alternatively, there is the need for high sensitivity film which can be used to image low energy radiation, for example beta rays (β^-) from tritium for ARG visualization. In this case, special films are now used in which the silver is on one side and there is no emulsion coating which would attenuate the low energy beta rays (1). Special films, and intensifying screens are used for mammography studies, to reduce patient dose. Since geometrical factors play a major role in determining the resolution (sharpness) of ARG images, close apposition between the imaged sample (radioactive tissue section) and the film is required.

When sensitivity is marginal, long contact times are needed. The time required for a given film can be reduced in several ways:

(1) Increasing the amount of nuclide given to the animal,

(2) Pre-irradiating the film to eliminate the low intensity heel of the curve, so that you start to work on the linear portion of the H(intensity x time) and D(density) curve, or

(3) By use of an intensifying screen and double-coated film so that radiations that penetrate the emulsion and which ordinarily would be lost, can elicit additional light from the screen, adding to the film exposure.

(4) Lastly, exposure in the cold will reduce fading of the latent image during long exposures.

2.1.2 Sensing systems

2.1.2a Densitometers. Manual densitometers have been used in sensitometry for many years. These are the typical systems used for quality control of film processing systems, including radiographic instrumentation. Measurement of the attenuation of light transmitted through a film in comparison to calibration step wedges is the standard method. Accurate quantitative data are obtained for points in the image sampled with such equipment. The light source sampling aperture, and sensitivity of the sensor are uniform (positionally invariant) and permit accurate comparisons from the few points one ordinarily samples. If it is necessary to make many such measurements over large areas, to preserve spatial as well as intensity resolution, then more automated methods are used.

2.1.2b Flying spot scanners. These devices typically utilize a scanning light beam which moves over the image, and intensity of transmitted light is recorded over sequentially sampled regions. High cost, high performance electromechanical systems of this type were developed for physics application, i.e. nuclear track analysis (2). Simpler electro-optical systems now are available commercially and are used for most applications (3).

2.1.2c Television camera-based systems. Vidicon (videoconverter) television pick-up tubes are the most commonly used means of transducing 2-D images into electrical signals which will drive a TV display. Vidicon tubes originally employed Sb_2S_3 as the photosensitive material. The Plumbicon contained PbO rather than Sb_2S_3, and more recently Chalnicon tubes are used, which contain $CdSe\text{-}As_2S_3$ (4). These changes in tube design have resulted in increased sensitivity (wide dynamic range), improved spectral response (covering IR through the UV) and diminished lag, which provides a more faithful representation of the scene.

The TV camera records and displays a 2-D image from 2 or 3-D objects and hence can be used for real time inspection, and measurement of moving as well as stationary objects. The output of such devices is displayed on TV monitors with between 525 and 1024 lines/frames, depending on spatial resolution requirements. Typically TV systems operated at 30 frames (60 interlaced fields) per

second and provide a flicker-free display on black and white or color cathode ray tube (CRT) monitors. The major problems with TV recording and display systems are noise sensitivity, and differing resolution along X and Y directions due to the scan raster pattern.

2.1.2d Mosaic-array sensors. Linear arrays of photodiodes or charge storage devices - charge-coupled devices (CCDs) and charge injection devices (CIDs) - are used for imaging transmitted or reflected light from stationary objects (5,6). The linear arrays view an object (positive or negative film) mounted on a rotating drum. Alternatively, linear translation of the film (or transducer) can be used to acquire data from a 2-D scene. Such devices are available as components or as assembled systems whose output can be displayd directly as TV images, or can be digitized for subsequent analysis. Blemishes, i.e., non-uniformities in the response characteristics of different elements in these arrays, are always present and require either digital normalization or analog masks for correction. These systems have the advantage of better spatial sampling properties than TV pick-up tubes. These devices have further advantages in that they can perform real time analog signal processing operations, as well as lending themselves readily to digitization for computer analysis. Two dimensional CCD and CID cameras are also available as complete systems. Increasing use of such devices is occurring and more sophisticated processing options are emerging. Commercially available CCD camera systems have gone from 32 x 32 (1976) to 488 x 380 (1980) and arrays up 1024 x 1024 are expected in the next year or two. Linear arrays are now available at reasonable prices in 2048 x 1 assemblies, which are used for high quality digitization of images for facsimile transmission and for film image analysis. By utilizing scanning translations of film or the linear array, 2048 by arbitrary size images can be acquired, displayed, digitized, and processed. Sensor materials will become available in the near future that will yield an improved spectral response compared to silicon. Materials, such as GaAs, are now being developed for CCD devices. Hitachi Corp., for example, is offering a 512 x 512, fully compatible with U.S. Television Standards (NTSC), and larger arrays are anticipated.

2.1.3 Digitizers. The spatial and intensity resolution that can be achieved in a particular study is limited by factors inherent in the scene and the film recording system. Analog to digital converters (ADC) are used to transduce voltage levels from analog sensors to digitized values.

Spatial resolution for TV imaging systems is determined by the number of samples taken across the image, and the size of the field of view. By optical magnification, one can vary the size of

the region represented by a given pixel. The sampling mesh can be adjusted by varying the number of bins into which the data are distributed. This is determined by the number of TV lines (y axis) and the number of samples taken along each line. Intensity resolution is limited by the number of levels into which the signal is divided. Digitizers with 8-12 bits resolution are commonly used which permit the construction of array sizes, or intensity levels, with 256-4096 different intervals.

TV frame digitizers are typically of two types. The first type is slow, but low in cost, while the second is fast, operating in real time and more expensive. The slow digitizer takes advantage of the fact that each of the 525 horizontal lines in a TV image has a 62.5 microsecond retrace time. Thus, if one samples and digitizes the signal level in one picture element at the beginning of each horizontal line, in sequence, and then advances through the image column-by-column it will take n(1/30) seconds to digitize the whole image, where n is the sampling mesh size chosen. Thus, an image can be digitized into a 256 x 525 mesh (actually only 480 horizontal lines are seen on a 525 line system) in 256/30 or approximately 8 seconds. This slow data rate permits easy acquisition and storage of the digitized image from stationary objects using low cost micro or minicomputer systems without special purpose components, besides the computer-based digitizer itself.

The second type of digitizer operates in real time. If one divides an image into 256 x 480 elements and samples it in 1/30 second, the system must digitize, and acquire more than 4 million 8 bit bytes/second. This requires fast data buffers in addition to fast ADCs and the usual computer hardware for imaging applications. This is the type of system that is used ordinarily for digitization of the output of CCDs and for real-time radiographic applications.

2.1.4 Processors. Image processing and enhancement can be achieved with analog, digital, and hybrid systems. Analog systems are usually fast and can handle large array sizes. Digital systems tend to be slower and more costly, but have greater flexibility. Actually, most systems have both analog and digital components and are best categorized as hybrids.

Simple optical data processing can be achieved very cheaply using TV cameras. The image from a single film viewed by a TV camera can be processed using contrast enhancement, background erase, and windowing with the resulting image displayed in black and white, or color. By the use of calibration step wedges in the field, intensity levels can be coded into pseudo-color intervals for visual "quantitative" comparisons within as well as between

films. Two different films taken at successive intervals can be viewed by 2 different cameras, whose outputs can be subtracted to display differences. In this fashion, images before and after contrast injection are used to display perfused structures without background common to both films. Similarly, ARG images taken with different labelled compounds can be displayed to reveal differences in metabolism of two compounds in the same tissue section.

More sophisticated optical data processing can also be accomplished using coherent light sources (lasers) and optical paths which permit spatial and frequency filtering of images (7). Optical filters designed in digital systems can perform mathematically prescribed operations and the results of these operations can be recorded on film. To avoid delays and problems in film processing, the "processed" images can be viewed in real time on TV monitors when the optical path is projected onto a TV camera, instead of film. Further, the ability to project video images into a coherent optical path for optical data processing is under development and such systems could have a significant impact on real time image processing of digital as well as analog (film) images (8).

Analog processing can also be achieved with CCD and CID systems. By varying the weighting factors between adjacent elements in linear arrays, 1-D smoothing or averaging can be achieved; by varying weighting factors between adjacent rows and columns in 2-D arrays, real time fourier transform operations can be performed (9). The ability to use CCD, and CID buffers for data collection, storage, and real time processing makes these element attractive for use in modern image storage and processing systems.

The development of fast, high performance, low cost microprocessors has revolutionized modern instrumentation. Because of the low cost involved in the cost of computer components, it is now economically feasible to construct dedicated systems for single applications, such as film analysis. The costs of software development at present greatly exceed the costs of hardware. Hence, until well-engineered commercial dedicated systems become available at low cost, many users will find it preferable to share the use of a larger computer developed for general purpose image processing applications. Specialized biomedical image analysis systems are now available in many institutions. A typical hospital-based nuclear medicine laboratory contains one or more general purpose digital computers which are used for image collection and processing and which can be used for film analysis. The film analysis system we are using is basically a nuclear medicine computer, and is described in Section 2.2.

The recent development and growth of digital radiography systems suggests that in the near future many hospitals will be equipped with high quality TV analysis and display systems for the conduct of routine radiographic studies. Since these systems, in general, digitize the output of a TV camera coupled to the back end of an x-ray image converter (image intensifier tube), it is clear that provision could be made easily to switch a second TV camera viewing a film into the data path when the x-ray system is not being used, to utilize the real time digitization, analysis, and display capability of the digital radiography equipment. The availability of such equipment should greatly expand the range of applications and use of film processing in radiological and allied studies.

2.1.5 Storage. Storage of large numbers of images is a difficult problem. Film itself, is the most commonly used high quality image storage medium. With the high cost of silver, logistic, and cost problems in storage, accessioning and distribution of films, alternative media are being sought for medical diagnostic and research purposes.

Analog images can also be stored on video tape (reels or cartridges), or on video discs. The former have large capacities, and are most useful for acquiring and storing cinematic images. This is due to the fact that the noise content of a single image (still frame) is often too high for direct viewing. Once recorded on video disc or tape the noise is a permanent part of the study. Since the noise from the TV system is random, this source of artifact can be removed from TV camera/film analysis systems by summing together and signal averaging multiple digitized images from a single high quality film record. In this way, TV-film analysis systems can produce relatively noise-free images.

Video discs permit rapid random access to single images in large files, whereas it takes a much longer time to move from the first to the last recorded image on a typical tape file. Further, the ability to stop-frame and digitize an image, and do subtraction studies, for example, is far simpler and more accurate when high performance video discs are used for video image recording. Such capabilities are useful for ARG and other imaging applications.

Higher fidelity can be retained by storing the data directly in digital form. Temporary storage in semiconductor or core memories is useful, prior to storage on a permanent archival media. Such archival materials include floppy discs (cheap, and can be used for individual patient files), or various types of disc cartridge systems. These can contain many studies and pro-

vide the capability of random access and permit retrival of large numbers of images stored in compact form.

Permanent, non-reuseable laser-encoded files have been used as an archival media for very large systems. Simpler systems used mylar tape, and a laser written hole pattern encoding the digital data. Currently, similar systems are being developed using multiple layer disc packs in which the laser-induced hole pattern determines the image element values. In one such system under development (Phillips), the information is digitized and recorded on a disc, from which it can be displayed in video format or can be retrieved in digital form for further numerical analyses. Several such discs could be on-line simultaneously. The capacity of each disc is of the order of 1,000 megabytes, equivalent in storage capacity to fifty 1200 foot magnetic tapes and the cost/disc should be less than $200. Thus, such systems could provide the capacity, and quality needed for archival purposes for clinical and research purposes at reasonable costs.

2.1.6 Displays. The development and wide dissemination of high quality x-ray computed tomography systems has brought state of the art display systems into medical use for the first time. Interactive systems which permit the operator to carry out different display processing options and to recover quantitative information from user-defined regions of interest now are a standard part of radiological image analysis systems, including low cost nuclear medicine data acquisition systems. These provide black and white and color displays, and with specialized peripheral devices and interfaces can be used to acquire and display images from various sensors, including film analysis systems. Modern computer architectures often include general or special purpose array processors which enhance the speed and complexity of operations that may be performed in the display processing systems, with little or no dependence on the main frame computer itself. Such systems will be of importance in correlative imaging situations, where images from different devices are registered so as to display concordant and discordant features.

2.2 Description and Characterization of a TV-Sensor Based System.

2.2.1 System Description. The Brookhaven National Laboratory, Medical Department TV-film analysis system is illustrated in Figure 1.

2.2.1a Hardware. The TV camera is a Chalnicon (Hamamatsu Model C1000-01) 1), coupled via a camera control unit (Hamamatsu Model M999-05) to a Digital Equipment Corp. PDP 11/34 via a DMA

1. Hamamatsu Systems Inc., 332 Second Ave., Waltham, Mass. 02154.

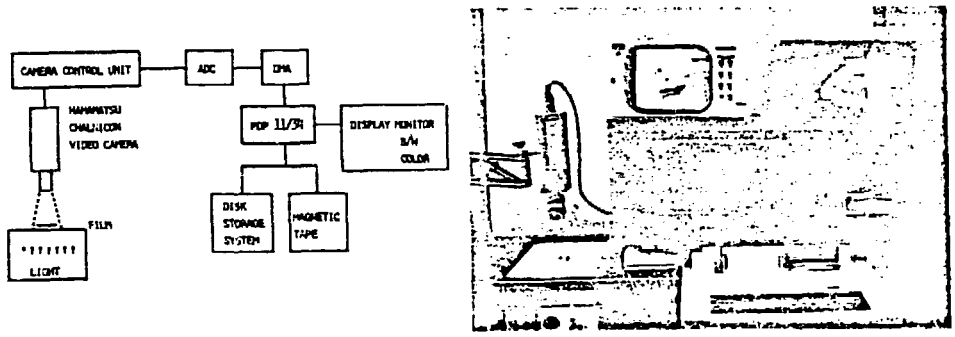

Fig. 1. Schematic diagram (left) and picture (right) of TV-film analysis system in Brookhaven National Laboratory, Medical Department.

interface (DR11B). An adapter ring permits the camera to be used with standard 35mm photographic lenses. By proper choice of lens and distance, the size of the field viewed can be adjusted for all film sizes one is likely to encounter. The camera control unit provides focus and gain controls for the TV camera, low level bias adjustment and variable digitizer mesh sizes (256^2, 512^2, and 1024^2).

The light box constructed for this application consists of eight tungsten filament light bulbs controlled by a variable autotransformer in a box covered with ground glass.

The display system used is a Unibus device which buffers 256 x 256 x 18 bits of data, and displays images up to 256 x 256 x 16 or 512 x 512 x 4, with 2 overlay planes (Computer Design and Applications - CD&A - Model MDP-3) 2). The display system is coupled to an array processor (CD&A Model MSP-3) for enhancing display system performance. Images are displayed on black and white and color TV monitors simultaneously. The color-coded images reveal more levels than can be perceived on the black and white, while the black and white displays provide an immediately recognized intensity scale that complements the color display.

2.2.1b Software. An RT-11 operating systems is used with Gamma-11 (DEC) and more recently Delta-11 (CD&A) has been added. The options used for film analysis procedures are:

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2. Computer Design and Applications, Inc., 375 Elliot Street, Newton Upper Falls, Mass. 02164.

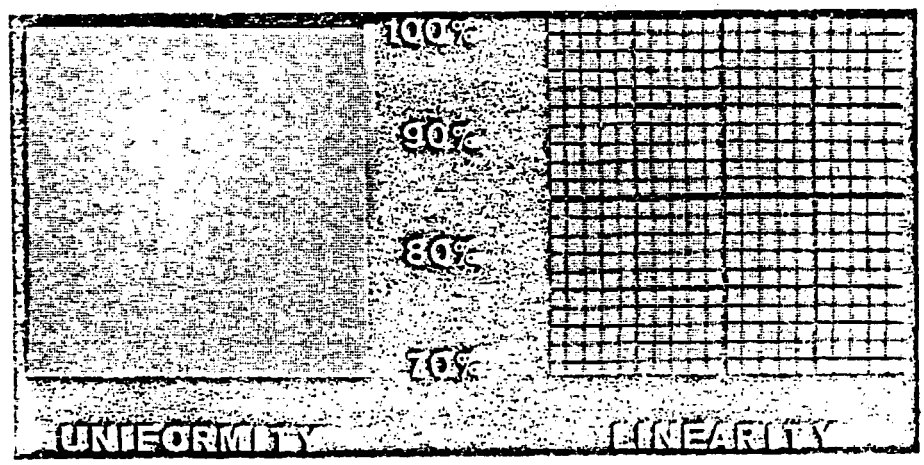

Fig. 2. Uniformity and linearity of TV-film analysis system.

(1) Variable array size digitization.

(2) Signal averaging by multiple integration (to improve signal to noise ratio).

(3) Display of the magnified image obtained from a small region with high resolution (1024 X 1024 sampling).

(4) Imaging system distortion corrections (uniformity, linearity, blurring).

(5) Registration of two images to compare and manipulate these images pixel-by-pixel. For double-isotope ARG analysis, blurring or resolution recovery of the digitized film image is done to permit spatially-congruent assessment of tracer content. This is important for example, when a blurred F-18 image is compared to a sharper C-14 image.

(6) Quantification of film density in each pixel, using a standard curve with linear interpolation between adjacent values.

(7) Histogram equalization displays are used to present images with wide variations in gray scale, while quantitative displays use linearized scales.

These utility programs operate under the RT-11 system, but some data analyses which need larger memory are performed on a 32 bit computer, VAX 11/780, to which the data are transferred via magnetic tape.

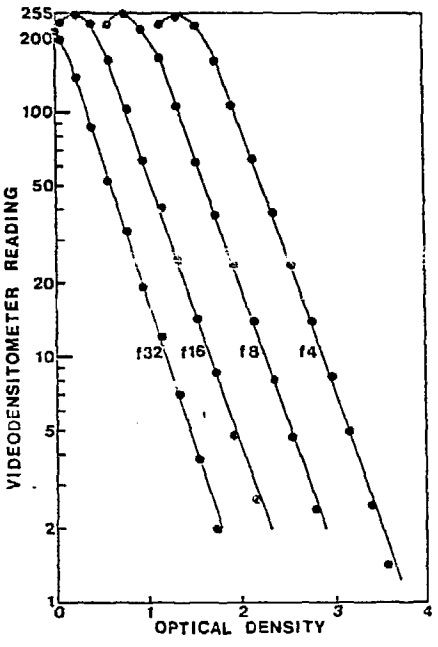

Fig. 3. Relation between optical density and digitized light intensity.

2.2.2 Performance characteristics.

2.2.2a Uniformity and linearity. The uniformity and the linearity of this system are illustrated in Figure 2. The coefficient of variation of the digitized number using a uniform intensity source (light box) is less than 2% when signal averaging is used (16 to 32 integrations). Intensity distortion is characteristic of the sensor response, and to a lesser extent non-uniformity in the illumination system. These need to be adjusted computationally to provide a flat field response. The maximum intensity distortion is approximately three times the coefficient of variation and the major distortion in our system is seen as a decreased mean response level in the lower right side of the field. Spatial linearity was measured using linear graph paper with reflected light. Measured spatial linearity distortion is less than 1% with a standard photographic close-up lens, and manufacturer specifications quote linearity as better than 0.2%.

2.2.2b Spatial resolution. The spatial resolution of the system depends on the focal length of the lens, the lens to film distance, and the array size used. The maximum spatial resolution of this system obtained by digitizing a 1 x 1 cm field into a 1024 x 1024 array, provides a 10 x 10 micron pixel size, which exceeds the resolution needed for ARG studies. Studies with TV test

patterns show that the system can resolve between 8 and 16 lines per mm.

2.2.2c Light intensity response. Figure 3 shows the relation between optical density and digitized light intensity measured from a calibrated intensity step wedge. The response is linear for a broad range of optical densities when different lens openings are used. The response characteristics of the system rarely require digitization with more than a single lens opening.

2.2.2d Stability. A usual procedure involves 16 integrations to reduce noise from the video camera system. This requires 70 seconds for acquiring and signal averaging a 128 x 128 image array. The stability of the system was assessed by repeatedly digitizing the output of the light box for many hours. After initially turning on the system, it took approximately 10 minutes for a stable mean and standard deviation to be recorded (density recorded in the center of the field of view). Short term transients were not seen. Long term drifts, which ranged up to 2%, were observed over several hours. Since the procedure involves reference to calibration data with each image processed, these slow drifts do not interfere with the accuracy or precision of the study analyses.

3 METHODS AND APPLICATIONS

3.1 Film Enhancement

There are many ways to enhance films if one has the option of taking additional exposures. When one is dealing with unique images, this option is not available and it may be important to recover information by various tricks. The problem may arise because of under/over exposures, from sources of distortion which are inherent in the imaging system, or from subject related artifacts, such as motion. Each of these can be corrected, at least in part, by appropriate methods.

It is possible to extract additional information by methods which enhance the film image per se. Thus, neutron activation will render the developed silver grains radioactive, and then contact ARG (the activated film is used instead of a tissue section to produce the ARG) may reveal additional detail not perceived in the original negatives themselves (10). This method requires the availability of thermal neutron sources and is time and labor intensive in terms of the processing requirements. In addition, if other elements in the film are activated, high background levels will limit the enhancement achieved.

Autoradiography has also been performed on films using ^{35}S-labelled compounds which react with the developed silver grains in the film (11). Contact ARGs reveal detail in the second film exposed to the low energy beta rays from ^{35}S which could not be perceived in the original images. Figure 4 shows that a low contrast (low dose) X-ray can be contrast-enhanced chemically (ARG) to provide a xerograph-like image, without added radiation exposure to the patient. The method is time consuming and requires the use of radioactive chemicals and hasn't yet found wide application although very good results have been obtained with under-exposed satellite photographs, and medical images (mammograms, and angiography studies) (11-14).

Another very promising method avoids the need for radioactive solutions, and does not require the ARG processing step. This involves the reaction of a fluorescent dye with the silver remaining on the film. By exposing the processed film to UV light, the fluorescent distribution can be imaged on film, or can be viewed with a high resolution lens-coupled video camera for real time processing and viewing of the image (15). In this system, processing time and complexity is small and as such could be useful for recovery of useful information from under-exposed images. It has been shown that the method can be useful if background fog levels are low, and care is taken in processing the original films, to avoid artifacts.

If one analyzes film without physical or chemical enhancements, there are analog and digital techniques which may be used to improve contrast and resolution. These include:

(1) Optical data processing - By using different band pass filters in the optical path, filtering operations can be accomplished which:

(a) Remove characteristic artifacts, (such as scan lines)

(b) Compensate for blurring due to imaging system distortions, and

(c) Recognize patterns for which characteristic masks have been created.

(2) Contrast enhancement and background erase.

These operations can be carried out using analog or digital techniques. By adjustment of background cut-off, one may eliminate background fog or intensity levels below a threshold value.

Fig. 4. Low contrast X-ray mammogram (left) and radiochemically-enhanced image (right).

One may display all the intensity levels that can be viewed on the display media (TV or film) for selected intensity bands through the image where additional structural detail is desired. Similarly, one can recover information at the lowest or highest levels where the film process is least efficient (the tails of the H&D curve).

3.2 Autoradiography (ARG)

The autoradiographic method has been used widely for many years, and a large literature exists on methods and applications (16-20).

The ARG method enjoys new popularity because it provides data not easily obtained otherwise. Small accumulations in parts of organs cannot easily go unnoticed in studies using these techniques. Further, improved sample preparation equipment for whole body sectioning, now makes it simpler to conduct such studies. Further impetus comes from the need to evaluate biodistribution (including dosimetry) and to assess the potential utility of new compounds developed for use in clinical nuclear medicine. The fact that nuclear medicine scientists skilled in these procedures are actively involved in these studies should assist in promulgating these methods for use in a growing number of applications. Previously, most tracers used in diagnostic nuclear medical imaging studies emitted low energy (100-200 KeV) gamma rays which did not lend themselves readily to ARG. The development of new positron-emitting short radioactive half-life agents has revived interest in ARG for various reasons.

Information on biodistribution at multiple times is needed for:

(1) High resolution biodistribution data,

Fig. 5. Anatomical picture (left) and ^{14}C-2-deoxyglucose autoradiogram (right) of transverse section (mouse brain).

(2) Dosimetry,

(3) Improved understanding of normal physiological, and biochemical mechanisms,

(4) Effects of drugs/disease on distribution and metabolism, and

(5) Correlations between blood flow and metabolism.

Some examples of ARG studies which provide these types of data are illustrated from our work and from the literature. The ability to localize different compounds, one at a time, in the body requires no new techniques. The ability to map two or more processes, simultaneously, is now being explored in several laboratories (21-26). Quantitative densitometry systems (26-28) make it possible to quantitate the relative distributions of two (or more) tracers simultaneously, and to assess the effects of various perturbations on metabolism (29). In addition, methods now exist for doing ARG on samples containing stable isotopes which make it possible to do new types of studies in human patients. Examples of different approaches will be presented for single, dual, and multitracer studies. Variation in physiological state and difficulties in cutting exactly comparable sections through experimental and control animals, contribute to the error in such studies. In Section 3.2.2, the use of dual tracer techniques will be shown to be a means of minimizing these errors.

3.2.1 Studies using a single tracer

3.2.1a Metabolic studies. ARG studies involve the production of a film record of radioactive emissions from a thin tissue section (typically 10-30 μ thick). Som et al. (28) in our lab have developed procedures for whole body ARG in collaboration with Fand and McNally (30). The images are a 1:1 mapping and hence visual inspection reveals major correspondences. Figure 5 shows images of ^{14}C-2 deoxyglucose (^{14}C-2DG) in mouse brain, next to a photograph of the anatomical section from which it was derived. The histological section provides information on the anatomical structures which are spatially congruent with the ARG image of the radioactivity distribution. Given

(1) that fiducials can be identified in both sets of images, and corresponding locations in the two representations overlaid, and

(2) that calibration data permit density to be transformed into μCi content, and

(3) blood glucose levels and clearance of the injected ^{14}C-2DG are measured, then it follow that regional glucose metabolic rates can be computed for different body regions. Sokoloff and his colleagues pioneered in the development of this method. Wolf and his colleagues at BNL developed the ^{18}F labelled 2-fluoro-2-deoxyglucose compound (^{18}F-2DG) which permits the use of this method in patients (using PET) as well as for ARG in animals. When data for different brain regions are calculated from such studies and then summed, the results are in close agreement with independent estimates from global measurements of total brain glucose metabolism.

From studies, such as these, regional cerebral glucose metabolism (RCGM) has been quantitated for different brain structures in normal animals. Changes from these values are assessed by studying groups of animals, each exposed to either mock procedures or experimental agents, and differences between RCGM assessed based on statistical tests of differences between means of different groups of experimental subjects. Additional data can be extracted from studies such as the above. These include:

(1) 3-D anatomical mapping of organs, and/or organelles established from a series of slices taken at multiple levels.

(2) Histochemical procedures can be used to localize and possibly to quantitate the distribution of metabolites in the various organs (for example, glucose content may be mapped following glucose oxidase treatment of sections) (31). Such analyses would be

fostered by the use of color sensitive TV camera systems, since stained regions have characteristic colors. Alternatively, color filters could be used which increase contrast selectively for particluar colors viewed by a black and white camera.

(3) The effect of different drugs, treatments, or disease, can be studied in animals and analyses based on pattern changes.

(4) Radiation dose distributions in selected organs can be assessed from sequential slices taken at different times following injection into different animals. This will provide information on mean dose as well as the range of doses in normal and abnormal circumstances.

(5) By chemically analyzing tissue plugs sampled from regions of interest in particular sections, the chemical form of the label can be assessed.

The analysis of chemical form of the injected tracer is clearly a factor of major concern for understanding the biology underlying the observed images. For volatile compounds, such as ^{14}C-labelled chloroform ($^{14}CHCl_3$) it is possible to distinguish metabolite distribution from the parent compound ($^{14}CHCl_3$) by the use of two temperatures in the processing of the materials. The initial AGR sections are made with tissues kept at low temperatures throughout the processing. This records the location of the volatile parent compound, plus non-volatile metabolites. By letting the specimens warm up and degass a second ARG image reveals only the fixed metabolites. Thus, one can separately analyze the distribution of $^{14}CHCl_3$ plus metabolites, and metabolites alone, and by difference determine the distribution of unmetabloized $^{14}CHCl_3$ that was present at the time the animal was sacrificed (32).

3.2.1b Blood flow. Since metabolism is a convolution of blood flow with subsequent metabolic pathways (transport and change) it is necessary to assess blood flow and metabolism independently if the two processes are to be separated (20,21). Such studies can be accomplished readily by the use of labelled 10-15 micron diameter microspheres, which are trapped in perfused capillaries. Gallium-68 is a very useful agent for such studies since it is a short-lived positron-emitter, which can be obtained readily from a long-lived generator system. For assessment of regional pulmonary perfusion, peripheral vein injections are made. For systemic distribution, intracardiac injections of microspheres are necessary. For the latter purposes, studies in rats (or animals larger than mice) may be needed where left heart injections are more easily made.

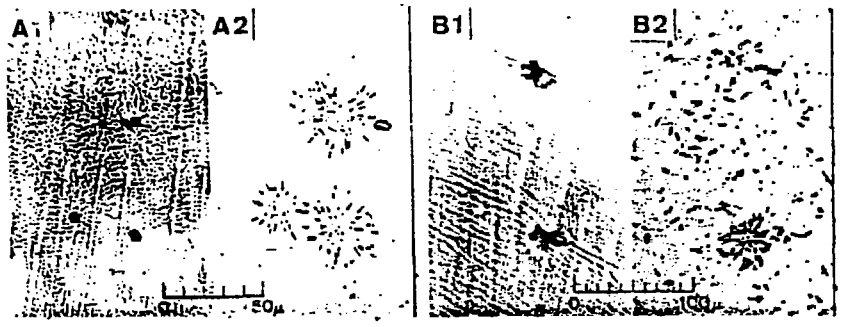

Fig. 6. Microautoradiographic images of deuterium (A2 and B2) in human erythrocytes (A1) and transformed human lymphocytes (B1).

With the development of increasing public concern about radiation exposures, potential benefits can be derived from the use of non-radioactive tracers. Various stable tracers can be used for tissue distribution studies. Tissue distribution studies can be done in animals using sample counting (neutron activation analysis or x-ray fluorescence) or ARG techniques. Some such studies can be done in patients in easily sampled tissues (e.g. blood, and urine) as well as in tissues from which biopsy samples are to be collected for medically-justified reasons. Examples of stable tracer studies with labelled compunds that could be conducted include the ARG assessment of the distribution of deuterium-labelled compounds in blood cells and boron-labelled compounds in tumors.

Deuterium ARG takes advantage of a $D(T,n)^4He$ reaction. At BNL, the uptake of deuterated thymidine into human lymphocytes in vitro, during DNA synthesis was confirmed using 200 KeV tritons from a Van de Graaf accelerator (33). The alpha particle emitted from the triton activated deuterium nucleus has a maximum energy of 4.7 MeV. A 6 μ teflon absorber which intervenes between the sample and the alpha particle detector (cellulose nitrate plastic) absorbs part of the alpha particle energy, and all of the tritons. Thus, the radial pattern of tracks etched in the plastic around individual cells reflects the deuterium content of the cell. Figure 6 shows results achieved with this method. The method is complex and requires relatively high concentrations of deuterium, but could be useful when unique data are needed and when radiation dose needs to be minimized or avoided.

A useful analog to these techniques for mapping alpha particles in tissue, has been developed at Harwell (34). Their interest was to be able to assay and localize the dose from alpha-emitters in bone (^{235}U and ^{239}Pu). The technique involves

alpha-particle "shadowing" of dewaxed specimens (also necessary for neutron activation images). Shadow images (tissue "thickness" images) are obtained by exposing the LEXAN plastic film to alpha-radiations transmitted through the specimen from a high intensity alpha-source (^{239}Pu). They then expose the samples to a thermal neutron beam. The fissions induced in the ^{239}Pu and ^{235}U nuclei produce densely ionizing fragments whose tracks are easily identified in the ARG images. The long exposures needed for standard ARG of the spontaneous alpha radiations from the low levels of ^{235}U, or ^{239}Pu encountered, was so long that fading of the latent images made direct ARG too insensitive. The method developed using LEXAN plastic track etching provides higher sensitivity and is able to measure high normal and elevated levels in human tissue samples (34).

3.2.1c Tumor metabolism studies. The distribution of radiolabelled tracers can be assessed in animals using ARG. Packer et al. of our group at Brookhaven National Laboratory have studied the biodistribution of over 30 radiopharmaceuticals thought to be tumor-seeking agents (35). Several animal models have been used. Initially their work was with the Greene melanoma in hamsters and more recently with the Harding-Passey melanoma (36) in mice. The biodistribution studies were often erratic and it was not until they started using autoradiography and obtaining melanin analyses, that they learned that pigment-affinic radiopharmaceuticals were distributed unevenly within the Greene melanoma. The distribution in the Harding-Passey melanoma was quite uniform; therefore, we now use autoradiography in the Harding-Passey melanoma for screening new radiopharmaceuticals. The results of these biodistribution studies, and autoradiography, Fand (30), have been published (37).

Neutron capture therapy (NCT) involves the administration of compounds which can be labelled with an element which has a high cross-section for neutron capture followed by prompt emission of an alpha particle - $^{10}B(N,\alpha)^{7}Li$ - and relies upon the availability of compounds which are concentrated heavily in tumors. If the range of the alpha particle is sufficient, i.e., the site of attachment is within range of the radiosensitive tumor cell nucleus, then such therapy can be clinically useful. It is important to have compounds which localize in the tumor, and not in normal radiosensitive structures which are present in the neutron-irradiated field. Early trials with NCT of human glioblastoma multiforme brain tumors failed due in large part to the poorly-localizing compounds used. A significant effort is being made in many laboratories to develop ^{10}B (and to a lesser extent Li and Gd) compounds which have suitable properties for similar applications. Fairchild et al. at Brookhaven National Laboratory have been attempting to label melanoma seeking agents with boron.

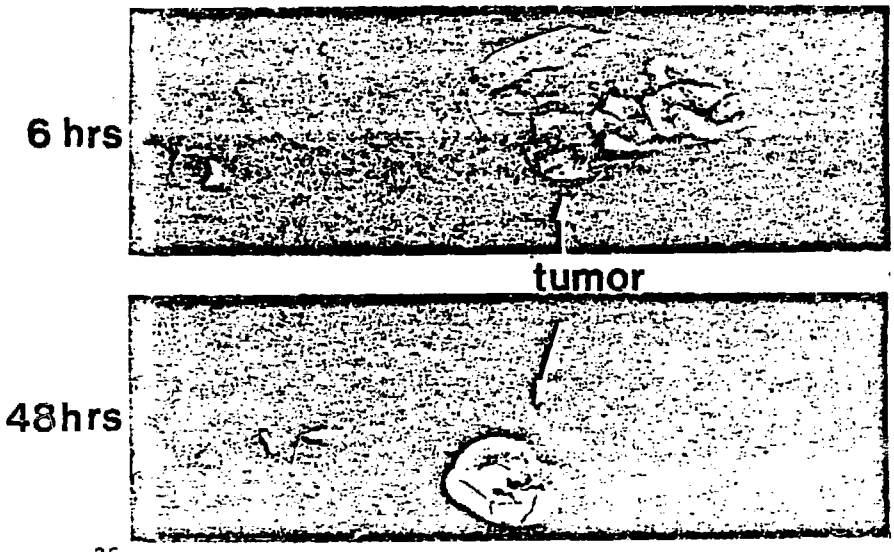

Fig. 7. ^{35}S-chlorpromazine ARG in Harding-Passey melanoma (mice).

The assessment of biodistribution of several of these compounds, including chlorpromazine is being accomplished with ARG in our laboratory in support of this effort. In the absence of ^{10}B-labelled compound, we have assessed the distribution of ^{35}S-labelled chlorpromazine in mice with a transplanted melanoma. The result is illustrated in Figure 7. The uptake of the ^{35}S-labelled chlorpromazine is more than 10 times higher than other agents studied, and if the borated compound behaves similarly, the prospects for its utility in tumor therapy would be significant. When the ^{10}B-labelled compound is available ARG studies will be carried out using techniques developed in Japan (38).

3.2.2 Studies using multiple tracers. It is possible to image multiple radioactive tracers in the same sample if either energy or their radioactive half-lives differ significantly. The short-lived positron-emitters can be studied in combination with longer-lived ^{14}C labelled compounds by taking advantage of $t_{1/2}$ differences. Thus, a sample containing ^{68}Ga ($t_{1/2}$ = 68 min) and ^{14}C ($t_{1/2}$ = 5730 yrs) when imaged for 8 hours immediately after sample preparation will reveal the distribution of the ^{68}Ga with very little contribution form the longer-lived ^{14}C. A second film imaged for 2-4 weeks thereafter, will reveal the distribution of the smaller amount of ^{14}C given to the animals.

Alternatively, different penetrations of the radiations can be used to separate different radiations. Thus, low energy beta rays from ^{3}H-labelled compounds can be separated from higher energy beta radiations by use of differential absorbers. Thus,

Fig. 8. Dual isotope ARG (mouse brain). Separate images of ^{18}F-2DG and ^{14}C-2DG distributing in single mouse.

^{14}C and ^{3}H can be imaged simultaneously on 1 film. A second exposure using an intervening plastic film whose thickness is sufficient to attenuate the ^{3}H beta ray, images only the ^{14}C, and the difference images then can be ascribed to the ^{3}H (39). A combination of ^{14}C, ^{3}H, and a short-lived β^+ emitter could also be imaged for 3-isotope ARG studies.

3.2.3 Simultaneous injection of two tracers. ARG studies have been conducted in our laboratories with ^{18}F-2DG (^{18}F has a 110 min half-life) and ^{14}C-2DG in the same animal (Fig. 8). The study reveals a close correspondence between the biodistribution of these two compounds. An even more critical comparison would have been to compare the biodistribution of stable fluorine-labelled ^{14}C-2DG to show whether and to what extent the fluorine label is responsible for changes in biodistribution. Studies involving organ sampling of such a compound have been reported which show no major differences (40), but we have not verified these with ARG.

The separate measurement of blood flow and metabolism in relative terms in the same animal can be accomplished using 2 tracers, simultaneously. Absolute quantification will be more difficult, but possible in principle. The simultaneous injection of ^{68}Ga-labelled microspheres and ^{14}C-2DG injected into the left atrium (or ventricle), provides systemic biodistribution data which permits assessment of the degree of coupling of blood flow and metabolism in normal animals. Such an experiment permits analysis of changing patterns in animals exposed to stress, with different diseases and following different treatments.

The analysis of distribution and metabolism of vasoactive amines in the lung can be assessed with ^{14}C-labelled compounds, along with intravenously injected ^{68}Ga microspheres to study pulmonary pathophysiology correlates. Cardiac studies using ^{125}I labelled fatty acids could be correlated with regional metabolism of ^{18}F-2DG in the same tissue samples, and distribution patterns compared to other animals with disease, different treatments, etc.

The biological behavior of tumor localizing agents used in nuclear medicine can be assessed by studying the biodistribution of ^{67}Ga-citrate, for example, in tumor models where a second agent is also given. In such an experiment, for the second tracer, some animals would receive ^{18}F-2DG (for metabolic correlations) others would get ^{68}Ga microspheres (as a blood flow control) while others could receive ^{125}I IUdR or ^{3}H-thymidine (for cell replication rate comparisons). Additional studies can be readily performed on treated tumors (radiation, chemotherapy, etc.) to assess metabolic effects.

3.2.4 Sequential injection of two tracers. Most of the dual tracer studies described above were designed to identify differences in two processes in normal and disease states by analysis of the major biological variables simultaneously, i.e. blood flow and metabolism. If one finds that the difference appears to involve blood flow changes primarily, then a second set of experiments could be carried out to verify the results. An animal injected with a long-lived flow tracer (for example, ^{131}I-microspheres) could be exposed to the stress, drug, or disease, and a second microsphere tracer (Ga-68 for example) given to determine in the same animal if the previously-observed pattern could be substantiated with each animal serving as his own control. This information could help document the vascular effects in radiation therapy about which much debate exists. It should be noted a large number of microspheres would have to be given into the arterial supply of the irradiated field.

The use of film analysis systems makes it possible to quantitate these changes with reasonable precision. An example of such a study, which we have performed, involved the effect of the tranquilizer, chlorpromazine on the metabolism of glucose in mice. Control mice were given ^{14}C-2DG followed by a saline infusion and then ^{18}F-2DG. Experimental animals received ^{14}C-2DG and then chlorpromazine was administered (dose = 12 mgm/kgm) and 60 minutes later ^{18}F-2DG was injected. The animal was sacrificed 60 minutes thereafter and the relative distribution of the two labelled deoxyglucose tracers studied. The ARGs for two animals are shown in Figure 9 along with the quantitative assessment of the results. The quantitation was achieved by digitizing standards for the ^{14}C and ^{18}F studies from which a calibration curve is created relating

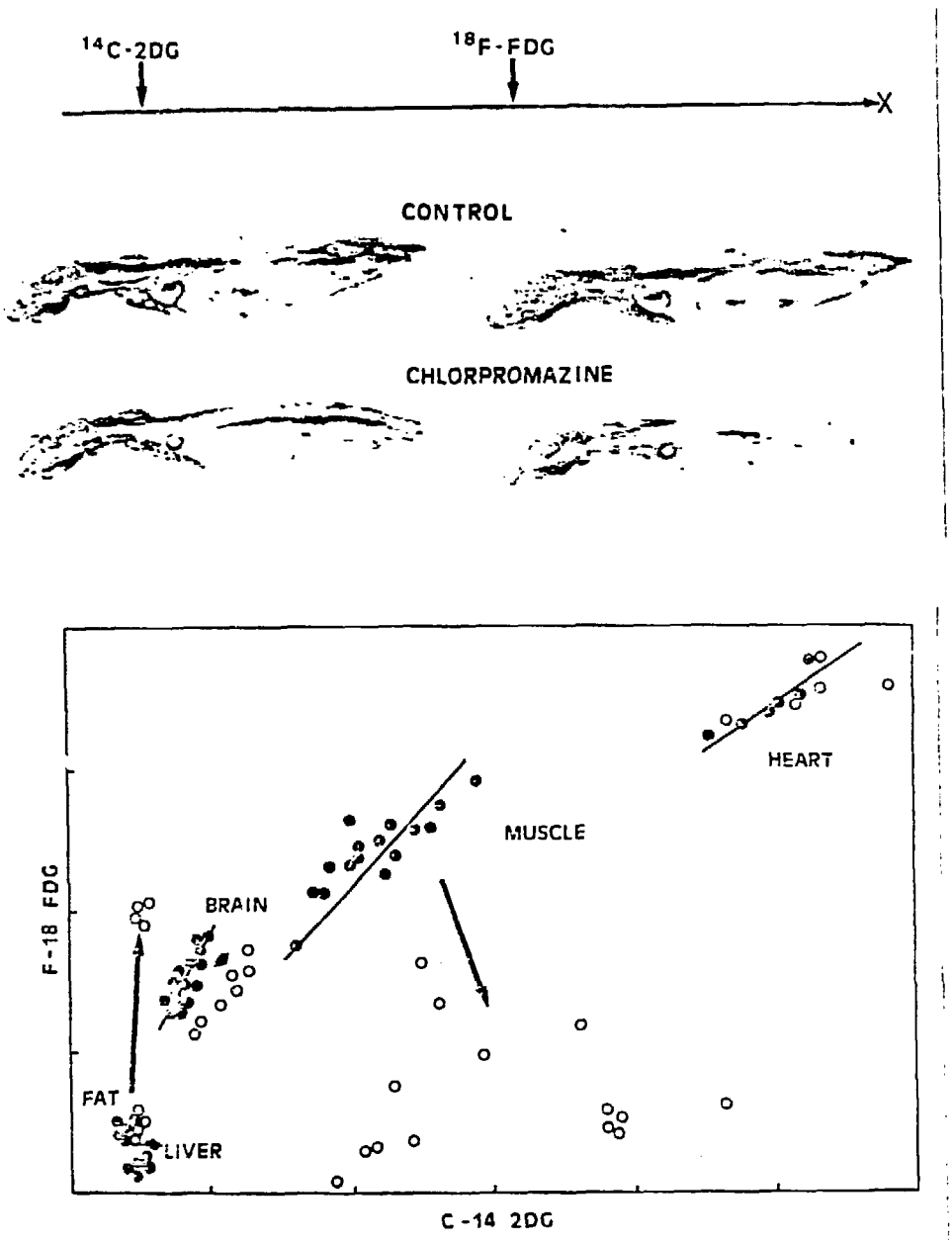

Fig. 9. Dual isotope ARG. Effect of chlorpromazine (CPZ) on glucose metabolism in mice. Upper: ARGs of control and CPZ treated mice. Lower: Quantification comparing ^{14}C-2DG and ^{18}F-FDG disribution in control (solid circle) and CPZ treated (open circle) mice.

optical density and μCi content of a pixel. The images of the animal recorded on the ^{14}C and ^{18}F films are analyzed and the activities in selected regions of interest in the image of these two nuclides are recorded and plotted (Fig. 9). It is clearly seen from the ARG and from the graph relating the change in tissue isotope content that the chlorpromazine had a profound depressing effect on metabolism in voluntary muscles, but not the heart, and that while the distribution decreased in skeletal muscles, the fat content of ^{18}F-2DG increased following chlorpromazine. Such a conclusion requires use of quantitative data to determine the magnitude of the effect, since differences in the photographic process, per se, preclude such judgments.

3.2.5 Studies involving 3 or more tracers. Extending ARG methods to 3 tracers would make it possible to add one more dimension to the analytic judgments and would increase the statistical power of the experimental design. Thus, a smaller number of observations would be needed to reach a given conclusion, since interanimal variations would be reduced. The problem is to ensure that experimental accuracy is not compromised and that the occasional loss of samples that occurs does not equal or exceed the efficiency gain. We have not yet carried out 3 isotope studies but plan to do so. An example of such a study could be to assess the effects of fast neutron therapy (14 MeV) on early changes in pulmonary blood flow. Thus, ^{125}I labelled microspheres could be injected prior to neutron hemithorax irradiation, followed one hour thereafter by ^{131}I-labelled microspheres, followed in six hours by ^{68}Ga-labelled microspheres. The evolution of changing patterns could be seen on the irradiated side and controlled with respect to the distribution to the unirradiated side. The time spans over which such studies can be carried out are limited by the tracer half-life, and by the in-vivo stability of the injected compounds.

3.2.6 Other applications of film analysis systems. The prime impetus for this review was to indicate the techniques and potential role of ARG for nuclear medical applications. However, it is clear that there are a wide variety of other medical applications for such systems.

Film imaging and analysis techniques are most useful when signals come at rates that are too high or too low for other modes of acquisition. For light photography, the photon fluxes are too high for other recording media, without loss of spatial and contrast resolution. Until recently, when higher performance measurement systems were developed, the same could be said for radiographic studies. The data rates in nuclear medicine imaging procedures are sufficiently slow that image data can be collected digitally. Hence, film analysis rarely is needed for nucler medical patient studies. For ARG studies, film is an ideal measure-

ment media since a separate "system" (film) can be committed to a single patient (slide) for weeks or months without interruption or undue cost.

There are significant applications for film analysis systems which have been used for radiological research and which could be useful for patient diagnosis. Examples include the following:

(1) Enhancement of low contrast films. Where dose reduction is sought, films could be deliberately underexposed to minimize radiation dose (if subsequent enhancement would permit retrieval of needed data). It could also be used to enhance an archived low contrast film which did not reveal a lesion, whereas a current film is read as positive. The judgement as to whether the lesion was present at the earlier time might be assisted by enhancing the earlier film (41).

(2) Low pass filtering could be used to assist the radiologist in screening films, particularly when looking for low contrast large objects, such as lung nodules in chest films (42,43). If systems were readily available and easy to use, such techniques would be used routinely.

(3) Measurements of dimensions can be useful in classification of disease based on organ size/shape, structural anomalies, and temporal changes chronicled. Successful classification of valvular heart disease has been accomplished based on cardiac size/shape (44). Temporal changes in cardiac chamber size and wall motion are useful in the classification of coronary artery disease. Irregularities in the caliber, dimensions, and pulsatile character of coronary artery blood flow can be assessed from contrast dye injected into the arteries per se. Attempts are now being made to extract similar data from intravenous contrast injections, which if successful would be of great clinical value. (Such studies, however, will probably require digital radiography systems, and will not rely upon film analyses.)

(4) Measurements of textural features in chest x-rays have been useful for the classification of pneumoconioses using objective criteria (45). Similar methods are being used to diagnose osteoporosis from spine x-ray films (46).

(5) Progression of arthritic involvement of joints has been quantitated using sequential x-rays. Color coding of density levels in reproduced films (using a calibrated step wedge) is a sensitive means of displaying quantifiable changes without loss in spatial resolution. Thus, the spatial distribution of these changes can be perceived readily. Quantitative methods of assessing bone mineral changes from x-ray films are well estab-

lished. These procedures measure bone mineral content globally and do not preserve high resolution spatial information (47).

(6) Blood flow to organs can be assessed from cine recorded contrast angiography studies. Differential renal blood flow has been measured as an average value (48) and as a pulsatile time varying flow profile (49). The temporal variations in flow, and the changes in vascular dimensions at different phases of the cardiac cycle provide additional information of pathophysiologic importance not available from the average flow values per se.

Microscopic analyses offer a wide range of options for quantification, many of which have been extensively explored. Representative examples of application areas include the following:

(1) Cell size/shape characterizations, and counting,

(2) 3-D structural reconstructions from sequential sections,

(3) Histochemical/structural correlations (multispectral analyses),

(4) Chromosome karyotyping and anomaly scoring,

(5) Sperm counting, and motility studies, and

(6) Cytological screening.

4 CONCLUSIONS

The different components that can be used in modern film analysis systems have been reviewed. TV camera and charge-coupled device sensors coupled to computers provide low cost systems for applications such as those described in this review. The increasing availability of digital radiography systems can be used for film analysis if they are available after hours for such applications.

There is a generally recognized need for the development of unified viewing stations where the results of different procedures can be compared. Until digital systems are developed in each center, film can be used to permit comparisons between analog and digital images from comparable structures provided by different imaging modalities.

The ARG method provides an important tool for medical research and is especially useful for the development of new radiopharmaceutical compounds. Biodistribution information is needed for estimation of radiation dose, and for interpretation of the

significance of observed patterns. The need for such precise information is heightened when one seeks to elucidate physiological principles/factors in normal and experimental models of disease. The poor spatial resolution achieved with current PET-imaging systems limits the information on radioreceptor mapping, neurotransmitter, and neuroleptic drug distribution that can be achieved from patient studies. The artful use of ARG in carefully-controlled animal studies will be required to provide the additional information needed to fully understand results obtained with this new important research tool.

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