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A densitometer for quantitative autoradiography

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A low cost spot densitometer system is described. This system is useful for quantitative autoradiography of local cerebral glucose utilization, blood flow, receptor binding and other applications requiring densitometry on films. The densitometer can be used alone or interfaced to a microcomputer.

The densitometer consists of a photographic enlarger, a digital multimeter, and the densitometer electronics. We have described how to construct, test and use the densitometer and how to interface the densitometer to a microcomputer.

The advantages of this system are: (1) the ability to enlarge the image for accurate measurements from 'small' areas; (2) a completely unobscured image during measurement; (3) low cost and (4) ease of use.

Introduction

Quantitative autoradiography has become a useful technique for studying many properties of the nervous system, including local glucose metabolic rates (Sokoloff et al., 1977), cerebral blood flow (Sakurada et al., 1978), and neurotransmitter receptor binding (Penney et al., 1981a,b). There are two basic variations of this technique. The first involves grain counting on emulsion-dipped slides and the second involves measurement of density (densitometry) on films which have been exposed to tissue containing a radio-labeled ligand. The main advantage of the densitometric over the grain counting technique is that tissue radioactivity concentrations can be determined much more rapidly.

The degree to which a film is exposed to light or a radioactive substance determines the density of silver grains on the developed film. A densitometer measures the amount of light which passes through the film compared to the amount of light which is transmitted through air. The unit of measurement is in Optical

* Reprint requests should be sent to G.W. Dauth. A more detailed description of the construction of the densitometer is available upon written request.

Density (O.D.) units:

$$O.D. = \log (I/F)$$

where I is the amount of light transmitted through air and F is the amount of light transmitted through film.

Several instruments are available commercially for measuring O.D. Although moderate in cost, commercial spot densitometers are difficult to use because the light sensor is usually mounted on a swing arm which obscures the film from the operator during the measurement. Moreover, these instruments make it difficult to measure accurately the O.D. of small structures. Some commercial instruments include video digitizers and scanning microdensitometers, however, these components are expensive. For example, a complete system consisting of a scanning microdensitometer, a minicomputer and image display hardware can cost over $100,000.

The densitometer which we have developed is a cost-effective alternative to the commercial systems. We have used this device for the past 3 years (Penney et al., 1981a,b; Penney and Young, 1982). The densitometer has proved to be highly reliable and the numerical results are comparable to those obtained from a scanning microdensitometer system (Goochee et al., 1980). Our densitometer has several advantages over commercially available instruments: (1) the densitometer, including a photographic enlarger and a digital multimeter, can be assembled for less than $1000; (2) the densitometer does not obscure the image during measurements; (3) our system allows the operator to enlarge the image so that accurate measurements can be made within small areas; and (4) when the densitometer is interfaced to a small microcomputer, all conversions can be performed automatically and measurements can be made at rates up to 1/s.

System and circuit description

The basic system consists of 5 components: the densitometer electronics; a photographic enlarger; a digital multimeter; an AC line voltage regulator; and a variable transformer. The voltage regulator and transformer are optional, but provide a stable and controllable voltage for the enlarger lamp. The densitometer can be interfaced with any computer which has an analog to digital converter. Alternatively, the densitometer can be used without a computer by using the display on the digital voltmeter.

The densitometer circuit consists of 3 stages (Fig. 1). The first is the detector circuit which includes a PIN silicon photodiode (D1) and an amplifier (IC1) configured as a current-to-voltage converter (Gage et al., 1977). Increasing the incident light intensity increases the current through the diode. IC1 converts the photodiode current to a voltage.

The next stage in the circuit (IC2) is a low pass filter with a cutoff frequency below 60 Hz. The filter stage is necessary because the PIN photodiode can respond to changes in the light intensity within as rapid a time as 1 ns. Consequently, the diode can detect 60 Hz fluctuations from the incandescent enlarger lamp. The filter

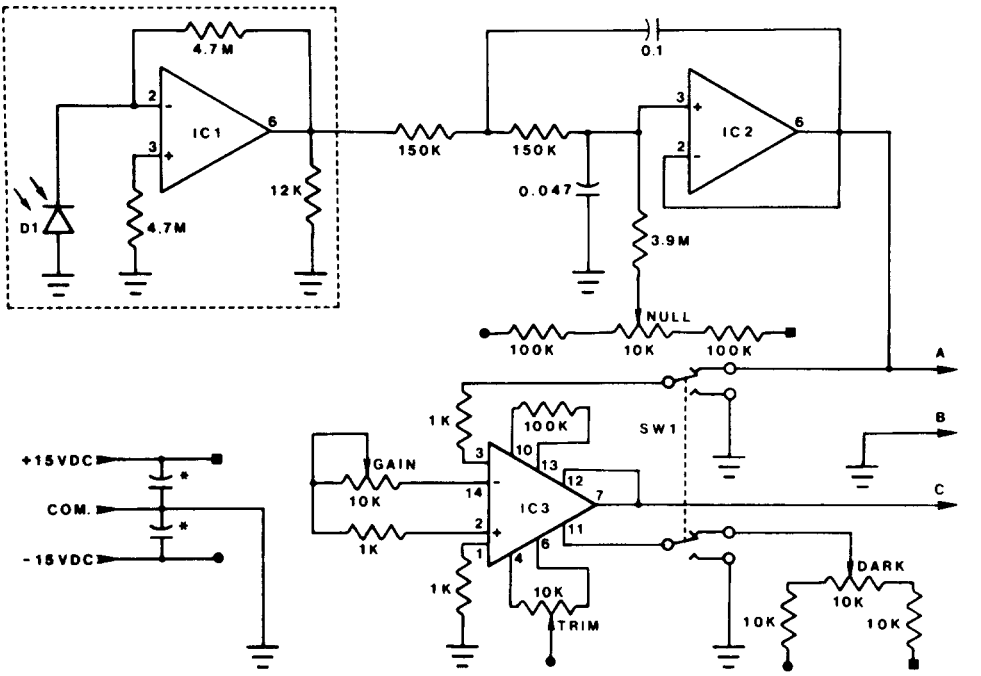

Fig. 1. Circuit diagram of the densitometer. Closed circles indicate connections to −15 VDC and closed squares indicate connections to +15 VDC. Power supply connections to the integrated circuits are omitted for clarity. The numbers associated with IC1–IC3 are the pin numbers for the integrated circuits. D1, MRD500—Mororola PIN Silicon Photodiode; IC1, LF355 Motorola FET Op Amp; IC2, 72741 Texas Instruments Op Amp or equivalent; IC3, AD521 Analog Devices Instrumentation Amplifier. The NULL, TRIM and DARK controls are 15 turn trim pots; the GAIN control is a 10 turn pot with locking dial. All pots are linear taper. All fixed resistors are 1/4 W 5%. SW1 is a double pole, double throw switch. All capacitances are in microfarads, rates at 30 WVDC or higher. The components within the dashed box should be mounted on a separate circuit board and connected with a shielded cable (see Fig. 2). * These capacitors are electrolytic, 10–100 μf, and they should be used if the power supply is poorly regulated or if there is ripple on the supply.

stage also contains a NULL adjustment (Fig. 1) which is used to balance the input to IC2 (measured at point A in Fig. 3) when the diode is in complete darkness.

The last stage (IC3) is an instrumentation amplifier which provides the DARK reference level and GAIN controls (Fig. 1). These controls are used to determine the output voltage range of the densitometer. The output of the densitometer is measured at point C (Fig. 1).

Densitometer construction

Construction of the densitometer is straightforward; only the essential details will be described. First, the photodiode should be in close proximity to the current amplifier in order to reduce the effects of electrical noise. Thus, the components

Fig. 2. The completed circuit boards ready for installation. The small circuit board contains the PIN photodiode and its amplifier. The syringe used to mount the detector board has been press-fitted on the diode. The twisted pair of wires on the left of the main board are connected to the gain control (Fig. 1) and the three twisted wires existing from the bottom of the main board are connected to the power supply. Note the position of the switch and the three trim pots on the top of the main circuit board. The switch is used to secure the main circuit board to the chassis box.

within the dashed box in Fig. 1 are mounted on a small circuit board, separate from the remainder of the circuitry (Fig. 2). Second, the photodiode should be collimated. We used a 10 mm section from a 1 cc plastic syringe to mount the detector and to provide collimation (Fig. 2). The finger grips of the syringe should be epoxied to the inside of the top of the chassis box used to house the electronics. The inlet hole on the top of the chassis box is the same diameter as the inside of the syringe. An aperture plate is made from a small piece of thin brass sheet with an appropriately sized pin hole; we used a 1.5 mm aperture in our system. The interior of the syringe barrel, back side of the aperture plate and inside of the pin hole should be covered with a flat black paint to eliminate reflection. Since the chassis box serves as a projection screen, the top of the box and the aperture plate should be painted white.

The instrumentation amplifier has a linear response over an output range of ± 10 V. Given this constraint, the final setup of the densitometer will depend on the input voltage range of the analog to digital converter (ADC) used in the computer system.

The ADC should have a minimum resolution of 8 bits. A unipolar rather than a bipolar converter is preferable because the data conversion routines are easier to write.

Using the densitometer

Initial setup of the densitometer requires adjustment of several components to maximize the system response and linearity. First, with SW1 set in the CALIBRATION position (i.e. pins 3 and 11 on IC3 grounded), D1 occluded, and GAIN set at maximum, the voltages at points A and C are set to 0.000 V by adjusting the NULL and TRIM potentiometers, respectively. The adjustment of the DARK potentiometer should be made to provide a DC offset voltage to match the system output range to that of the ADC. The DARK adjustment is made with SW1 in the READ position (i.e. inputs connected to pins 3 and 11 on IC3) and D1 occluded. The DARK voltage is measured at point C and is independent of gain. These adjustments, once made, should be stable, although verification at regular intervals is advisable. With SW1 set in the READ position, adjustment of the gain setting and film density reading are performed. Adjustment of the GAIN potentiometer is made each time a new film is inserted into the densitometer or if any of the following are altered: magnification; focus; f-stop of the enlarger lens; voltage applied to the enlarger lamp or the size of the detector aperture. The gain is adjusted so that the lowest density of interest (usually film background or empty film carrier) produces the minimum output voltage acceptable to the ADC. Increasing film densities will produce corresponding increases in output voltage to the maximum as specified by the reference voltage supplied to IC3 (DARK adjustment). If the densitometer responds in the opposite manner, simply reverse D1 in its socket.

To measure regional optical density of an autoradiogram, place a glass carrier containing the film on the negative stage (Fig. 3). Select the desired magnification, light intensity and f-stop. Focus the image and adjust the gain setting as described above. Move the film so that the area to be measured projects onto the aperture. The digital multimeter will read the voltage at point C and provide an indication of film density. Alternatively, an ADC under computer control can digitize the voltage at point C. If the densitometer is interfaced to a computer, the program can compute O.D., and if radiolabeled standards were exposed along with the tissue, the program can convert density to radionuclide concentration. The computer program used with our system also computes local cerebral glucose utilization rates as described by Sokoloff et al. (1977).

Fig. 4 illustrates one of the advantages of our densitometer: the image can be enlarged to measure accurately the density in small areas. The measurements made from the hippocampus in the low power image clearly result from measuring overlapping areas of high and low density, and the resulting numerical value reflects this overlap. More accurate measurements can be made from the high power images. The measurements from the amygdala on both the high and low power images demonstrate that the system provides similar results when measurements are made

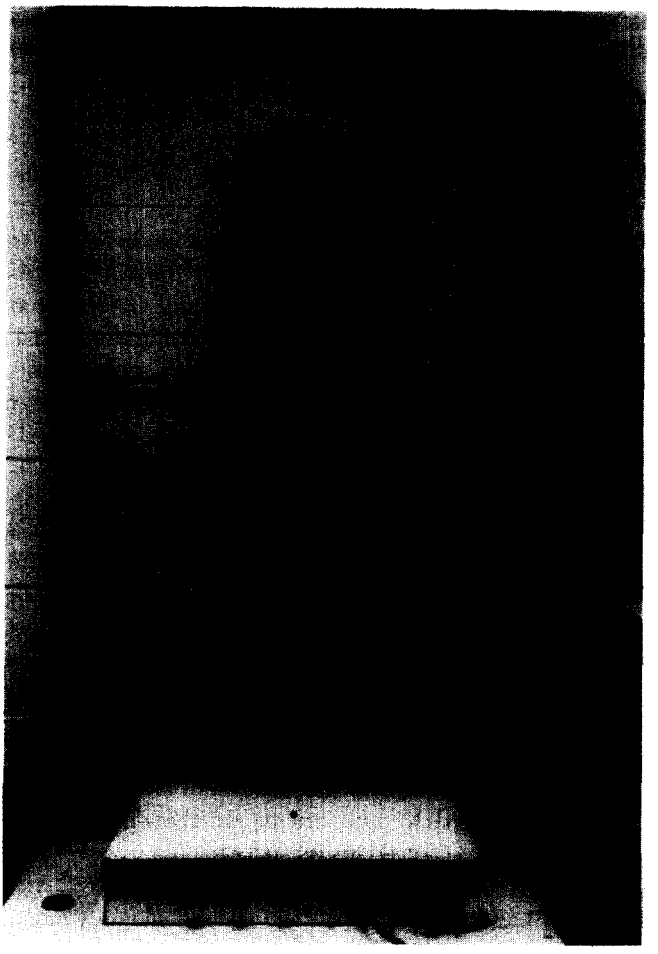

Fig. 3. The densitometer set up for use. The aperture is located at the position of the dot on the top of the densitometer chassis box. The aperture plate is secured with tape. The digital multimeter can be seen in the background. The open arrow points to the glass carrier which contains the autoradiogram. The small box with the * contains one of the switches which controls the computer and the other (not shown) is a foot switch. The variable transformer which controls the voltage to the enlarger lamp is in the upper right of the photograph.

from areas of homogeneous density regardless of the magnification.

The spatial resolution of our system depends on many factors, including aperture size, lens focal length and magnification set on the enlarger. The O.D. resolution of this system also depends on several factors, including the ADC selected and how the system is set up. Our densitometer has a range of 0.00 to 2.0 O.D. units. The same range can be achieved by keeping the light source as bright as possible. We use a 150 W enlarger lamp run at 90–100 VAC and a good quality f 2.8, 50 mm enlarger lens.

Table I contains measurements made on our system from a Kodak neutral density step wedge. The wedge was calibrated on an Optronics scanning microdensitometer.

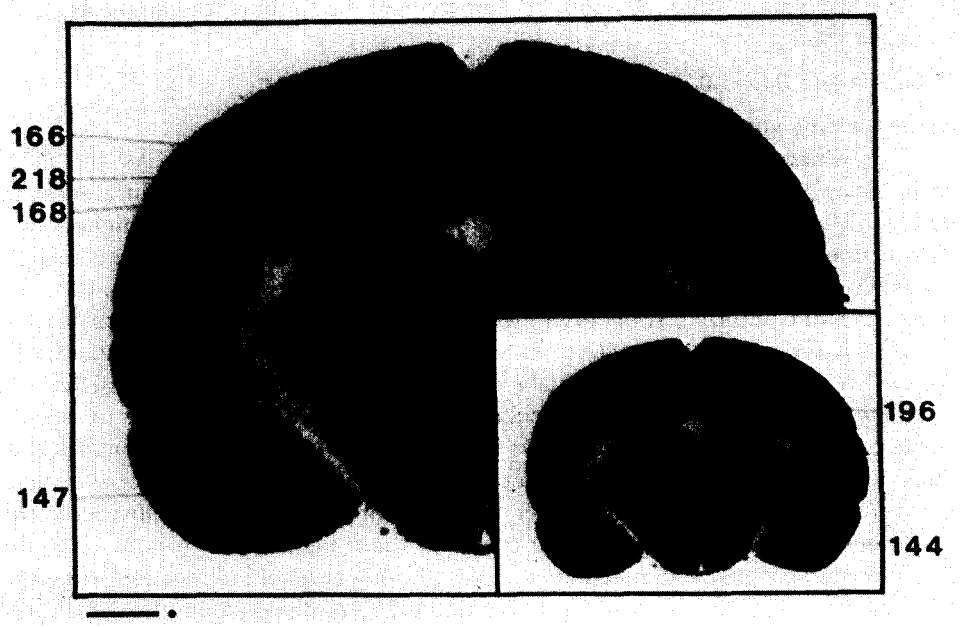

Fig. 4. An autoradiogram of a section of a rat brain from a rat injected with [^{14}C]2-deoxyglucose. The large image is magnified 11× and the small (inset) is magnified 5×. The same magnifications were used when making the measurements listed in the figure margins. The leader lines point to areas from which the measurements were taken. The units of measurement are nCi[^{14}C] per gram of tissue. The bar at the lower left indicates the width of the rat brain at 1× magnification (15 mm) and the dot to the left of the bar represents the size of the aperture on the densitometer (1.5 mm). Note that increasing image magnification helps eliminate partial volume effects in heterogeneous regions (hippocampus) of the film without affecting readings from areas of more uniform density (amygdala).

TABLE I

VOLTAGE READINGS AND CALCULATED DENSITY VALUES FROM A KODAK NEUTRAL DENSITY STEP WEDGE

O.D.$_S$ from the spot densitometer.
O.D.$_O$ from an Optronics Scanning Microdensitometer.

Voltage	O.D.$_S$	O.D.$_O$
0.67	0.06	0.03
2.35	0.28	0.24
3.26	0.46	0.42
3.88	0.65	0.61
4.28	0.84	0.80
4.56	1.06	0.99
4.71	1.24	1.17
4.79	1.38	1.34
4.86	1.55	1.53
4.91	1.74	1.71
4.93	1.85	1.88
4.95	2.00	2.00

The spot densitometer was adjusted for a 0.00–5.00 V range as described above. The readings were taken from the digital multimeter and the formula for computing O.D. from the voltage was:

$$\text{O.D.} = \log (5.00/(5.00 - \text{V}))$$

where V is the voltage.

A drawback of our system can be appreciated from Table I. As the O.D. approaches 2.00 O.D. units, the ability of the system to resolve fine differences in density decreases. The solution to this problem is to ensure that the highest film density does not exceed about 1.70 O.D. units. Since the film background on a single coated X-ray film (Kodak SB-5) is about 0.20 O.D. units, approximately 1.5 O.D. units can still be utilized. If the full range is required, the film background instead of the glass can be used as the zero point. If the films contain radionuclide standards, the O.D. values will be relative but the nuclide concentrations will be absolute.

Allow approximately 1 h for the enlarger lamp and the lamp housing to reach a stable operating temperature before use in order to prevent the system from drifting. Once the lamp has reached a stable operating temperature, the densitometer does not show any appreciable drift over a period of several hours. Obviously the densitometer should be run with the overhead room lights off; however, since the densitometer is highly collimated, it will tolerate a low level of indirect ambient light. We run the system with a small light box placed 2–3 m distant from the densitometer.

The densitometer we have described is a cost-effective apparatus for the investigator who needs to do quantitative autoradiography on films. The electronic components cost less than $150; a good digital multimeter can be purchased for less than $150; and the Bessler 45MX enlarger can be purchased for $600–$700. We have used our system for over 3 years and have found it to be reliable. The only maintenance which has been necessary for the densitometer has been replacement of the enlarger bulbs and routine cleaning.

The densitometer can be easily interfaced to a microcomputer which has an ADC. The most difficult aspect of programming the microcomputer is the polynomial regression analysis used to convert O.D. measurements to the concentration of the nuclide in the tissue. Fortunately, several algorithms have been published for polynomial regression analysis both in Pascal (Miller, 1981) and Fortran IV (Nguyen, 1981).

The curve obtained from the computer must be monotonic over the range of the standards and data should not be used which are beyond the end points of the standards. To ensure that the curve is monotonic, we display the entire curve along with the data points for the standards either as a graphics plot on a CRT screen or a printer. If the curve is not monotonic, it is recalculated using a lower order regression. A third or fourth degree regression will usually provide the best fit.

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