Digital Autoradiography: Design, Development, and Evaluation of a Solid-State Image Analyzer

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A digital image analyzer was developed for high spatial resolution analysis of autoradiograms. The system uses a linear array of charge-coupled devices operating under microcomputer control to scan and digitize autoradiograms into matrices of up to 1,500 × 2,000 pixels with 256 gray levels. The digitized images can be converted from gray scale to pseudo-color and displayed on a high resolution color monitor. Software was developed to facilitate quantitative analysis of autoradiograms produced in single and multiple tracer studies. Because of the high output linearity and accuracy of the solid-state detectors, the system was found to digitize autoradiograms significantly more precisely and accurately than previously described autoradiographic analyzers.

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Quantitative autoradiography is a powerful tool for measurement of tracer distribution in nuclear medicine. Tracers used in nuclear medicine can be labeled with many electron and positron emitting radionuclides including hydrogen-3, carbon-14, fluorine-18, titanium-45, gallium-68, technetium-99m, indium-111, iodine-123, iodine-125, iodine-131, and thallium-201 administered to animals (*1*). The animals are killed and organs of interest are removed and frozen. The frozen organs are sectioned into thin slices using a cryomicrotome, the sections are mounted on glass or plastic slides, and the sections are then dried. The dried sections are placed against film and the decay of the radionuclides produces exposures which are proportional to the local radionuclide concentrations. These exposures cause visible film darkening to occur during development and the amount of darkening is a function of tracer concentration and duration of exposure. Autoradiograms thus represent high spatial resolution maps of tracer concentration.

In order to best utilize the information that autoradiograms contain, one must have a method of obtaining measurements of film density with high precision, accuracy, and appropriate spatial resolution. Precision describes the size of an incremental step in system output as a fraction of the range of output values of the image. Accuracy refers to how well a measurement can be reproduced.

Beginning with early studies and extending to many present studies, quantitative analysis has been performed by measuring film density using a photomultiplier tube-based, small aperture, densitometer. This can be done either manually (*2,3*) or with computer assistance (*4,5*). Although this method can be precise and accurate if a high quality densitometer is used, it is tedious and the number of measurements per section are therefore limited. It is impractical to generate tracer concentration maps of entire sections since images produced with many tracers have over 40,000 resolvable regions per cm^2 (*1,6*).

Recently, systems have been developed which can create digitized maps of density from entire sections. These include scanning microdensitometers, video camera digitizers, and very recently described, solid state detector scanners (*7–17*).

The video camera systems have precisions and accuracies which limit the detection of differences in tracer concentration to ~4–5%. The rotating drum type of scanning densitometer (RDD) had measured coefficients of variation in tracer concentration determination of 6–12%. The solid-state systems, silicon diode and charge-coupled device array cameras, can probably

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measure tracer concentrations within 2–3%. Although these systems and their commercial counterparts can be used in some types of autoradiography, they cannot digitize autoradiograms to the level of inherent film response uniformity, which is >99%.* Therefore, a more precise and accurate image analysis system could yield potentially more precise and accurate tracer concentration maps. This is of particular importance in quantitative multiple tracer autoradiography where cross-contamination must be subtracted on a pixel by pixel basis from two or more images without significant introduction of error (*18*).

We undertook the task of developing an image analysis system specifically optimized for digital autoradiographic analysis. The system was designed from the ground up for high precision and accuracy of measurement of tracer concentration without excessively long scan times.

METHODS

We first compared the two major types of solid-state light detector arrays, silicon diodes and charge-coupled devices. These types of detectors are both available in either linear arrays or matrix arrays. Because of limitations in the number of pixels available in matrix arrays when we began this project, we chose to explore linear arrays in more detail.

Linear arrays of both silicon diodes and CCDs are available with very high signal/noise ratios, dynamic ranges, and stability. The major difference between the detectors is that CCDs use analog shift registers for signal transmission, while silicon diode arrays use video lines. While the details of this difference are beyond the scope of this report, the effect is that the CCD arrays can have ~17 times the inherent sensitivity of the silicon diode arrays. We felt that this would allow shorter scan times with the CCD arrays using the typical illumination levels of autoradiography, without necessitating the use of multiple video lines or complex amplification circuits.

Mechanisms to move the linear CCD array so as to map out a matrix were obtained commercially.† Amplification and A to D conversion circuits were developed so that the response of the detectors best matched the density ranges of autoradiographs of common tracers. A variable gain circuit was developed which allowed the system to respond to the low density ranges of autoradiograms of common cerebral blood flow and metabolism tracers (*20*), as well as the higher ranges found in some whole-body tracer studies (*11*).

After preliminary tests of a prototype that was built around a minicomputer (DEC PDP 11) (*19*), we decided to develop the system around a data transmission structure (bus) developed by IBM for microcomputers. Moderately priced, complete 16-bit central computers are available which use this bus, and data can be transmitted with comparable speed as with more costly minicomputers.

A direct memory access interface was designed to map the data from the camera into a dual ported 1,000 kbyte memory. The system is thus referred to as a memory-mapped charge-coupled device (MM-CCD) scanner. Data contained in the dual ported memory is continuously outputted to a high resolution color monitor through a set of three 256 by 256 look-up tables. A trackball is used to control the look-up tables for interactive contrast enhancement and for region of interest analysis. Dynamic random access memory (RAM) totaling 3 mbytes was interfaced through the bus for programs and image storage. Industry standard 1.2 mbyte floppy disk drives, which could hold one 1,024 × 1,024 image or four 512 × 512 images, were used for long-term storage.

Programs were written in C and assembly language for typical image processing requirements of single and multiple tracer quantitative autoradiography. These included programs to convert densitometric images directly to functional images, routines to calculate average values or histograms in regions of interest, and routines for pixel by pixel analysis of different images in multiple tracer studies.

RESULTS

Using DMA data transfer, an autoradiogram can be digitized into a 512 by 512 matrix in ~2 sec, or a 1,024 × 1,024 matrix in under 5 sec. After the input of experimental data and kinetic equations, conversion of densitometric images to functional images representing tracer concentration, blood flow, glucose metabolism, or receptor density can be performed. Using a programmable front end look-up table, this conversion can be performed while the autoradiogram is scanned, without any increase in scan times. Data from a 512 × 512 image can be transferred to or from RAM in ~2 sec. Once in RAM, images can be corrected for illumination nonuniformity using true multiplicative correction.

TABLE 1
Summary of Comparison of Autoradiographically Important Performance Characteristics of a VC, RDD, SC, and MM-CCD*

	SR	SN	DR	MTF	GA	PU	PL	PS	GR	HR	ST
VC	50μ	20–30	60–100	>30%	Fair	Poor	Fair	Good	60–100	None	<10s
RDD	50μ	50–100	>100	>30%	Good	Good	Good	Varies	>100	None	>5m
SD	50μ	>200	>500	>70%	Good	Good	Good	Good	256	None	1m
SC	50μ	>200	>500	>70%	Good	Good	Good	Good	128	None	3s
MM-CCD	50μ	>200	>500	>70%	Good	Good	Good	Good	256	2/1	2s

*Specifications were obtained from references describing the use of the systems (*7,10,12,15*) and from the manufacturers.

Pseudocolor representations of gray scale images can be created with 256 displayable colors from a palette of over 16 million. Once the user has created a desired color scale, images can be windowed and contrast enhanced.

The reproducibility of the system with respect to densitometric measurement was determined by sequential image scanning. The system gain was first set so that an autoradiogram with an optical density range of from 0.2 to 0.8 was represented by over 150 gray levels, which would allow precision of tracer concentration measurement to within 1% (*20*). The autoradiogram was then scanned repeatedly over 2 hr. The first image was subtracted from the later images on a pixel by pixel basis, an absolute difference image was created, and coefficients of variation between pixels in different images were measured. Differences averaged less than 1 part in 256 for the early scans and increased to less than three parts in 256 over the 2 hr. This translates to accuracy of tracer concentration measurement on a pixel basis to within 2% (*20*).

Autoradiographic images from a ^{18}F-fluorodeoxyglucose study were scanned using a video camera system[‡], rotating drum densitometer[§], and MM-CCD scanner (Fig. 1). Histograms of the images showed that the precision of the MM-CCD scanner was 2–3 times those of the other systems. Images from this study are shown in Figure 2.

The system was used to scan and digitize autoradiographs from a triple label study comparing the distributions of [^{14}C]iodoantipyrine (IAP), [^{123}I]isopropyliodoamphetamine (IMP), and [^{201}Tl]diethyldithiocarbamate (DDC) (*21*). The very high precision and accuracy of the MM-CCD scanner allows detection and evaluation of subtle differences in uptake mechanisms which have not previously been appreciated.

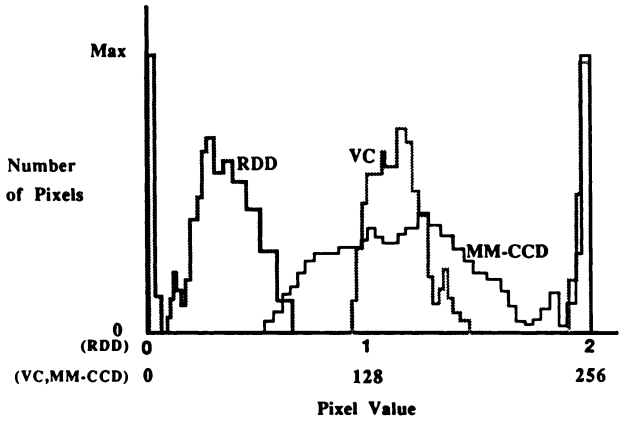

FIGURE 1
Illustration of the high precision of density measurement of the MM-CCD scanner. A fluorodeoxyglucose image was scanned with a VC densitometer, RDD, and the memory-mapped charge-coupled device scanner. (Units represent the entire range that the systems produce. Because the output of the rotating drum densitometer is in optical density rather than transmittance, its scale is in the opposite direction as those of the other systems.) The difference between the highest and lowest values in the image as a fraction of the total output range is significantly greater with the MM-CCD scanner, making precision greater.

DISCUSSION

Autoradiographic images reflecting biodistribution and kinetics of tracers used in planar imaging, single photon emission computed tomographic imaging, and positron emission tomographic imaging can be produced with great spatial detail. The past several years have seen the development of several types of systems which can be used for quantitative analysis of these autoradiograms. Initially reported systems used commercially available front ends which were originally designed for applications other than quantitative autoradiography. They included video cameras (VC), which had been intended for television, and scanning densitometers (RDD), which had been constructed for general film analysis. Although they were adequate for some types of quantitative analysis, they were not optimal. The CCD camera system (SC) reported in 1984 (*12*) offered the advantage of high speed digitization, but its precision was not significantly higher than some video camera systems and it did not allow illumination uniformity correction. The scanning silicon diode array system (SD) also developed in 1984 (*15*) offered the potential for higher performance, but it had somewhat long scanning times. Also, because the camera was not originally designed for the relatively low gray scale range which is optimal for some autoradiograms (*20*), the potential benefits of the very high dynamic range of the detectors were not fully utilized. Table 1 summarizes the performance characteristics of the MM-CCD scanner compared to examples of previously reported systems.

When using these types of systems to digitize autoradiographs, precision and accuracy are largely determined by the following group of important parameters. Spatial resolution (SR) is determined by the size of the image and the dimensions of the matrix into which the image is digitized and is usually defined in terms of the dimensions of the smallest resolvable region. Dynamic range (DR) is defined as the maximum output of the system divided by the smallest resolvable increment in response above peak noise. Signal/noise ratio (SN) describes the average system output divided by peak noise. Along with dynamic range, to which it is related, it defines the highest potential precision and accuracy that a system can have. Modulation transfer function (MTF) describes the response of a system at a light-dark interface. Units are in percent change in output of the scanner divided by the change in the input. When MTF

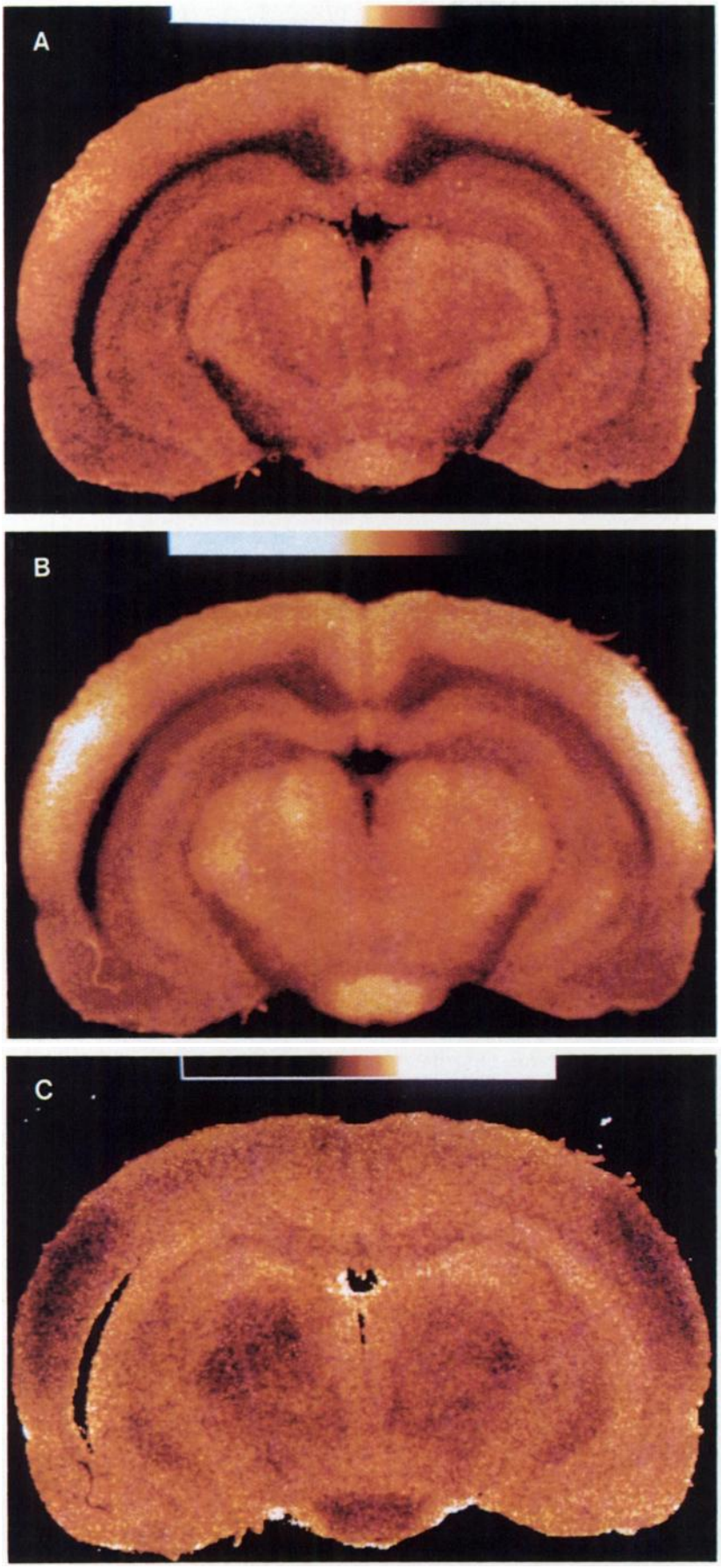

FIGURE 2
Examples of digitized images from a dual tracer autoradiographic study comparing cerebral glucose metabolic rates (LCMRgl) measured with 2-^{14}C-glucose (upper image) and [18F]fluorodeoxyglucose (middle image) using a "hot iron" color scale. The images were created by transforming the digitized autoradiograms into metabolic images on a pixel by pixel basis. The lower image represents the ratio of metabolic rates, LCMRgl glucose/LCMRgl fluorodeoxyglucose. Glucose underestimates LCMRgl by over 30% in areas of high metabolism, as illustrated by the low ratio values, presumably because of loss of ^{14}C through CO_2 production.

is <100%, "graying" will occur at a light-dark interface yielding erroneous density values. Geometric accuracy (GA), describes how well the shape of scanned objects is preserved and how well an individual pixel can be repeatedly located. Photometric uniformity (PU) describes how well the system preserves densitometric response across the image. Photometric linearity (PL) describes how well the response of the system linearly reflects light input. Photometric stability (PS) describes how response is preserved over time. Gray scale resolution (GR) describes the number of true steps into which the darkness of an image is divided. Headroom (HR) describes the difference between the amount of light which yields maximum system output and that which saturates the detector. It is limited by the dynamic range divided by the number of gray levels of the system. Scanning time (ST) describes the time necessary to digitize an image. Table 1 compares values of these parameters for examples of different types of digitizers reported for use in autoradiography.

The memory mapped charge-coupled device scanner developed in this study was designed specifically for autoradiographic analysis. Using the MM-CCD scanner, tracer concentration can be measured very rapidly with a precision to <1% and accuracy to within 1–2%. This represents a substantial improvement in performance over previously reported systems for use in autoradiography. While the significance of improvement may be debatable in some single tracer studies, it is extremely important in multiple tracer studies.

NOTES

*Kodak, Inc., NMB film, technical specifications.
†Scientific Imaging, Inc., Santa Clara, CA.
‡Carl Zeiss, Inc., Thornwood, NY.
§Optronics International Inc., Chelmsford, MA.

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