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Video and scanning microdensitometer-based imaging systems in autoradiographic densitometry

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The characteristics of computer-assisted imaging devices (CIDs) incorporating charge-coupled device cameras, vidicon cameras and scanning microdensitometers (SMDs) are compared. Advantages of vidicons include their low cost, the option of real-time imaging, continuously variable optical magnification and the wide choice of available vidicon elements. Disadvantages include densitometric non-linearities and, with some vidicons, lack of dynamic range. A CID comprising a laboratory minicomputer, image processing device, vidicon and SMD scanners and our 'PANDA' image analysis routines is described. Software corrections for vidicon errors are evaluated by comparing the densitometric accuracy of the CID receiving input from the vidicon camera and from the SMD. The scanners exhibit similar densitometric accuracy and linearity, and acceptable between-session repeatability. It is concluded that densitometric precision of a corrected vidicon scanner is not the limiting factor for quantitative autoradiographic densitometry.

Introduction

Autoradiographic densitometry substitutes an easily obtained optical density (OD) measure for measures obtained by more tedious procedures of counting grains. Densitometry is used with the [¹⁴C]2-deoxyglucose (2-DG) method for determination of local cerebral glucose utilization, for determination of local rates of protein synthesis, for regional localization of other radio-labelled ligands in brain and whole-body autoradiographs, and is applied to quantitative immunocytochemistry and cytometry (see Ramm and Kulick, in press for bibliography).

A common method of densitometry is to move an autoradiograph under the aperture of a microscope-mounted photometer. A spot reading is taken and the autoradiograph is then moved until the next area of interest lies under the aperture. Although this technique has been implemented at relatively low cost (Haas et al.,

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1975; Bryant and Kutyna, 1983), it has two major disadvantages: (1) sampling speed is very slow, limiting the number of samples that can be taken and the adequacy with which sampled regions are defined; (2) visualization of the autoradiograph, and therefore placement of the sample aperture, is difficult. Manual densitometry is particularly cumbersome in comprehensive mapping studies, for example, the measuring of glucose utilization throughout the nervous system.

A computer-assisted imaging device (CID) incorporates a scanning digitizer which transforms autoradiographic or other gray-scale data into one intensity and two positional image dimensions, stored as an array of numbers in a dedicated image memory. From the data array, the image can be recreated for display or subjected to image processing algorithms. A CID is well-suited to comprehensive densitometric mapping. Rapid density sampling is performed with computer-generated apertures (windows) of various sizes and shapes. The image, stored as an array of picture elements (pixels) can be displayed, contrast-enhanced, color-coded etc., without actually changing the data values. Thus, the visibility of subtly delineated regions can be improved for display. Finally, because the entire image is directly accessible to the computer or to a specialized image processor, the CID can subject large data bases to image processing algorithms.

The CID consists of 4 major components (Fig. 1). These are an image acquisition device, a general purpose processor, an image processor and image display monitors. The characteristics of CID configurations are treated elsewhere (Ramm and Kulick, in press). Three factors dictating performance of the image acquisition device include:

(1) *Dynamic range*. The scanner has a dark threshold below which no output is generated, and a saturation threshold corresponding to maximum output. The difference in OD between the two thresholds is the dynamic range. The dynamic range of the scanner should at least contain the range of densities found in the material to be analyzed.

(2) *Sensitivity*. The ability of the scanner to discriminate gray levels is dependent upon its noise characteristics (see below). A scanner should be capable of providing at least 6–7 bit (64–128 gray levels) densitometric resolution (using an integrative noise reduction procedure).

(3) *Shading*. Shading is a measure of photometric non-uniformity observed in scans of a homogeneous image. It imposes a major limitation upon the ultimate accuracy of a vidicon scanner.

Optical microdensitometers such as the scanning microscope photometer (SMP) or scanning microdensitometer scan the object to be measured past a photometer aperture. In the case of the SMP, the object lies on a stepping microscope stage the position of which is registered within a microprocessor. At each step of the scan, a reading of aperture position and of the integrated OD under the aperture is taken and stored. SMP systems (e.g. Leitz DADS-560) have wide dynamic range, high sensitivity and high precision. They are, however, very slow (typically about 20 min to scan a 512×512 pixel image). Further, because mechanical and optical components of an SMP are expensive the image processing and display capabilities of a moderately priced unit are rather limited.

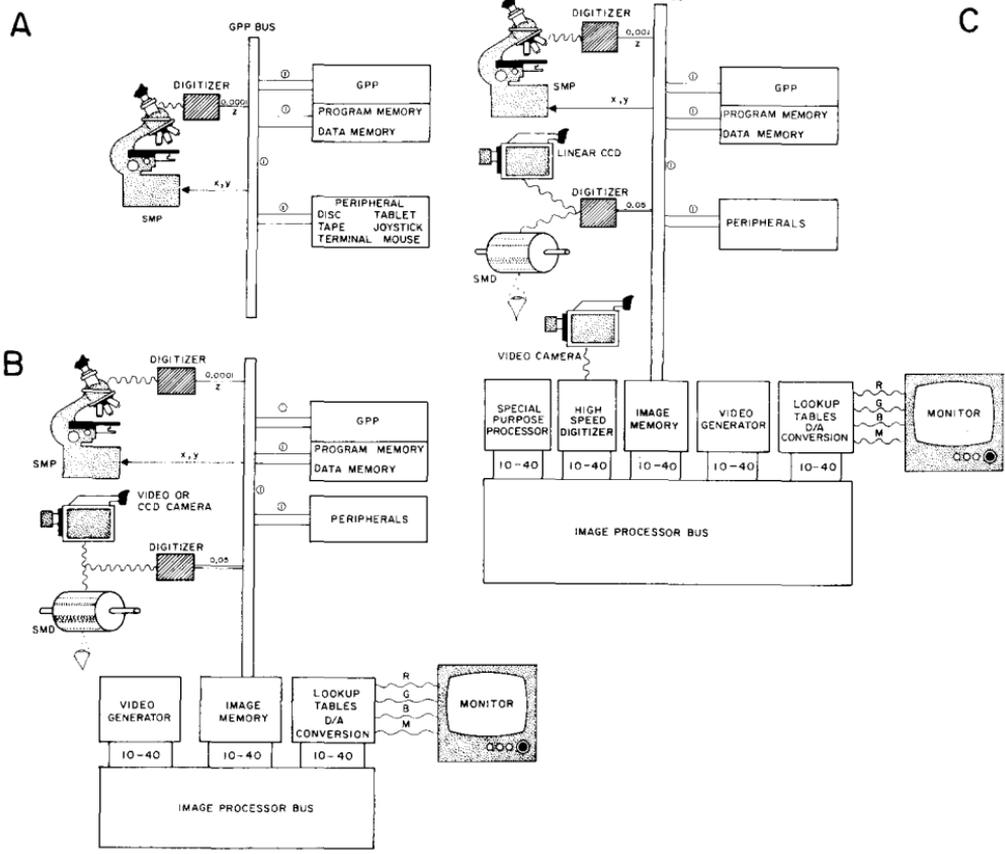


Fig. 1. Configurations of CIDs. A: the computer-assisted densitometer/photometer. Numbers associated with the bus indicate approximate bandwidth (million operations/s). Analog signals are represented with wavy lines, digital signals with straight lines. Positional drive signals (x,y) generated by the general purpose processor (GPP) direct the stepping stage of the scanning microphotometer (SMP). Density data (z) are read following each step of the stage and stored with x,y coordinates in the GPP data memory. As there is no image refresh memory, the image is accessible as numerical data only and is limited to the addressable memory space of the GPP. Data acquisition speed is limited by the step rate of the stage and the read rate of the photometer head. B: the basic CID, including an image refresh memory. Data signals from any scanner are placed on the GPP data bus. Typically, the scan process permits a $512 \times 512 \times 8$ bit image to be acquired in 1-10 s. The contents of image memory are accessible to the data memory of the GPP at a rate limited by the GPP bus bandwidth. Image memory is refreshed and displayed at a rate dictated by the video generator, in which synchronizing and drive signals for all imaging components originate. Data from image memory are routed through lookup tables, which map density data onto colors (R,G,B) or monochrome (M), and permit rapid manipulation of color and contrast. The D/A converters provide an analog signal to modulate the guns in the display monitors. All functions of the image processor are carried by an independent bus of wide bandwidth. C: a CID capable of real-time digitization and rapid image processing. Image data from slow-scan devices (SMP, SMD, linear CCD) is placed on the GPP bus. With such slow-scan devices, GPP bus bandwidth is not the primary rate limiting factor. The video camera and high speed digitizer can, however, provide data at rate much faster than the GPP bus can accept it. Therefore, these devices are interfaced directly with image memory via the image processor bus. This feature and a fast digitizer permit storage and display of the image at video frame rates. Similarly, the special purpose processor is connected to the image data path by the image processor bus, permitting rapid manipulation of the contents of image memory (e.g. frame summing).

Sophisticated scanning microscope systems (e.g. Cambridge Quantimet 720, Zeiss IBAS) allow a choice of vidicon scanner or photometer heads. This arrangement provides the densitometric characteristics and flexibility (see below) of any vidicon-based CID.

A scanning microdensitometer (SMD) contains a rotating drum (e.g. Optronics P1700) or a flat plate (e.g. Perkin-Elmer) upon which the film sheet is mounted. On the drum scanner, a light source and detector move past the rotating film on a lead screw. On a flatbed scanner, the film is moved between a fixed illumination source below and the collector above. Various sizes of aperture can be selected to scan the film at fixed resolutions. SMDs are accurate, have wide dynamic range, high sensitivity and excellent spatial linearity. Their major disadvantages are high cost (> \$40,000), a slow scan rate, the limited set of available resolutions (aperture settings) and the fixed ultimate resolution limited by scan step accuracy.

The line-scan CCD camera uses a specialized integrated circuit consisting of a linear array of individual photodiodes. A lens forms a focal plane image across which the photodiode array is carried by a lead screw. The line-scan CCD camera exhibits wide dynamic range, good response to low light levels and linear response to incident illumination (unity gamma). Typically, it produces a high-precision 1024×1280 pixel image in less than 2 min, and can perform less accurate scans at higher speeds. CCD area arrays (e.g. Fairchild CCD3000) which scan the image electronically and have the speed of video cameras are also becoming available. The array CCD, like the vidicon, permits real-time imaging. The resolution of array CCD cameras is presently limited because of the difficulty and expense involved in producing large, error-free photodiode arrays. In the relatively near future, however, array CCD cameras may replace vidicons in many applications.

Although CCD cameras can be very cheap, those which have been packaged as precision scanners incorporate manufacturer-supplied diode-response compensation and software. Such devices (e.g. Eikonix EC 78/79), while relatively expensive (approximately, \$20,000 for camera and support electronics), promise the densitometric performance of a SMD (but see below) at a price midway between that of the SMD and of the vidicon camera.

Because SMPs are slow, SMDs expensive and low-cost CCD devices upoven, vidicon camera scanners are used in many CIDs. We have used CIDs incorporating both vidicon cameras and SMDs for analysis of data from 2-DG autoradiographs. We analyze most data with the vidicon scanner, primarily for reasons of convenience. Other advantages of vidicon scanners include: (1) their low cost, (2) the image may be viewed in real time, (3) continuously variable image resolution is selected by optical magnification, and (4) application flexibility results from the wide choice of available vidicons.

Opposed to the advantages of the vidicon are its densitometric and spatial non-linearities, and the lack of dynamic range characteristic of some vidicon cameras. The practical effects of these factors upon the densitometric precision of the CID have not been described. For this reason, vidicon-based CIDs have often been limited to procedures not requiring quantitative densitometry (Gallistel et al., 1982). We will briefly discuss the sources of error in densitometric CIDs, and means

of postprocessing error correction. We will then test the validity of our error correction procedures with a comparison of identical data read by a vidicon camera and a scanning microdensitometer.

Vidicon errors

All vidicon and CCD cameras exhibit some inherent shading; i.e., equal levels of incident illumination do not produce equal signal values from all areas of the raster. These deviations are typically less than 10% of a vidicon's dynamic range and vary with the intensity of incident illumination. Sources of shading error include the illumination source, camera lens and camera electronics. Some form of hardware and/or software correction for shading must be implemented.

For our purposes, we have defined as acceptable maximum shading of less than $\pm 1.5\%$ in any area of the raster used for densitometry. This error level must not be exceeded throughout the density range in which the data lie. It is the demand that shading error remain small across a wide range of image densities which is the single most difficult aspect of vidicon densitometry. The level of residual shading is ultimately dependent upon the correction algorithm and can probably be brought to less than $\pm 1\%$. However, the 1.5% criterion is easily achieved and is not inappropriate to the sensitivity of the autoradiographic technique (see Ramm and Kulick, in press).

A potentially significant source of vidicon error lies in the dependency between signal output and intensity of incident illumination. The relation is expressed as $I = KL^\gamma$, where I is signal current, K is a constant and L is the level of incident illumination. The power, γ , varies from about 0.65 for the antimony trisulphide vidicon to unity for vidicons using most other photoconductors. The 0.65 γ vidicon has wide dynamic range (approximately 350:1), but exhibits some lag and burn in, and compressed response and degraded densitometric resolution at high illumination levels. It also requires a relatively high level of incident illumination. For these reasons, we use a camera incorporating a Toshiba cadmium selenide vidicon (Chalnicon). Although this photosensor has a dynamic range of only 100:1, it is characterized by low dark current, near unity γ (Fig. 2), low lag and negligible burn in. It also requires a lower level of incident illumination than does the trisulphide vidicon. These advantages, we feel, outweigh the dynamic range limitations of the Chalnicon. Although we have not used the Panasonic Newvicon (cadmium and zinc telluride), we understand that its characteristics are similar.

A third major source of vidicon error is camera-generated noise. The analog vidicon output (typically 1 V peak-to-peak) contains signal and, also, a white noise component generated in the photoconductor and electronics of the camera. For accurate quantification, signal amplitude must be sufficiently large to negate the effects of noise. Therefore, the signal-to-noise (S/N) ratio required for a given purpose is determined by the number of gray levels into which the analog signal is digitized. As the number of levels comprising the digitized signal (bit density) increases, the voltage band assigned to each level and the interlevel increment decreases relative to the noise signal. At some point, the interlevel increment becomes small relative to the video noise level. The result is error in the discrimination between adjacent shades of gray.

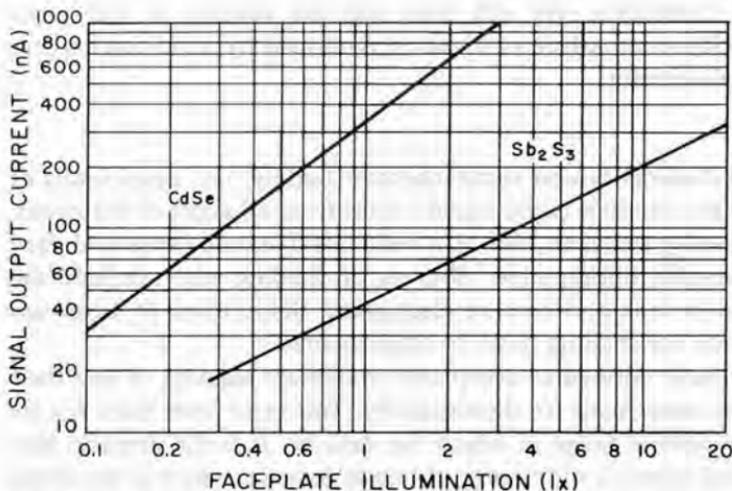


Fig. 2. Light transfer characteristics of antimony trisulphide and cadmium selenide (Chalnicon) vidicons. The trisulphide vidicon ($\gamma = 0.65$) exhibits progressively compressed response at high levels of incident illumination. The unity-gamma Chalnicon exhibits higher sensitivity and superior linearity at all levels of faceplate illumination. (Adapted from data provided by Hamamatsu and Toshiba.)

The required S/N ratio of the camera is a function of the gray level resolution. Unfortunately vidicon cameras exhibit relatively low S/N ratios, typically about 40 dB root mean square when operating at a bandwidth of 8 mHz (S/N ratio decreases with increasing bandwidth). It can be calculated that this S/N ratio is appropriate to the discrimination of only 16 levels (4 bits) of gray (with a confidence level of 99.7%). Integration in space or time is necessary to increase the effective S/N ratio of the camera. Spatial integration requires averaging of pixels. Temporal integration requires the averaging of frames, a facility offered by many image processing devices. As frames or pixels are averaged N times, the root mean square amplitude of noise is decreased by a factor of $1/\sqrt{N}$. Theoretically, averaging of 100 frames (or pixels) acquired by a vidicon with a 40 dB S/N ratio allows a 60 dB S/N ratio to be achieved. This S/N ratio is appropriate to 8 bits (256 levels) of gray level resolution.

Modulation transfer function (MTF)

The response of any scanner, including the SMD and SMP (see Gallistel and Nichols, 1983), to an input image is a function of the spatial frequency of the input. A vidicon presented with successively finer spatial frequencies of sine waves on a luminant field should ideally produce a sine wave voltage which varies in frequency but not in amplitude. In fact, however, as spatial frequency increases modulation of the vidicon output decreases in non-linear fashion (Fig. 3). The practical consequence of this effect is a decrease in the visible contrast and measured density of very small regions in the autoradiograph.

To avoid non-linearities due to the MTF, higher optical magnifications may be

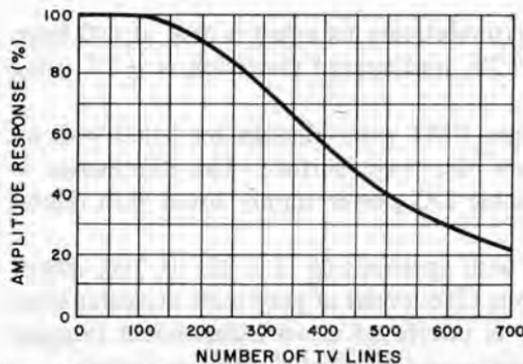


Fig. 3. Contrast transfer of the Hamamatsu N1453 Chalnicon. As image spatial frequency increases, there is decreased output from the camera. However, there is a range (up to about 150 lines) in which image contrast is faithfully rendered. Most autoradiographic features lie in this range. (Redrawn from Hamamatsu Technical Bulletin SC-5-3)

necessary in densitometry of small regions. An advantage of the vidicon is the easy selection of an optical magnification which displays the maximum amount of autoradiograph while minimizing contrast degradation. The measured area should be sufficiently wide to fall in the spatial frequency range where close to 100% contrast is obtained. Our vidicon, for example, responds with 100% contrast to a line with a thickness equivalent to 1/100 of the height of the photoconductor surface (100 tv lines in Fig. 3). The densities of narrower lines are underestimated. Our system represents the photoconductor as an image containing a 512×512 array of pixels. Thus, features measuring approximately 5 pixels in size or larger produce full contrast. Therefore, we select optical magnification so that the smallest features to be analyzed occupy at least 5 pixels in the image. In rat brain, for example, few features are less than $150 \mu\text{m}$ wide. For this reason, we usually analyze rat brain autoradiographs at a magnification of $30 \mu\text{m}$ /pixel. This magnification allows full contrast densitometry and the convenient display of an entire section on the monitor.

An experiment

We have discussed criteria for selection of an optical scanner, and have suggested that a vidicon camera has significant advantages for quantitative autoradiographic densitometry. We now report a direct comparison of the performance of a CID receiving input from a vidicon camera and from an SMD. We also report, informally, the results of scans performed with a CCD camera.

Materials and Methods

Scanning devices

The Hamamatsu C1000 video camera is fitted with external drive, a N1453 1" Chalnicon and a 55 mm micro-Nikkor lens set at f8. Horizontal resolution of the

camera is 700 lines. Amplitude response (modulation transfer) is 60% at 400 lines. Shading (before software correction) is 9.7%, and spatial distortion is $\pm 1\%$ using EIAJ test chart C2.

Illumination is provided by an Olympus PMT macroilluminator fitted with an opal glass diffuser mounted 2 cm below the stage surface. The illuminator is powered by a variable voltage, highly stable DC power supply fitted with digital readout of lamp voltage.

The SMD, an Optronics P1700, scans with apertures of 12.5, 25, 50, 100, 200 or 400 μm . An integral digitizer provides 8 bits (256 levels) of gray-scale discrimination on a 0–2D logarithmic scale. The SMD is interfaced to an independent imaging system consisting of a VAX 11/780 computer and NORPAK graphics system.

The image processor and host computer

The analog video output, or the digitized SMD output, is fed to a Grinnell GMR 270 Display System (512×512 visible resolution, 30 Hz refresh format). The display system contains an 8 bit digitizer (bypassed with SMD input), which can fill one of the two independent memory planes in $1/30$ s. A processor card allows for true pipelined operations on one or more display channels in $1/30$ s. Addition, subtraction, and various logical functions can be directly executed on 8 bit quantities. There are facilities for zooming images by factors of 1, 2, 4 or 8 and zoomed images may be translated at any zoom factor. Also present (selectable) are one 12 bit monochrome lookup table or three 8 bit D/A converters with color lookup tables. Image data is routed through the lookup tables and combined with video synchronization to provide three 8 bit video signals to drive the RGB color monitor, or one 12 bit signal to drive a B/W monitor.

A sample window is generated by a quad independent cursor card and moved by a joystick control unit or Summagraphics mouse. Gray-scale or pseudocolor images are displayed on a Hitachi HM-2719 19 inch color monitor with resolution of 720 pixels horizontal and 540 pixels vertical.

The host computer, a PDP 11/23, is fitted with dual floppy and dual RL02 hard disc drives, 224 kilobytes of memory and a direct memory access interface to the GMR 270 unit. The control terminal is a DEC VT100.

Data were analyzed using the PANDA (Program for Analysis of Digitized Autoradiographs) routines developed in our laboratory (Ramm and Frost, in preparation). PANDA includes sophisticated facilities for image digitization, analysis and display. The routines of interest in the present case provide convenient procedures for linearity and shading corrections.

Initially, the operation of the camera linearity correction and the ability of the camera to provide a ratio scale of % transmission was tested with scans of photographic film. The video camera scanned a calibrated photographic step tablet (Kodak), before and after application of linearity corrections and calibration to a film density standard.

The video camera and SMD then scanned a set of Kodak Wratten #96 neutral density filters. These filters contain a dispersion of fine carbon particles in gelatin and are particularly suitable for inter-device comparisons as they exhibit very low

light scatter (minimizing the effects of device specularly). The density range (5.2–80.0% transmission, 0.10–2.00 D) was selected to include densities likely to be found in autoradiographic data.

The SMD performed a continuous scan of the filter series with an aperture of 400 μm . Portions of each filter were then selected, scanned at 100 μm and stored on magnetic tape. The SMD was then turned off, turned on again, and allowed to stabilize before the next scan. This procedure was repeated a total of 5 times. The taped data were subsequently transferred to the PANDA system where each step of the filter series was sampled with a window of 10×10 pixels.

Linearity correction was applied to camera data by storing system response to each filter and the actual transmittance value for each filter. Linear interpolation on the resulting curve of read density for true density input was used to correct subsequent samples for deviation from linearity.

Shading error was established on a defocussed blank field at 50% transmission. Using the stored error matrix, a proportional correction was applied to all sampled image data. Residual shading at 50% transmission was $\pm 1.5\%$ over the entire image. In the central 100×100 pixel region of the field shading was less than 0.3% at 50% transmission, and was less than 1% at 10% and 80% transmission. Finally, a system densitometric reference point was established at 31.6% transmission (0.50 D). No corrections were applied to SMD data.

Following these calibration procedures, 256 digitized frames of the filter series were averaged. Transmission values were read (10×10 pixel window) in the central 100×100 pixel portion of the field at a magnification of 100 $\mu\text{m}/\text{pixel}$. The camera and illuminator were then turned off, and the entire calibration and scan procedure was repeated 5 times.

Finally, we attempted to place the performance of the various scanners in practical context by examining the extent to which film noise limits the detection of differences in autoradiographic labelling. Three sets of 20 μm sections of brain homogenates were made. The 4 homogenate samples in each set differed in label concentration in fixed steps of 1%, 2% and 5%. Each homogenate set was placed on Kodak SB5 film, which was exposed to an OD of approximately 0.8 by the sections. The films were scanned with a Perkin-Elmer PDS 1000 SMD with a square aperture of 25 μm .

Results

The corrected vidicon achieved a highly linear response during scans of the photographic step tablet (Fig. 4). In scans of the neutral density filters, both scanners exhibited random variation at any given pixel of less than 0.1% on repeated acquisitions of an image. Neighbouring 10×10 pixel windows of carbon filter did not differ by more than 1% and usually varied by much less. In camera scans of the filters, windows separated by more space (e.g. 100 pixels) varied by up to 1.5%.

Both scanners provided a comparable level of densitometric accuracy (Table I). The regression line of the observed video readings upon the actual transmission

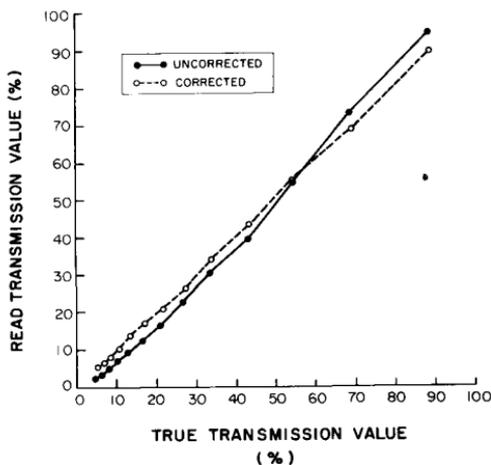


Fig. 4. Linearity of an uncorrected and corrected vidicon-based CID. The device scanned a Kodak photographic step tablet spanning the density range contained in most autoradiographic data. The Chalconic exhibits adequate dynamic range, good linearity before correction and excellent linearity after the correction procedures are applied.

values has the equation $y = -0.33 + 1.00 x$. The regression line of the observed SMD readings upon actual transmission values has the equation $y = -0.20 + 1.01 x$. In both cases, the correlation between the observed and true values is essentially perfect ($r = 0.999$). Although the repeatability of the camera measurements was good, repeatability of the SMD was consistently superior, as demonstrated by the smaller S.D.s at all SMD transmission values.

We also evaluated performance of the Eikonix model 78/79 scanned linear photodiode array by making repeated scans of a single film standard using the

TABLE I

RESPONSE OF A VIDEO CAMERA AND SMD TO WRATTEN NO. 96 NEUTRAL DENSITY FILTERS

Both scanners read the filter series 5 times and were turned off between each scan of the series. In addition, the camera error corrections were re-established between each scan. Thus, the standard deviations provide an estimate of session-to-session repeatability attainable with a corrected video camera and uncorrected SMD. Note that accuracy of the scanners is comparable. Repeatability of camera is deemed acceptable, that of the SMD is excellent.

True density		Camera density (\pm S.D.)	SMD density (\pm S.D.)
D	%	(% transmission)	(% transmission)
0.10	80.0	80.1 (1.06)	78.8 (0.16)
0.20	63.0	63.8 (0.67)	63.2 (0.10)
0.30	50.2	50.4 (0.56)	49.0 (0.08)
0.50	31.7	31.5 (0.49)	32.2 (0.13)
0.70	20.0	20.2 (0.55)	19.8 (0.09)
1.00	10.0	10.9 (1.20)	10.5 (0.04)
1.50	3.2	3.9 (0.52)	3.2 (0.03)
2.00	1.0	0.8 (0.31)	0.8 (0.00)

manufacturer's proprietary shading and diode equalization corrections, a pixel size of $60\ \mu\text{m}$, no averaging and digitizing at 12 bits and storing the most significant 8 bits. Adjacent pixels on the uniformly exposed standard showed a standard deviation of approximately 1.5 units (out of 256). The same pixel on repeated scans varied less, exhibiting an S.D. = 0.41.

Our scans of film exposed to standards made from tissue homogenates revealed pixel to pixel variability somewhat higher than for SB5 film exposed to uniform light or to plastic radiation standards, perhaps because of the rough surface texture which is a characteristic result of cryostat sectioning. The pixel values in each standard (excluding regions containing artefact) exhibited standard deviation values ranging from 3.2 to 6.6 units (out of 256). Thus, the standards that differed by 5% (approximately 10 units) were discriminated from one another with fair reliability without averaging of neighbouring pixels. The 2% standards required averaging of considerable areas (about 10 pixels square) for reliable discrimination and the 1% standards could not be discriminated over areas much smaller than 70 pixels square (approximately $2\text{mm} \times 2\text{mm}$).

Discussion

The highly stable values observed for any individual pixel show that sensitivity at individual pixels was appropriate to 8 bit densitometric resolution. The slightly greater variability observed over neighbouring pixels was also evident in digitized images of a defocussed blank field. This variability did not, therefore, originate in the carbon filters but in some aspect of the imaging process. At the level of neighbouring windows of moderate size (containing more than 2 pixels), system sensitivity remains high, appropriate to 7–8 bit resolution with either scanner. The greater variability observed in non-adjacent areas of a camera scan can be attributed to imperfect operation of the shading correction algorithm. At the level of widely separated windows, system sensitivity was appropriate to 6–7 bit resolution when using vidicon input.

Although neither the vidicon nor SMD exhibits perfect densitometric accuracy (Table I), a comparable level of accuracy is available with either scanner. The superior repeatability of the SMD may be important in some applications requiring high precision densitometry. Further, the demonstrated performance of the vidicon camera is about optimal while SMD performance could be improved by software calibration to a range of densities lying in the specific film to be scanned. This procedure works well with the vidicon camera (Fig. 4) and should yield very accurate measurements if used with the SMD.

The line-scan CCD camera performed about as well as the scanners tested above, with some of the convenience of the video system (e.g. continuous magnification selection but no real-time digitization) and without requiring user software correction for linearity, shading or noise. It appears, on the basis of our informal tests, to be a reasonable alternative to the other scanners. However, we are not at present certain as to the ultimate shading accuracy of the CCD camera. If the proprietary

shading correction remains valid over a broad range of optical densities, this scanner with its wide dynamic range offers some performance advantages over the vidicon and a price advantage over the SMD.

There remains the question as to whether any scanner used for autoradiographic densitometry requires measurement capability more accurate than that of a vidicon. An autoradiograph is an analog record of radioactivity distribution in underlying tissue. The most important characteristic of an autoradiographic densitometer is its ability to perform consistent measurements at a level of precision at least equal to that of the autoradiograph. The individual investigator must decide whether his autoradiographs and preparation techniques provide an analog record which taxes the 6–7 bit precision of vidicon densitometry, and whether the expense and trouble of using a SMD are justified. We suggest that in a well-configured CID, vidicon performance exceeds the sensitivity of the autoradiographic method. Methodological sensitivity is limited by aspects of the autoradiographic procedures (e.g. variation in section thickness, calibration of radioactivity standards) and properties of autoradiographic materials (e.g. film noise and sensitivity).

From our measurements of actual autoradiographs on SB5 film, we conclude that the combination of surface nonuniformities of cryostat sections and SB5 film noise limits the discriminability of density differences smaller than 1% over areas smaller than about 4 mm². Film areas of this size are rarely homogenous, and are larger than many discrete brain regions. Therefore, the level of precision obtainable from the autoradiographic representation of many brain regions is not appropriate to an 8 bit density resolution. Although other films may exhibit less noise than SB5, the precision limit imposed by noise and other errors is likely to remain more stringent than any imposed by a vidicon scanner operating with software correction. In sum, we suggest that the dynamic range, accuracy and sensitivity of an appropriately corrected vidicon are suitable for quantitative autoradiographic densitometry.

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