A Semi-Automatic Computer-Microscope for the Analysis of Neuronal Morphology

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Abstract—Quantitative microscopic studies of individual neurons of the central nervous system, especially of their elaborate dendritic and axonal patterns, can be done only with great difficulty using conventional biological microscope instrumentation. In order to simplify and speed the gathering of such data, a computing light microscope has been developed. This instrument functions as a unit under the control of the investigator examining the histologic preparations. It is capable of measuring accurately distances in all three coordinate axes. Measurement of the length of dendrite branches is performed by means of a chord approximation. Computation is performed by means of conventional electronic analog techniques. Chord distances are computed according to the Pythagorean theorem by means of squaring, summing and square rooting. The initial coordinates of the chord are held in capacitor hold-circuits. The input to the computer section of the instrument is, by means of linear-motion transducers, fixed to the stage of the microscope along the three coordinate axes. There are two output devices, 1) a digital printer which prints on tape the distance measurements in micra (μ), and 2) a plotting board on which is drawn a two-dimensional projection (in the plane of section) of the neuron. The distances measured range roughly from 3 to 100μ, the accuracy of the measurement is ±1μ or ±9 per cent, whichever is greater. Analysis times are reduced from the approximately 24 hours required by camera lucida techniques and hand calculation to 30 min with this new instrument.

I. Introduction

QUANTITATIVE studies on the structure of the nervous system have not played a major role in the development of neuroanatomical sciences as a whole. One of the factors may be that instrumentation has been, and still is, a severe limitation to the amount and variety of quantitative morphologic data that can be gathered within a reasonable time. In general, the morphologist's instrumentation, while sophisticated and often of excellent quality from the optical standpoint, is deficient in its ability to permit the rapid acquisition of sizable amounts of numerical information about stereometric properties of the object under study.

In order to facilitate the quantitative studies of neuronal morphology being carried out by one of the authors (Van der Loos), an analog computer integrated with the mechanical stage of a light microscope has been developed which permits rapid and accurate measurement of microscopic distances associated with individual nerve cells and which, moreover, yields accurate reproductions in two dimensions of the investigated cells. The instrumentation is presumably of a more general interest to biologists (and probably workers in other fields as well) whose work involves measuring and plotting of microscopic distances.

The use of analog computer techniques appears to be somewhat novel in the field of quantitative microscopy. In this paper, a description will be given of the computer-microscope and of its relation to the type of neuron analysis previously carried out by hand. In a subsequent paper[1] emphasis will be on the quantitative morphologic methods in neuroanatomical research and on the contributions the computer-microscope has to offer to this field.

II. The Neuroanatomical Problem

The microscopic preparations on which the analyses are carried out are 100μ thick sections of the cerebral cortex of various mammals. The blocks of tissue from which these sections are cut first undergo a treatment by virtue of which only approximately 2 per cent of all the neurons available (Sholl [3]: about 2.5 per cent; Van der Loos [4]: 2.05 per cent) are stained a deep black. Each neuron that is stained, however, is stained in its entirety, that is, cell body and dendrites as well. The staining of the axon is often incomplete. Because of the elective property of this so-called "Golgi method,"[2] relatively thick sections can be used which are not so densely black as to become opaque. The use of thick sections enables the investigator to study a considerable portion of the total dendrite length of each nerve cell. To clarify this point, it may be recalled that dendrites are offshoots from the nerve cell body; in the material studied (mammalian neocortex) there are on the order of six dendrites to each nerve cell. They branch mostly dichotomously in all directions, but tend to be confined to a volume of tissue of given shape which may vary from one cell type to another. The fraction of a neuron's dendrite length that can be viewed in a 100-μ thick section is on the order of 55 per cent. For a discussion of

Manuscript received August 15, 1964. This work was supported by U. S. Public Health Service Research Grant NB-04012, from the National Institutes of Neurological Diseases and Blindness; and by a grant received from the Joseph P. Kennedy, Jr., Memorial Foundation. Two brief reports on the computer-microscope have appeared, [1] and [2].

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[1] Van der Loos, H., and E. M. Glaser, Semi-automation as an aid in quantitative neuroanatomy, to be published.

[2] Named after its inventor, the foremost Italian neuroanatomist Camillo Golgi who, in 1875 (see Golgi, [5]) published his technique. The modification used in this and previous studies is a variant (Van der Loos, [6]) of Cox's [7] modification.

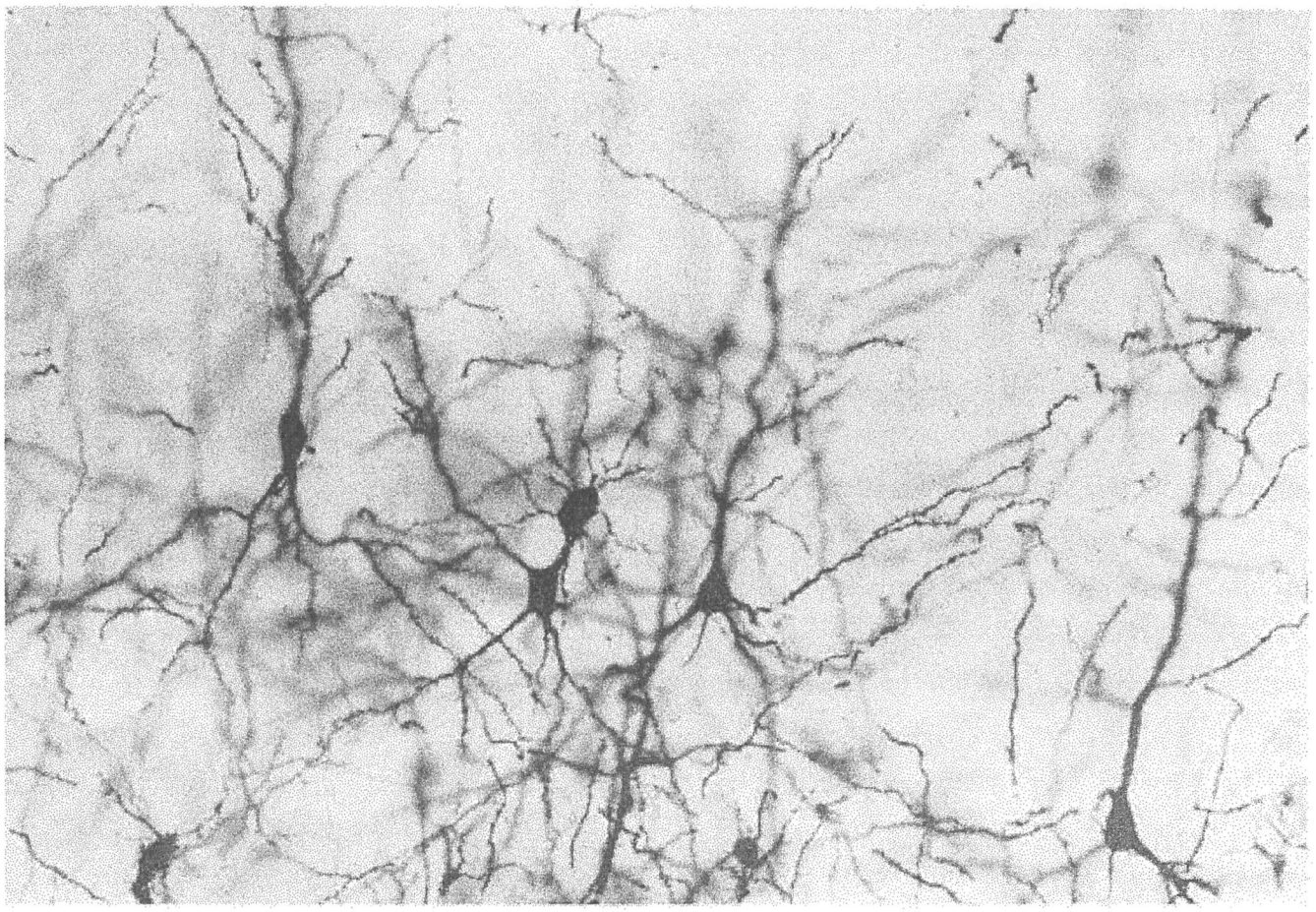


Fig. 1. Photomicrograph of Golgi preparation of rabbit visual cortex. This field contains examples of four of the five principal cortical cell types. Magnification 500×.

this and other aspects of the quantitative methods used, see Van der Loos, [4], [6], [8].

An illustration of the appearance of a Golgi preparation is given in Fig. 1. It is to be noted that the detailed study of the individual neurons as depicted in this figure requires, in the scope of this work, the use of highest possible resolution, high magnification optics.[3] When the analysis was done manually, accurate camera lucida reproductions were made at a magnification of 500×. These reproductions were drawings of the dendrite systems of the nerve cells under investigation. Each drawing represented, in fact, the projection of the dendrite system in a plane perpendicular to the optical axis of the microscope. The desired raw data from this material are the lengths of the individual dendrite branches, or rather, the chord approximations of these lengths. They were determined by measuring the distances between dendrite origin and dendrite branch point, between two consecutive branch points, and between dendrite branch point and dendrite ending, respectively. Examples of such dendrite branches are indicated in Fig. 9(b) by means of *a*, *b*, and *c* in the order given above.

A typical hand drawing, as alluded to earlier, is reproduced in Fig. 5(a). The numbers represent the depths, in micra, in relation to a horizontal plane of reference. Since the magnification of the drawing is known, these depths, together with the branch length projections, permit the application of the Pythagorean theorem. Thus, the three-dimensional length of each branch is calculated.

In making the camera lucida drawings, the diameter of the field of vision was 80μ. The dendrite systems studied have, however, an extent of up to 640μ. In addition, the focal depth was kept as small as possible (1.35μ). Thus, while drawing, it is frequently necessary not only to change the position of the microscope stage and the position of the drawing paper, but also to continually change focus. In this manner, which could justifiably be designated clumsy, the average time needed for the analysis of one neuron was approximately 24 hours.

The time-saving aspect alone makes the computer-microscope an extremely welcome addition to our instrumentation; for an analysis of one neuron yielding the same information as the hand analysis just described takes, on the average, 45 min and no more than one hour.

[3] The microscope used is the Leitz Ortholux. It was chosen because of its sturdiness and the quality of the fine movement of the focussing mechanism (excellent resettability with very little backlash). The instrument is equipped with a plan-apo objective 100× oil immersion lens (N.A. 1.32) and a pair of 25× periplanatic oculars. This combination yields a "lateral" resolving power of 0.2μ and an "axial" resolving power of 0.6μ.

III. The Semi-Automatic Computer-Microscope

A. *General System Description*

The microscope stage is equipped with infinite resolution, high-linearity linear-motion transducers[4] which yield output voltages proportional to the position of the microscope stage in all three dimensions (see Fig. 2). X and Y are the coordinates in the horizontal plane, normal to the optical axis of the microscope. Z is the vertical coordinate, parallel to the optical axis. The transducers are quite small and light in weight and their use in no way interferes with the operation of the microscope. A Leitz 144 stage was selected for relative freedom from "stage noise," i.e., motion components in coordinates other than the ones being controlled. The transducers are equipped with coiled springs which insure proper tracking of the wiper as the stage moves back and forth. With this installation, stage movements tend to become somewhat stiff and less easily controllable. To counteract this, the stage positioning knobs were provided with large collars. The microscope base[5] itself contains the coarse and fine adjustments for the movement in Z and is also equipped with a linear motion transducer. The distance between two points is determined by bringing them successively under the intersection of a pair of reference crosshairs in the field of vision. It is possible to select the origin of the coordinate system at any point that is desired, usually the center of the cell body being investigated. The voltage outputs make it possible to perform all measurements on an electrical analog basis accurately and rapidly. A block diagram of the system is shown in Fig. 3.

The computation of distances is performed as it was done in the hand operation; by using a chord approximation to the curvilinear dendrites whose lengths are to be determined. An additional reason for employing this type of measurement is that in following the path of a meandering dendrite, one is faced with the necessity of performing frequent changes in the focus of the microscope. These focal changes are largely unpredictable and, also tend to introduce a certain amount of uncertainty in the X and Y coordinates. These conditions, together with the plethora of intertwined neurons, even though only 2 per cent of them are stained, make it frequently necessary to retrace a segment of the dendrite in order to be assured that one has not strayed from the correct path, to a dendrite of another cell, for example. Therefore, a continuous and error-free tracking of a dendrite cannot be performed. One can, however, arrive at any particular point of the dendrite after a number of detours and backtracks. It is appropriate then to use only a starting point and a terminal point as the basis for a computation of the distance between two relevant points (see Section II). Of course, the accuracy of the approximation would improve as the distance between the points decreases (assuming perfect measuring equipment). If an analysis should require such a decrease, one could modify relatively easily the procedure somewhat in order to obtain the desired closer approximation to the real dendrite length. However, under the present circumstances this would slow up the analysis considerably.

In order to compute distance by the straight line approximation, it is necessary to obtain the coordinates of both the initial and the final points of the chord representing the measured segment of the dendrite branch. The final point coordinates are obtained directly from the linear-motion transducers, while the initial-point coordinates are obtained by storage of the transducer voltages acquired while the stage was focused on the initial point. Such storage is performed conveniently in analog-computer capacitor holding circuits.

Once the three-dimensional coordinates have been obtained, the distance between the final and initial points is found by coordinate subtraction and application of the Pythagorean theorem. The required squaring and square-rooting operations are performed with the aid of Quadra-

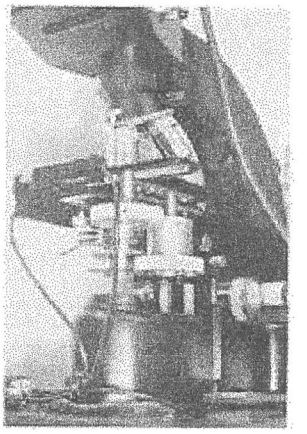


Fig. 2. View of linear motion transducer installation on microscope. The X and Y transducers are on the stage; the Z transducer is on the base.

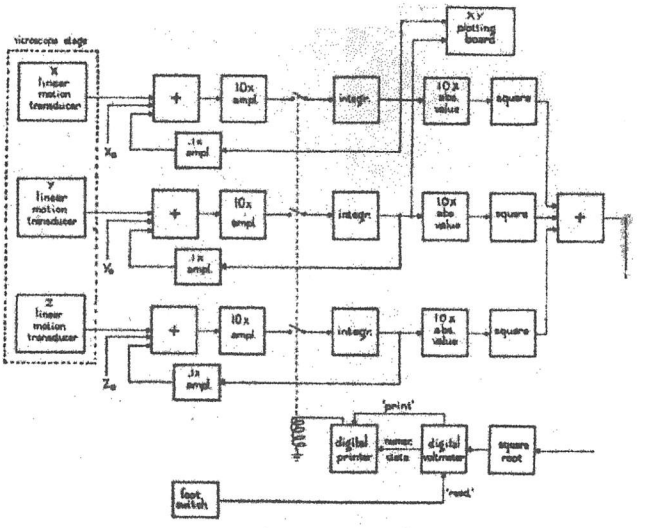


Fig. 3. Block diagram of distance computation system.

[4] Computer Instrument Corporation, Hempstead, N.Y., (Model 113).

trons.[5] These are nonlinear resistive elements located in the summing and feedback networks of an operational amplifier. The output of the triangulation, or distance-computing network is fed to an analog to digital converter and digital printer combination[6,7] which prints out the distance measured, along with an item count of each measurement made. It is possible at any time to obtain a subtotal from the printer so that one may, at the conclusion of the measurements on a single dendrite, for example, print out the total length of the dendrite. At the conclusion of the measurements on a particular neuron, one may also obtain the total length of all the dendrites belonging to that cell.

Along with the computation of distances along the dendrites of the cell, a two-dimensional 500× or 1000× drawing of the cell is made on a plotting board.[8] The plotting board in this situation operates in an incremental fashion. That is, it remains at the particular position representing a point already obtained on a dendrite until the microscope stage coordinates are changed to those of the subsequent point on the dendrite and the relevant distance computation has taken place. For each measurement, the distance computation, as well as the subsequent position change of the plotting board pen, takes place at a single command of the operator. The plotting board pen moves in a straight line from one point to the next. This is achieved by the installation of balanced, long time constant input circuits at the plotting board inputs.

The output of the computer section thus consists of distance measurements in the form of four digit numbers on an adding machine tape, as well as a plot of the dendrite system studied. The digital measurements are later rounded off to two significant figures, representing distances up to 99μ. It is possible for the investigator to correlate the three-dimensional numerical printer output with the two-dimensional plotting board output by writing item count numbers next to the drawn branch projections on the plotting board. This is done during the analysis itself and is not as cumbersome a procedure as it may initially seem. Needless to say, this operation could be automated with an additional investment in equipment.

Figure 4 is a photograph of the complete system.

B. *Modus Operandi*

The sequence of events in operating the instrument is as follows:

1) A reference point is chosen. Usually this is the center of the cell body.

2) Front panel zeroing potentiometers are used to null out the readings of the transducers at the reference point.

[5] Douglas Aircraft Company, Inc., Aircraft Division, Long Beach, Calif., (Type P, Model E).
[6] Cubic Corporation, San Diego, Calif., (Model V-46APS).
[7] Victor Business Machines Co., Chicago, Ill., (Digit-matic, Model 12.08-272).
[8] Electronic Associates Inc., Long Branch, N.J., (1100 E Variplotter).

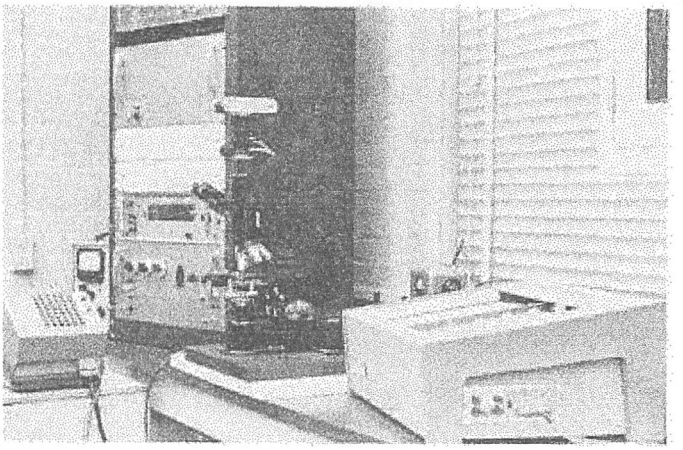


Fig. 4. Overall view of the computer-microscope.

This is done at the summing junction of the input amplifiers.

3) A reference point is chosen on the plotting board, by means of its centering potentiometers, in accordance with the shape of the dendrite system of the neuron to be mapped.

4) The microscope stage is moved to the first point to be mapped and measured.

5) The operator presses a foot switch. This produces the following sequence of operations:

a) The distance moved is measured and printed.
b) After printing is completed, the new coordinates are stored in the holding circuits, erasing the old ones, if any.
c) The plotting board pen moves to the position corresponding to the new X and Y coordinates.

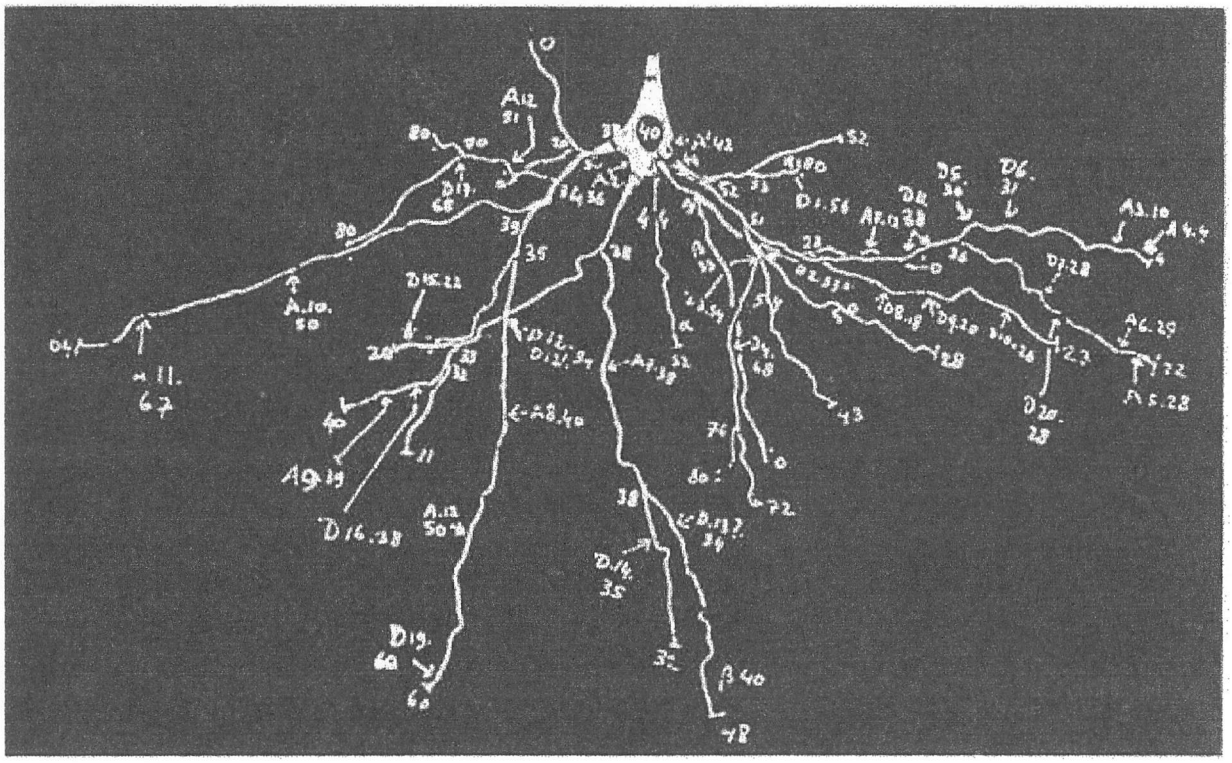
The end of this cycle is indicated by a signal light. The operator then continues the analysis by repeating steps 4 and 5 as often as is necessary.

It should be noted that a penlift switch enables the operator to raise and lower the plotting-board pen at will. He would raise it if, for example, he returns from a dendrite ending to an already passed branchpoint in order to then measure and plot the other branch originating at that point. In such return tracings the printer does not record the distance traversed.

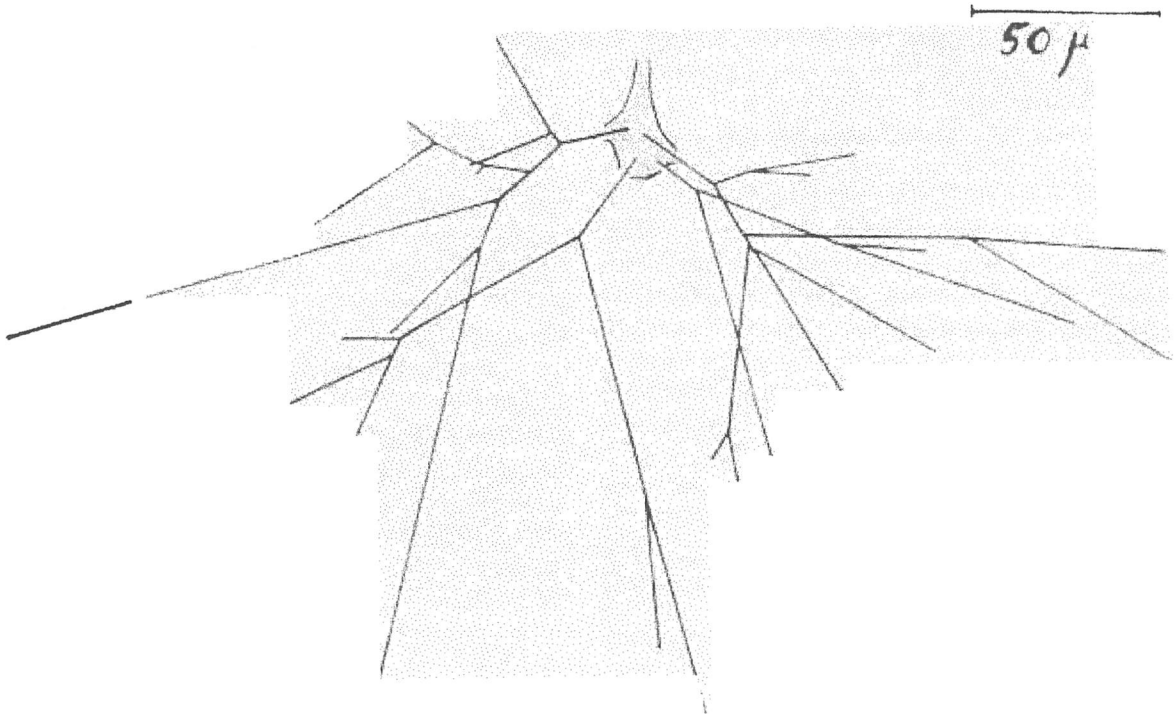
In Fig. 5, one may compare the results of the pictorial parts of two analyses of the basal dendrite system of the same cell; one carried out by hand (a), the other with the computer-microscope (b).

C. *Design Considerations*

1) *Linear motion transducers:* The nature of the measurement procedure is such that, per move, only relatively short distances along the dendrite are measured. The range of distances is from 3 to approximately 200μ. In a typical series consisting of 500 measurements, the range was 3-206μ. Approximately 65 per cent were between 5 and 40μ and approximately 25 per cent between 40 and 100μ. Originally it was felt that rotary potentio-



(a)



(b)

Fig. 5. (a) Camera lucida drawing of the basal dendrite system of a pyramidal cell in rabbit cortex. The numbers are the Z coordinates. Magnification 500×. (b) Computer-microscope drawing of the same dendrite system at the same magnification. The cell body has been drawn in by hand for illustrative purposes.

meters affixed to micrometers moving the stage in the three coordinate directions would be satisfactory for transducing position into voltages. Pertinent trials and discussions with the microscope manufacturer's representatives (Leitz)[9] disclosed that their biological microscope stages had too much inherent backlash in the rack and pinion mechanism to permit achieving the desired accuracy of measurement. Also, the ratio of linear stage displacement to angular rotation of the pinion was determined to be too large to yield accurate measurements unless a very large diameter potentiometer was used. For practical and economic reasons, therefore, it was decided to use linear-motion transducers fixed directly to the stage, bypassing the problem of backlash inherent in commercially available stage drives. (In fact, a specially designed stage, driven by micrometer spindles affixed to rotary potentiometers was considered a better choice but found to be too expensive.) The transducers used are infinite resolution carbon film potentiometers[4] which have a specified and measured linearity of 0.2 per cent, and a backlash of about 1μ. The total travel of these transducers is 20 mm. The terminal-linearity figure indicates that the maximum error in reading position at any point along the travel of the transducer is $0.002 \times 20\,000\mu = 40\mu$. Terminal linearity here is specified in terms of voltage deviation from the ideal straight line input-output plot as a percentage of the maximum output voltage. This figure in itself is not of great importance since incremental distances are being measured. The important aspect of potentiometer linearity is, rather, the "wavelength" of the linearity curve. This is indicated in Fig. 6(a) where a curve is shown relating the deviation of the transducer output voltage (reconverted back to equivalent "micra") from the ideal straight line.

It can be seen from this figure that the important quantities related to the potentiometer performance are the slopes of the curve between two consecutive points of maximum deviation. When the incremental distances being measured are much smaller than the wavelength of the curve, as is always the case here where the wavelength is of the order of 3000μ, the slope and the wavelength determine one component of the error incurred in the measurement. There is also an apparently random short wavelength fluctuation in the linearity curve superimposed on the larger, long wavelength fluctuation. The nature of this error can be seen in Fig. 6(b) where the potentiometer output in equivalent micrometers is plotted against input position of the wiper for a 100μ section of the potentiometer. The maximum deviation from the ideal straight line is 3μ and the "wavelength" associated with this variation is of the order of 10μ. It can be seen, therefore, that the measurement error involved is the sum of components produced by the long wavelength error and the smaller short wavelength random error whose maximum value is

[9] Laboratory for Applied Microscopy, E. Leitz, Inc., New York, N.Y.

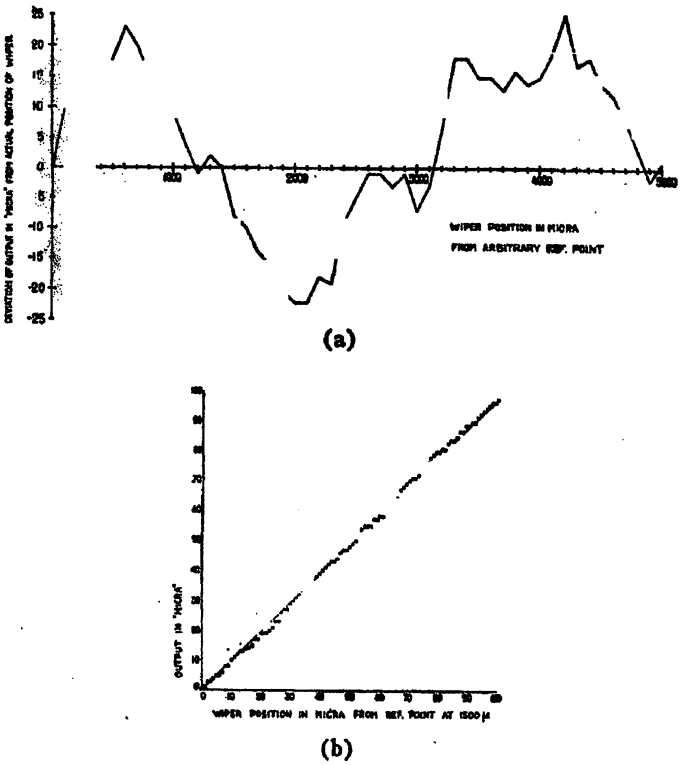


Fig. 6. (a) Deviation of linear motion transducer output from ideal straight line. Measurements were made at 100μ steps over a 5000μ interval. Sensitivity was 10 mV/μ. (b) Output of same transducer vs input over the 100μ interval between 1500 and 1600μ (in a) at 1μ steps. The straight line is drawn between the 0 and 100μ output values. Sensitivity 10 mVμ.

no larger than 6μ and whose standard deviation is close to 1μ. The long wavelength error component is given approximately by the product of the slope of the error curve and the actual distance being measured. In Fig. 6(a) the maximum slope between points of the curve 100μ apart is approximately 0.1. The average slope (absolute value) is approximately 0.03. A 50μ distance will thus be measured with an error which can be as large as 11μ (long wavelength error of 5μ and short wavelength error of 6μ) but which has a standard deviation (s.d.) which is on the order of 3.5μ (long wavelength error s.d. of 1.5μ and short wavelength error s.d. of 2μ) or 7 per cent of the measurement. This error is for one coordinate of the three being measured. The error in the total measurement of distance as obtained by squaring and square rooting according to the Pythagorean theorem is a much more difficult figure to obtain and will be discussed later in this paper from an empirical viewpoint. We note here, however, that the operation of squaring will tend to bias the sum of the squares to an average value which is larger than the true value even though the individual component measurements are unbiased.

2) *Amplification and subtraction:* The linear motion transducers are operated to yield a sensitivity of 10 mV/μ. This sensitivity is inadequate for driving the squaring and square-rooting circuits (see Fig. 3 for se-

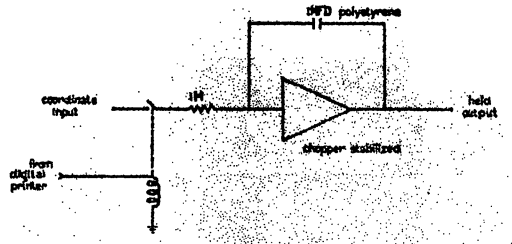


Fig. 7. Capacitor hold-circuit for storing coordinates of initial stage position.

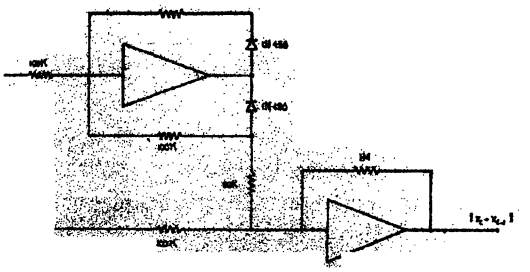


Fig. 8. Absolute value circuit.

quence of computer operations), and amplification is consequently required. The analog computing circuitry provides a gain of 100 to yield a sensitivity at the input to the squarers of 1 V/μ. The amplification is done in two stages, each with a gain of 10. The output of the first stage drives an integrating circuit with a time constant of 1 sec. The integrator is operated as a conventional capacitor hold circuit by means of a relay whose contacts are located in series with the input resistor (Fig. 7). When the relay contacts are closed, the circuit operates in the integrate mode and when the relay contacts are open, in the hold mode. The decay time constant of the circuit is a function of the leakage resistance of the condenser and the gain of the operational amplifier. A polystyrene condenser is used here in conjunction with a chopper stabilized amplifier.[10] The time constant of the circuit is therefore quite high. It has been observed that the deviation of the holding circuit amplifier output was less than 1 per cent after a 10-min time in the hold mode. This means that the time constant is at least 1000 min which is amply satisfactory for the present application since, in the usual operation of the instrument, the required holding times are only of the order of 15 sec.

The output of the holding amplifier for each coordinate is fed back through a third amplifier (gain 0.1) to the input amplifier. There are thus three inputs to the input amplifier: 1) the present value of the distance coordinate; 2) the negative of the held value representing the last measured coordinate value; 3) a reference value corresponding to the initial reference point (center of the neuron), as stated before (see Section IIIB). This reference value is set at the beginning of the measuring procedure and is held constant throughout the measurements made on the neuron. The other two inputs are constantly changing as the measurement procedure progresses, for every time the distance has been recorded, the final chord point of the previous measurement becomes the initial chord point of the next measurement.

3) *Squaring and square rooting:* The output of each of the input amplifiers is the difference, in voltage, between the present coordinate and the past coordinate measurement. The required measurement is the distance, in three dimensions, between the two consecutive chord points;

$$s = [(X_i - X_{i-1})^2 + (Y_i - Y_{i-1})^2 + (Z_i - Z_{i-1})^2]^{1/2}$$

This value is conveniently obtained in analog circuitry by means of the Quadratron.[5] This device was developed by Kovach and Comley [9] and employs temperature compensated varistors. The accuracy of the Douglas Quadratrons is specified to be 0.2 per cent of the full scale output of 100 V (squarer transfer function: $e_o = 0.01e_i^2$). Here, this corresponds to 100μ and so the error produced by squaring is less than 0.2μ. The operation of square rooting introduces a similar 0.2 per cent error. The total error produced by the triangulation circuitry will be a function of the magnitude of the individual coordinate distances. A chord whose components are 100μ in each of the three coordinates will be measured with a maximum error of about 0.3 per cent. On the other hand, a chord whose components are 10μ in each of the three coordinates will be measured with a maximum error on the order of 3 per cent. This error is smaller than that introduced by the linear motion transducers for distances smaller than about 50μ.

A necessary step before the squaring and square-rooting operations is the taking of the absolute value of the coordinate distances measured. This is performed by means of a circuit described by Howe [10] requiring two operational amplifiers per channel. The circuit is shown in Fig. 8 and provides a gain of ten.

4) *Analog to digital conversion and printout:* The output of the square-rooting circuit representing the three-dimensional measurement is fed, after being attenuated by 0.1, to an analog to digital converter. Attenuation is employed here to reduce sensitivity to the thermally produced fluctuations occurring throughout the system and appearing in the output of the square rooter. The particular instrument used here is a cubic digital voltmeter.[6] This instrument reads to four places with an accuracy of 0.01 per cent of full scale ± 1 digit. Its output is in turn delivered to a Victor Digitmatic totalizing printer.[7] Each measurement is printed out at each step of the measuring sequence. An item count is also recorded by the printer, automatically, so that the total number of measurements made can be easily determined. At any time it is possible to obtain a subtotal or a total of a given group of measurements made.

* Embree Electronics Corporation, West Hartford, Conn., (Model 1701 /s. These amplifiers, 16 in total, were used throughout the system.)

TABLE I

SYSTEM ERRORS

Source	Nature of error	Magnitude of error	Comments
Linear-motion transducer	Linearity Thermal drift Backlash	0.2 per cent (terminal) 100 ppm/degC 1μ	Carbon film element Carbon film element No error if point always approached from same side
Power Supply*	Short term drift Long term drift	0.002 per cent negligible	Zener diode voltage reference 45 min warm-up
Input Summing Junction	Resistor tolerances Resistor temperature coefficients	0.1 per cent 20 ppm/degC	Wire wound and metal film resistors Wire wound and metal film resistors
Holding Circuit	Leakage decay	Time constant greater than 1000 min	Polystyrene holding condensor
Operational Amplifiers	Gain variation (each channel)	Less than 0.4 per cent maximum	0.1 per cent resistors used
Squaring Quadratron	Departure from true square	Less than 0.2 per cent full scale	"Sine" wave error curve over full range of inputs. Maximum error 0.2 per cent full scale.
Square-Rooting Quadratron	Departure from true square root	Less than 0.2 per cent full scale	"Sine" wave error curve over full range of inputs. Maximum error 0.2 per cent full scale.
Plotting Board	Nonlinearity	0.075 per cent	Does not affect accuracy of computat.
Analog-Digital Converter	Conversion error	0.01 per cent full scale ± 1 digit	Last two digits used for round-off.

* Embree Electronics Corporation, West Hartford, Conn. (Model PS/200/35, master power supply; and SS/200/3.5, slave supply).

5) *Analog read-out:* Outputs of the X and Y holding circuits are also fed to the plotting board.[8] During the time the coordinates are being held, that is during the time that the search is made for the next point to be measured, the stylus of the plotting board is motionless. When a new set of coordinates is obtained, operation of a footswitch slews the plotting board pen to the new coordinate position immediately after analog to digital conversion of the distance has been completed. Between the outputs of the hold circuits and the input to the plotter are inserted variable attenuation networks which permit the plotting board to represent the dendritic field at any desired magnification up to 1000×.

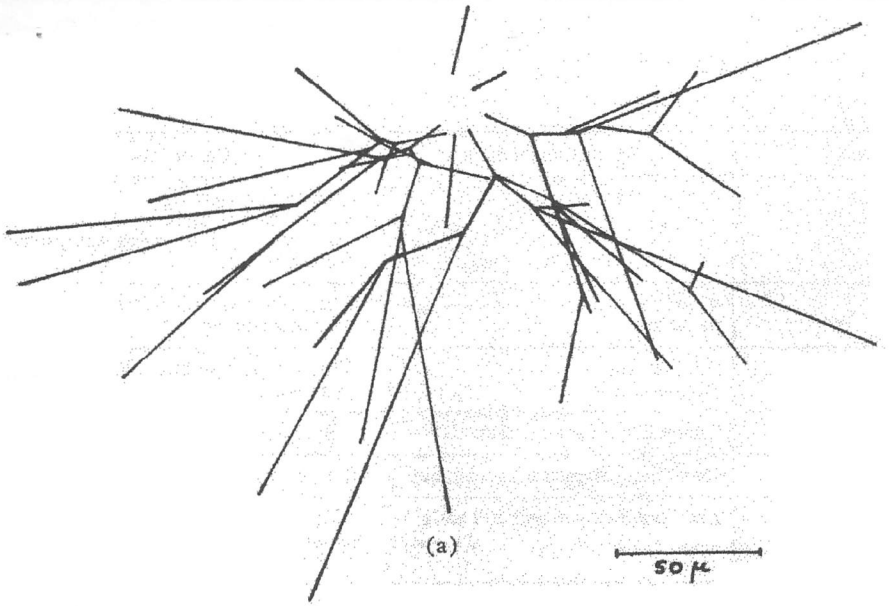
The input circuits to the plotting board are also provided with identical long time constant networks to insure that the path of the pen from one point to the next is a straight line. Only two dimensions are obtained on the plotting board, X and Y, the dimensions in the plane perpendicular to the optical axis. It is possible, however, to make one of these dimensions the Z, or depth, coordinate of the preparation, should that be desired, by a simple switching arrangement. During the time the neuron is being traced and measured, correlation between the numerical data and the two-dimensional plot is maintained by the operator who records the item count on the printing tape of each dendrite segment being plotted. It was judged to be economically unwarranted to perform this operation automatically.

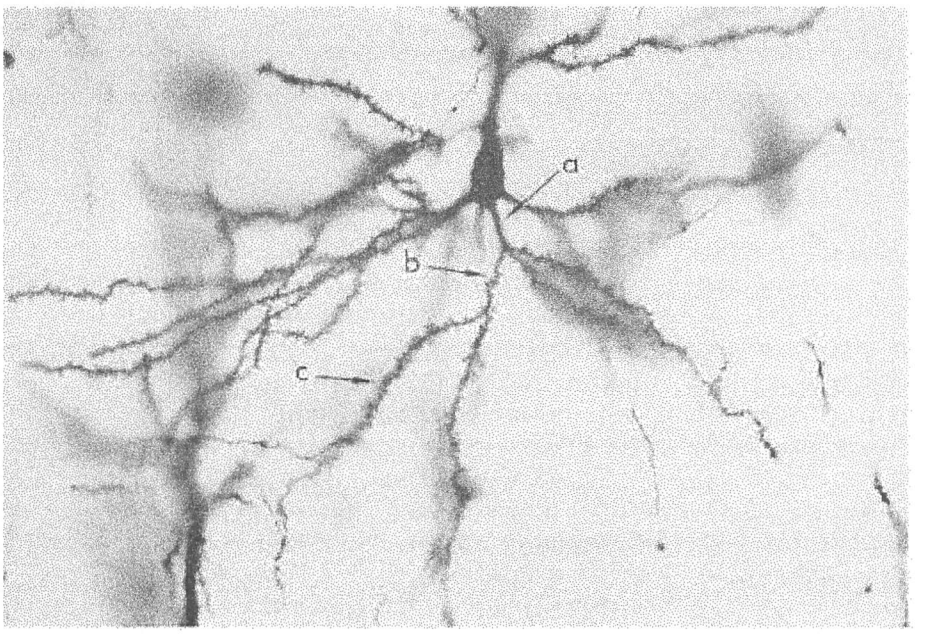
D. *Error-Analysis*

In Table I the source, nature and magnitude of the various errors inherent in the system are indicated.

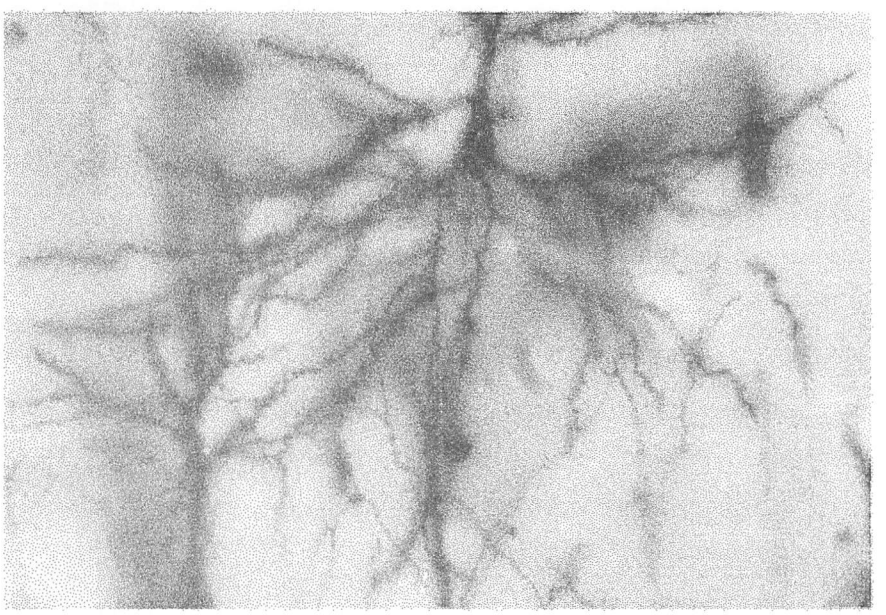
A detailed theoretical analysis of the manner in which these errors affect the overall accuracy of the system is quite difficult and beyond the scope of this paper. We note, for example, that the nonlinear operation of squaring and square rooting involved in distances, have a complex effect on the combining of the errors from the individual coordinate inputs. When, furthermore, the squaring and square-rooting operations are themselves subject to error, the complexity becomes extreme. It is nonetheless important that a measure of the overall accuracy of the system be obtained in order that its performance can be evaluated, and, especially, in order that the direction toward improved performance can be determined.

The following empirical test permits some evaluation of the overall performance of the computer-microscope under conditions similar to those of its regular operation. An accurate stage micrometer was oriented on the stage at an angle of 45° with respect to the X and Y axes. This micrometer is inscribed with 198 divisions spaced 10μ apart. Two types of measurements were made; those which included a 10μ distance traverse in the X-Y plane, and those which included a 20μ distance traverse in the X-Y plane. These two types of measurements were taken throughout the 1.980 mm total length of the stage micrometer in such a manner that three 10μ distances in the X-Y plane were followed by one 20μ distance. A 10μ traverse at 45° in the X-Y plane means a displacement of the stage equalling $(50)^{1/2}\mu$ in X and Y each. In order to give as little as possible bias to each of the coordinate axes in obtaining the resulting three dimensional value,





(b)



(c)

Fig. 9. Comparison of computer-microscope drawing (a) with photomicrograph (b) of same basal dendrite system. In (b), *a*, *b* and *c* mark examples of the three categories of dendrite branches, of which the lengths were determined (or rather: the chord approximations of their lengths, see section II. (c) A multi-exposure photomicrograph of the same dendrite system: many more of the cell's dendrite branches can be visualized, though at the expense of clarity. Also, the field of view is contaminated, markedly more so than that of Fig. 9(b), with quite a few dendrite and axon branches of neighbouring neurons. Magnification of (a), (b), and (c): 380×.

the stage was moved in Z over a distance of 7μ simultaneously with each 10μ displacement in the X-Y plane. These 7μ could be read from the calibrated fine adjustment dial of the microscope's focusing control. The true value of the total distance traveled, S_1, is $(149)^{1/2}\mu = 12.2\mu$. $\bar{S}_1$, the experimental mean, is 12.4μ; the standard deviation about S_1 is 1.5μ or 12 per cent of S_1.

In regard to the 20μ stage displacements in the X-Y plane, they consist of a move of $(200)^{1/2}\mu$ in each X and Y. For the same reason as given above with respect to the 12.2μ measurements, a movement of 14μ in Z accompanied each 20μ move in X-Y. The true value of each measurement, S_2, is $(596)^{1/2}\mu = 24.4\mu$ $\bar{S}_2$ is 25.2μ; the standard deviation is 2.2μ, or 9 percent of S_2.

In this test, after each length of 100μ in the X-Y plane (including six 10μ and two 20μ displacements) was traversed, the direction of movement in Z was reversed. This corresponded to a 70μ displacement in Z. 120 measurements of 12.2μ were made and 39 of 24.4μ.

We note here that before the above test each coordinate calibration was made at the output of the square rooter for X, Y, and Z movements. A series of 20 measurements of 50μ in each coordinate was made and the average taken in order to adjust the sensitivity of each channel properly. The errors in the 7μ and 14μ measurements taken in all dimensions simultaneously are probably explainable by the transducer error rather than errors incurred elsewhere in the system.

IV. Discussion

At the time of submission of this manuscript the computer-microscope has been in operation for 18 months, to our great satisfaction. As with any complex instrument, some routine maintenance is necessary. This involves regular checks of: 1) the accuracy of the transduction into voltages of the displacements of the linear-motion transducers, 2) the accuracy of numerical data processing by the computer section of the apparatus, 3) the faithfulness in representation of the two-dimensional stage movements by the plotting board, 4) the drift of the three holding circuits.

For each of these four adjustments, simple, standard procedures are used. An entire recalibration takes three-fourths of an hour. Only step 4) has to be carried out before each usage, probably because of the sensitivity of the holding circuits to thermal fluctuations. This adjustment is performed by means of three conveniently located trimpots and takes only a few minutes. It is performed after the instrument has reached a point of satisfactory thermal stability that is, after a 45 min warm-up period.

The computer-microscope is currently in use in two investigations, one which aims for a quantitative characterization of dendrite systems of the five main cell types that populate the mammalian cerebral (iso-) cortex; the other dealing with a quantitative assessment of dendrite growth.

A sample of our analysis taken from the former study is illustrated in Fig. 9. Figure 9(a) represents a machine-tracing of the basal dendritic system of a pyramidal cell of the (mature) rabbit visual cortex. Figure 9(b) presents a photomicrograph taken at identical magnification, of the same cell. Figure 9(c) depicts this cell more completely in a multiexposure photomicrograph.

In conclusion, it may be stated that the addition of linear motion transducers to the stage of a light microscope, of the type in use in biologic research, together with appropriate computational equipment, extends the capabilities of the instrument to the performance of quantitative measurements often held to be too elaborate and time consuming. Although the linearity and accuracy of the present instrument is limited essentially by the linear motion transducers, this is not a basic limitation. Improvement in the design of these devices can be expected. Alternatively, it should be possible to design micrometer driven stages, as are used on certain metallurgical microscopes, to which are attached multiturn potentiometers or digital shaft angle encoders. In the latter case a high-accuracy digital system would be obtained; in the former an improved analog system of the type discussed here. Both systems would employ holding circuits to preserve previous coordinate positions and, of course, triangulation circuits for distance computation.

Acknowledgment

The authors wish to express their appreciation to Dr. D. Bodian, Director of the Anatomy Department, School of Medicine, The Johns Hopkins University, for his encouragement, and to Dr. R. E. Cooke, Director of the Department of Pediatrics, The Johns Hopkins Hospital, and Dr. F. Richardson, of the same department, for their confidence in our project. Also, they are grateful to Mr. P. Tittel for his skillful assistance in the construction of the system. Finally, they thank Drs. P. Bartels and M. Nahmmacher of E. Leitz, Inc.[9] for their interest and valuable advice concerning the microscope "end" of the apparatus.

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