Quantitation of Biological and Other Data by Photoelectric Measurement of Areas.* (19916)

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The measurement of areas, regular or irregular, planar or projected, is frequently necessary in engineering and physical sciences. More recently, the method of area measurement has found increasing application in the computation of data obtained in various types of biological investigations and laboratory work. The usual method of area measurement calls for the laborious and tedious use of the polar planimeter. To avoid the disadvantages involved, it seemed desirable to develop an apparatus which would be suitable for the rapid and accurate determination of a wide range of areas (including very small ones), such as may be encountered in various laboratory technics in current use. A versatile instrument, utilizing the photoelectric measurement of intercepted light, and easily and inexpensively constructed in the laboratory from readily available components, has been devised. In simplicity, rapidity, and accuracy, its use is especially advantageous for the computation of the areas of electrophoretic patterns.

Description of apparatus. In order of arrangement, the essential components consist of a uniform light source, a condensing lens, a base plate surface at which the sample intercepts the light, another condensing lens, and a photoelectric cell connected to a galvanometer and a variable resistance.

The arrangement and design of the photometric apparatus are shown in Fig. 1. The light source consists of a 100-watt photo-enlarger-type (No. 212) lamp bulb in the Condensor Head (A) of a Kodak Precision Enlarger. The focusing (objective) lens, bellows, and the lower half of the condensing lens system, normally parts of this unit, are removed, leaving in place only the upper condensing lens (U). A 60 cycle, 120 watt voltage stabilizer of input 95-130 volts, output 115 volts (Raytheon Mfg. Co.) is required for the maintenance of constant voltage to the light source. The column holding the modified enlarger head is detached from its base and bolted to the leveled top of a light-tight, cabinet-type table base (B). In this top an aperture (O) 7¼ x 7¼ in. is cut. A recessed shoulder (S) ⅛ in. deep and ⅜ in. wide is machined along the perimeter of the aperture and serves to receive the various types of sample base plates (P) *e.g,* 8 x 8 in., which may be required. Usually, double thickness clear window glass may be used, or, for some applications ¼ in. metal sheet (Dural), the edges of which are machined to fit the aperture with upper surface flush with the table top. The metal base plate serves to hold area specimens on thin glass plates (G) (as in some applications, 9 x 12 or 8 x 10 cm). An aperture is likewise cut in the center of the metal plate, to permit rapid and reproducible placement of these glass specimen plates on a squared shoulder, recessed so that the glass plate is flush with the top surface of the holder. A finger indentation or tab of tape serves for the withdrawal of base plates and glass specimen holders.

The light source and base plates are centered and aligned. Sources of stray light are eliminated and possible reflecting surfaces are coated with Kodak Brushing Lacquer No. 4, dull black. *Flexaframe* (Fisher) assembly units (F), consisting of ½ in. rods, connectors, and feet, form the structural support for the focusing lens (L) and photoelectric cell (C) located below the table top. The vertical rods are aligned in proper position and fastened to top and floor of the cabinet by means of the foot units. The lens, 6 in. in diameter and 1½ in. thick at the center, of focal length approximately 10 in., is mounted in a properly leveled holder, in a centered position directly below the table top. With the light source in posi-

* The opinions expressed are the authors' and do not necessarily reflect those of the Navy Department.

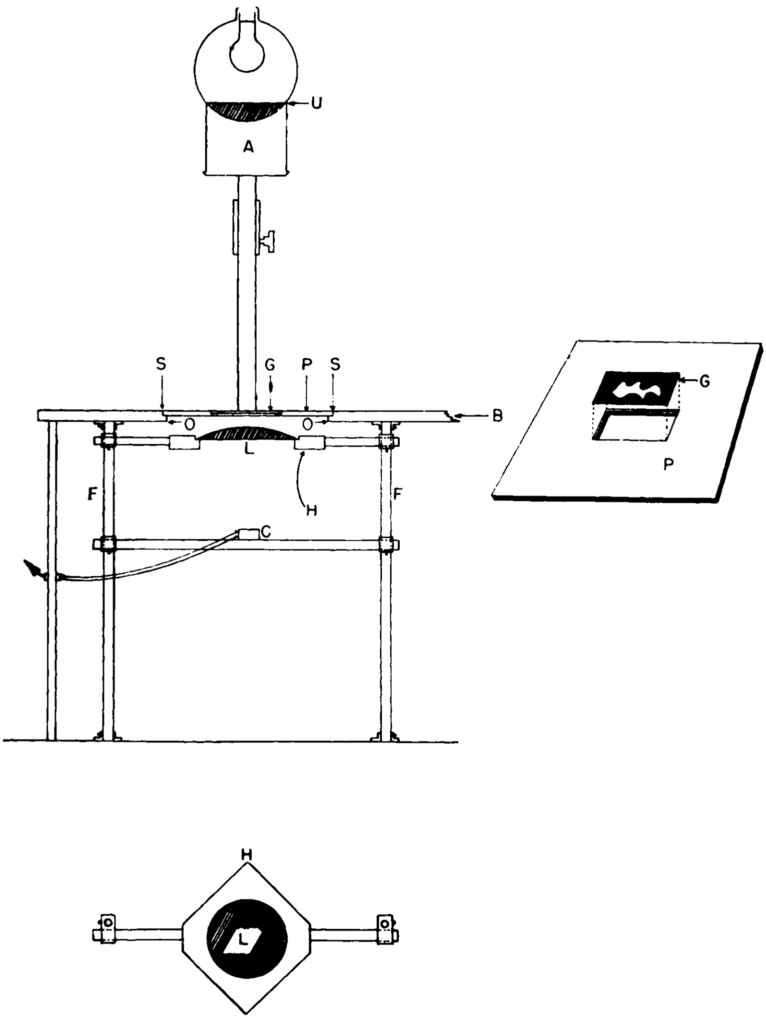

FIG. 1. Construction of areameter as described in text.

tion (usually at the highest level) about 37 in. from the table top, the photocell (Weston Photronic No. 594 RR or 856 RR) is fixed at a level (approximately 12 in. below the lens) such that the rectangular image of the condenser head aperture appears on the active surface of the cell in the maximum size circumscribed by the bakelite case.

The *photo current* generated by the cell is measured by a Rubicon galvanometer (Cat. No. 4625), sensitivity 0.006 μA/mm, resistance 1160 ohms, period 2.4 seconds, critical damping resistance, 7,800 ohms. The top of the galvanometer box is surmounted by an aluminum sheet metal cover with slides and back extended downward to shield against the effect of air currents, with a protecting piece of glass in front of the scale, for the same purpose. A rack and pinion device cemented to the front of the glass scale and operated manually by a knurled knob through the top of the box, permits fine control of scale movement to right or left.

A 180 ohm Helipot (Beckman Technical Laboratories) variable resistance, in parallel with the galvanometer, allows light current adjustments to a wide range of light intensities. A single pole double throw switch of high quality, is placed in the photocell circuit. In the neutral position, it serves to avoid wide swings of the galvanometer off the scale, whenever, as between readings, or when adjust-

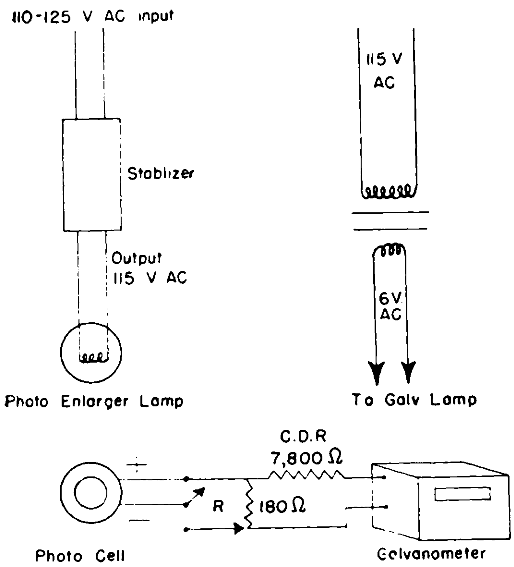

FIG. 2. Electrical circuits of areameter.

ments are being made for them, the cell is exposed to light of high intensity. The electrical circuit is shown in Fig. 2.

Procedure. The apparatus should be used under conditions such that environmental light does not, to any perceptible extent, affect the response of the photocell. To this end, there may be required a somewhat darkened room, or the use of a photographer's black cloth as a light shield between the enlarger lamp and the table top. The enlarger lamp is turned on, and usually allowed to attain a steady state over a period of 10 minutes. During this time, adjustment for dark current is made by covering the table top aperture with a sheet or flat piece of opaque material, closing the photocell switch to the galvanometer circuit position, and adjusting the galvanometer reading manually, exactly to zero.† A standard, known, transparent area masked by an opaque field, on a base plate corresponding exactly to that used for the specimen areas to be measured, is inserted in place and the galvanometer reading is adjusted to 100 electrically, by means of the variable resistance R (Fig. 2). The unknown specimen area is then immediately put in position, and the galvanometer reading observed. The full light current adjustment (the 100 setting) should be checked at frequent intervals between measurements of unknowns; the dark current zero should also be noted, although less often. To facilitate readings to 0.1 scale division a hand magnifier is used. Operational details and technics for various types of area measurements are best provided by the examples of experimental results given in the following.

Calibration and calculations. For the instrument described, the measured area must fall within the circumference, and hence be less than that (126.72 cm^2 or 19.64 $in.^2$) of a circle 5 inches in diameter. Measurements may be made of opaque (or less frequently, translucent) areas on a transparent background, or preferably, of transparent areas on a dark background. In effect, all measurements, whether of clear or opaque areas, are made by comparison with a ***known,*** transparent standard area set at a galvanometer reading of 100. Thus, the unknown area to be measured will be calculated as $Ta = R \cdot A/100 = Rm$, or $Oa = (100-R)\,A/100 = (100-R)m$ where Ta, Oa, and A denote unknown transparent, opaque, and known standard transparent areas, respectively, R is the reading of the unknown area, and m is the slope constant for all readings made against the known area with its galvanometer reading set at 100.‡ ***Permanent primary standards*** for calibrating the areameter over a range of

† It is good practice at all times when the instrument is not in use, to keep the switch in the position which short circuits the photocell. The photocell switch is closed to the galvanometer circuit only when galvanometer settings or readings are made.

‡ It is not necessary to have the transparent background area either known or calculable if measurements are made against a known, standard, opaque area provided the transparent background is kept constant and the same for any given set of measurements. The calculations for unknown transparent or opaque areas will then be made, respectively, according to the following equations:

$$T_u = \frac{R_{Tu} \cdot O_k}{S - R_{Ok}} \quad \text{or} \quad O_u = \frac{(S - R_{Ou})\ O_k}{S - R_{Ok}}$$

Where T and O are transparent and opaque areas, k and u are known and unknown, R is the appropriate reading, and S is the galvanometer setting for the transparent background, held constant for any group of unknown readings against a given opaque standard.

areas, and for testing the linearity of the instrument are made from 1/64 in. ground steel stock (Brown and Sharpe Mfg. Co.). A series of rectangular areas ranging from 0.402 to 38.712 cm^2 (0.0625 to 6.00 in.^{2}) $\pm$ 0.005 cm ($\pm$ 0.002 in.) in each dimension are machined from the same pieces of stock. These metal standards are then used directly as opaque areas, or as templates for tracing and cutting secondary standards on taped glass plates as described in the following. In the latter case, the area or its background is peeled from the glass to leave either a transparent or opaque standard silhouette of identical size and shape as the metal template.

Experimental results. Clean glass base plates§ 8 x 8 in. were covered on one side with black Scotch brand tape No. 22, 5 in. wide (Minnesota Mining and Mfg. Co.). Well centered on individual plates, circles from 12.70 to 2.54 cm (5 to 1 in.) in diameter, were cut with a sharp, pointed graver (X-Acto) and peeled off the glass surface of the plates, to yield a series of transparent circular areas. The 12.70 cm circle was set in place, covered with a piece of cardboard, and the galvanometer set at zero. The cardboard cover was removed and the galvanometer reading adjusted to 100. In succession, the other plates were substituted for the 12.70 cm circle and the readings noted.‖ Table I indicates that the results are in accordance with the theoretical requirement that when the illumination is uniform, the intensity of light transmitted through the base plate be proportional to the cross sectional area of the transmitted light beam. The observed galvanometer reading values plotted against areas lie on or close to the theoretical line passing through the origin.

TABLE I. Proportionality of Light Intensity to Area of Transparent Circles of Known Diameters.

Diam. of circle, cm	Galvanometer scale reading*	Calc. area, cm^2	Observed area, cm^2
12.70	100	126.72	—
10.16	64.3, 64	81.10	81.30
7.62	36.2, 35.9	45.62	45.68
2.54 + 5.08	19.8, 19.6	25.36	24.97
5.08	15.9, 15.7	20.26	20
2.54	3.9, 3.9	5.10	4.97

* Results of 2 experiments performed on different days.

In another test, the accuracy of area measurements and the reproducibility of fabrication of the standard metal templates were determined. A succession of individual 2.540 cm metal squares was placed on a taped base plate within a transparent circular area of 124.58 cm^2 (19.39 in.^{2}), the galvanometer reading of which was initially set at 100. Table II indicates these results, which could be obtained repeatedly, regardless of the placement of the templates within the clear area, whether centered, randomly scattered, or distributed fanwise along the periphery of the circle.

To test the versatility of the instrument especially in respect to the accuracy of estimation of smaller areas, measurements were made with various base plate standard areas set at 100 (Table III). As would be expected, the maximum accuracy for any area measurement was obtained by the use of a standard area approaching in size that of the unknown. Thus, a 6.425 cm^2 (1 in.^{2}) area was measured more accurately against a 3 in.^{2} standard set at 100, than against a 122 cm^2 (19 in.^{2}) standard (Table II). Opaque squares 1.606 cm^2 (0.25 in.^{2}) and 0.402 cm^2 (0.0625 in.^{2}) were measured to within one per cent of true value against a transparent 6.425 cm^2 area on tape background, set at 100. Indeed, the limitation as to the smallest areas accurately measurable, aside from the operator's ability to approximate fractional scale division readings, lies only in the accuracy with which a suitable small standard reference area can be made, and in the intensity of light available for measurement. Thus, by lowering the light

§ The most satisfactory glass for this purpose was that obtained by immersing exposed and otherwise useless photographic plates in hot soapy water to remove the emulsion.

‖ These measurements could be made by superimposing the glass plate of each of the smaller areas on top of the standard 12.700 cm circle area. In that case, the galvanometer reading would be set at 100 for the 12.700 cm circle area plate covered by a clear glass plate. Obviously, all of the glass used in any one series of measurements must be uniformly the same in quality and thickness. Transparent surfaces should be kept clean and free of finger marks, dust, etc., by the use of a fine linen or silk wiping cloth.

TABLE II. Measurement of Opaque Areas of Metal Squares on a Transparent Circular Background of Known Area (125.10 cm^2).

Opaque area on transparent circle		Galvanometer scale reading*	Opaque area, cm^2 Calculated	Opaque area, cm^2 Observed
0		100	0	0
2.54 cm opaque squares	(1)	94.8, 94.6	6.452	6.645
" " " "	(2)	89.5, 89.7, 89.6	12.90	13.03
" " " "	(3)	84 , 84.7, 84.3	19.36	19.61
" " " "	(4)	79 , 79.6, 79.1	25.81	26
" " " "	(5)	73.8, 74.3, 74.3	32.26	32.13
" " " "	(6)	68.8, 69.2, 69.3	38.71	38.52
" " " "	(7)	63.8, 63.9, 63.9	45.16	45.16
" " " " 1.27 " " "	(7) (2)	— 61.1, 61.1 ↓ ↑ ↓	48.39	48.65

* Serial readings of progressively changed areas, performed three times. Decreasing and increasing of opaque areas denoted by the symbols ↓ and ↑ respectively.

TABLE III. Effect of Choice of Standard on Accuracy of Area Measurement.

Stand. transparent area and galvanometer setting	Object in light path	Galvanometer scale reading	Area, cm^2 Calculated	Area, cm^2 Observed
56.33 cm^2 rectangle at 100	Opaque	54.3	25.81	25.81
		65.6	19.36	19.36
		77	12.90	12.97
		88.6	6.452	6.452
19.74 cm^2 circle " "	"	66.9	6.452	6.536
" " " " "	Transparent	24.7	4.903	4.878
56.33 cm^2 rectangle " "	"	3	1.613	1.677
19.74 cm^2 circle " "	Opaque	91.7	1.613	1.639
6.452 cm^2 square " "	"	74.7	1.613	1.632
56.33 cm^2 rectangle " "	Transparent	.9	.403	.516
19.74 cm^2 circle " "	Opaque	97.7	.403	.452
6.452 cm^2 square " "	"	93.7	.403	.406
" " " " "	Transparent	6.2	.403	.400
*1.613 cm^2 " " 50	Opaque	37.4	.403	.406
*1.613 cm^2 " " "	Transparent	12.4	.403	.400

* With light intensity increased.

source of the areameter along its supporting column as close as possible to the base plate, with the 1.606 cm^2 transparent area serving as a standard set at a maximum possible reading of 50, readings of 37.4 on 4 different opaque metal 0.402 cm^2 squares were obtained, giving an area to within 0.0032 cm^2 (0.0005 $in.^2$) of the true value (Table III).

A confirmation of the accuracy of the areameter results was obtained by other independent methods of area measurement. Irregularly shaped areas were randomly cut from cardboard and weighed against accurately cut squares of the same material. The results were not good, owing to the perceptible absorption of atmospheric moisture by the material. However, when thin .0061 cm (0.0024 in.) aluminum foil was used to make such areas, photoelectrically and gravimetrically determined values (ranging in size from 4.819 to 30.840 cm^2 (0.75 to 4.8 $in.^2$) were in agreement to within one per cent (Table IV). Comparison of photoelectric with planimetric measurements (also shown in Table IV) of irregularly shaped areas having the general dimensions of electrophoretic plasma patterns, likewise showed agreement of the expected order of magnitude.

Discussion. Although the principle on which it is based is not new¶ the apparatus

¶ In the course of this work, our attention was called to a photoelectric apparatus for area measurement of plant leaves reported by Mitchell(1) and subsequently modified by the American Instrument Co. Both models were constructed of more expensive materials, utilized a different optical arrangement, and neither would be suitable for the accurate measurement of areas less than 20 cm^2 (3 $in.^2$).

TABLE IV.
Comparison of Results of Area Measurements by Different Methods. Area in cm² measured by

Photoelectric areameter	Wt of sheet metal	Planimeter
4.807	4.848	—
19.10	19.03	—
12.13	12	—
7.007	7.039	—
23.94	23.68	—
30.91	30.97	—
11.42	—	11.61
17.42	—	17.55
17.68	—	17.68
19.61	—	19.81
16.45	—	16.58
13.23	—	13.10

and the generally outlined method for area measurement described in the foregoing have obvious advantages over other methods known to us or in use at the present time. Planimetry, whether direct or involving the tracing of enlargements of an original pattern, is time-consuming and fatiguing. A method which has been in use since the last century, namely that of weighing standardized sheet materials (2) may be highly inaccurate, as has been our experience in the use of paper or cardboard. Weighed thin sheet metal is feasible for occasional use but not practicable for any amount of work in the laboratory. Instruments such as the curve computer(3), differential analyzer(4), electro-mechanical integrator(5), and the Knorr-Albers microphotometric area determinator(6), more recently suggested for the measurement of areas in various types of physical and chemical studies, are all too expensive and too complicated for use by any but a few laboratories. Moreover, these instruments offer no advantage over the photoelectric areameter here presented, either in respect to accuracy or range and sensitivity.

The general application of area measurement as a method of quantitative analysis, appears to be increasing. In addition to the general and particular fields of chemistry, physics and biology represented by the foregoing citations, area measurements have been applied in analytical chemistry to the measurement of precipitates(7), the calibration of microburettes(8), the determination of fat as monolayer films(9), and in the quantitative estimation of α-amino acids by paper chromatography(10). Detailed methods and technics of measurements will be described in future communications from this laboratory concerned with work in electrophoresis, ultracentrifugation, and anthropometry. The simplicity, rapidity, and accuracy of the technic outlined in this paper may lead to the favorable consideration of area measurement not only for the purposes mentioned but for new uses also.

Summary. An apparatus utilizing the principle of the photoelectric measurement of intercepted light, and technics for its use in the rapid and accurate measurement of a wide range of areas are described. Application to a variety of quantitative physical, chemical, and biological measurements is indicated.

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