126. *Photogrammetric Representation of Golgi-Stained Nerve Cell*

Preliminary Report

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(Comm. by Tanemoto FURUHATA, M.J.A., Sept. 12, 1964)

The actual values of neuron size are particularly difficult to obtain, mainly due to the irregularity of the shape of the neuron soma and its three dimensional expansion. Until now, the estimation of the cell volume was based principally on the measurements of the major and minor diameters of the soma in Nissl-stained preparations. Gihl¹⁾⁻³⁾ demonstrated by means of a three dimensional reconstruction using Nissl-stained serial sections that the true shape of the cell cannot be established from its contour in one section alone. Schadé *et al.*⁴⁾ employed the three dimensional reconstruction with Nissl-stained serial sections to estimate the volume of the motoneuron.

In our histological analysis of the brain stem in kittens with the Golgi-Cox method, it has been found that a combination of reflecting illumination and ordinary substage illumination shows very well a relief of the soma surface and its relationship with the dendrites. By means of ordinary substage illumination, the nervous elements stained by this method are seen only in silhouette against a clear background (Fig. 1, a). Therefore, it is impossible to observe the real profile of soma and the spatial interrelationship of the soma and the dendrites, especially when the dendritic branches issuing from the soma are hidden by its shadow. However, the combined illumination shows these features clearly. In this case, the nerve cell soma and the dendrites appear a brilliant silvery or sometimes a gloosy brown, depending on a light intensity, due to the reflection of light by the granules of mercury plating of the cell surface (Fig. 1, b). Then, with this method of illumination, it was possible to obtain a pair of stereophotograms which are suitable for photogrammetry. In this article it will be reported that contour lines can be drawn over the neuron soma photogrammetrically. The cell volume and the surface area can then be estimated.

A brain stem of a kitten stained by the Golgi-Cox method and embedded in celloidin was cut in serial slices of 250 μ which are thick enough to contain the entire soma. The sections were dehydrated,

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Fig. 1. Photomicrographs of a cell of the bulbar and pontine reticular formation taken with the ordinary substage illumination (a) and with the combined illumination (b). *a* shows only its silhouette whereas *b* reveals the real profile of soma and the spatial interrelationship of the soma and the dendrites.

cleared in xylol, mounted serially on thin coverslips, covered with Canada-balsam and put in a special frame by which the balsam is kept off from touching the microscope stage when the section is turned over. As it will be described later, it was necessary for our purpose to invert the sections thus covered with balsam in order to observe it from the opposite side. The thin coverslip has an advantage over the ordinary slide for such a procedure. A special illuminator is prepared for reflecting illumination (Fig. 2). It consists of ordinary microscope lamp (a) and a microscopic condenser (b) to which a glass rod (c), about 10 centimeters long, is fitted, its tip being just at the focus of the condenser. A great advantage of this apparatus is that it supplies a light intensity sufficient for high power observation without any risk of overheating the specimen (g). After different methods were tried, the convergent method was found to be most appropriate for our purposes. A seesaw-like inclining stage (i) was constructed and fitted in place of the ordinary microscope stage.

Fig. 2. Diagrammatic sketch of the apparatus for microscopic stereo-photography. a: microscopic lamp, b: microscopic condenser, c: glass rod, d: camera, e: microscope, f: long focus lens, g: specimen, h: mechanical stage, i: seesaw-like inclining stage, j: substage lamp.

This stage can be inclined at six degrees to the right or the left of the vertical axis of the lens. A long focus lens (Leitz UM 32/0.30) (f) is used as the objective with HKW 10 $\times$ eyepiece (Nikon). After the stage is inclined to one side at the prescribed angle of six degrees by a bar, the specimen is held rigidly on the mechanical stage (h) attached to the inclining stage. A nerve cell to be examined is mounted in the center of the view field, with a certain point of its surface adjusted upon the cross mark which is ruled in the center of the view finder, and then photographed under the combined illumination mentioned above. Subsequently, a specially designed stage micrometer replaces the specimen in order to put a linear scale into the photograph by a double exposure. It is ruled on a silver-plated glass, so that it comes into sight as a bright figure, as shown in Fig. 2, a, b. Each size of the square is 150 μ long and its middle portion is divided into five units (one division 10 μ). The center of this square is adjusted upon the cross mark of the view finder. This is to fix precisely a spatial relationship between the image of the already exposed nerve cell and that of the micrometer being exposed. Next, by turning down the stage to the other side, the same operations are carried out. In this way, a pair of stereophotograms of a nerve cell viewed from one side can be obtained (Figs. 3 and 4, a, b). Then the specimen is turned over to get an equivalent pair of stereophotograms of another side of the soma surface in the same manner. In order to draw the contour lines on the soma with these pairs of photos, Wild's stereoautograph A 7 is employed. As a scale for

Fig. 3. Contour lines (c) drawn on a large neuron soma in pontine reticular formation of a kitten with a pair of stereophotograms (one side) (a, b). The broken lines show the limits between the soma and the dendrites.

depth, a piece of thin tin foil is used. Its thickness is established by means of Sip's universal measuring microscope. It is covered with Canada-balsam for the sake of uniformity with the histological sections in regard to refraction, and then photographed under the same conditions as the neuron. With the stereophotograms thus obtained, it is possible to regulate the depth for photogrammetry. The contour lines of both sides of the soma are seen in Figs. 3 and 4, c. The contour interval in this case is 2.5μ . The depth of each side was 35μ . Consequently, the total depth in this case is 70μ .

The measurements of the volume and the surface area of the cell body were carried out according to following formulas thought to give the best approximative values. It is impossible to determine precisely the places where the dendrites join the soma; the limits selected in this case as shown by the broken lines in Figs. 3 and 4, c. The formula used to calculate the volume is $\sum A \times H$, where A is the area surrounded by a contour line, and H is the contour in-

Fig. 4. Contour lines (c) of the opposite side of same soma with a pair of stereophotograms (a, b).

terval. These areas are measured with a planimeter. The value of the total soma volume thus calculated is $105,000 \mu^3$. The formula for calculating the surface area is $\sum \frac{L_1 + L_2}{2} \times \rho_1$, where L_1 is the length of a contour line measured by means of a curvimeter; L_2 is the length of a curve drawn parallel to the former at the distance ρ_0 , which is the mean value of the measured distances between neighboring contour lines; and ρ_1 is the length of the slope calculated from ρ_0 and the contour interval. The value thus obtained is $12,300 \mu^2$. Schadé *et al.* found that, among a number of equations used for determining the cells, the equation $1.04 \times 1/6 \pi ab \sqrt{ab}$ (a non-rotational ellipsoid in which the third axis is $\sqrt{ab}$) gave the best approximation for the motoneurons. The simple application of this formula to our case where a and b are 90μ and 55μ respectively resulted in a value of $188,000 \mu^3$. But, this equation is not directly applicable to Golgi-stained material, since it is impossible to measure the axis through the nucleolus due to the plating of the soma surface. In our cases,

the equation of rotatory ellipsoid, $2\pi\left(x^2 + \frac{xz^2}{\sqrt{z^2 - x^2}} \arccos \frac{x}{z}\right)$ for the surface area and $\frac{4}{3}\pi x^2 z$ for the volume where $x = \frac{b}{2}$, $z = \frac{a}{2}$, $x < z$, gave values nearest those obtained photogrammetrically: 13,400 μ^2 with respect to area and 142,000 μ^3 with respect to volume. In any way, it seems likely that the value obtained by photogrammetry is much smaller than the calculated one.

Summary. In our histological analysis of the kitten brain using the Golgi Cox method, it was found that the application of reflecting illumination provides a clear relief of the soma surface including its relationship with the dendrites. Utilizing this method of illumination, it was possible to represent faithfully the shape of the soma photogrammetrically. This has provided a means for precisely estimating the dimensions of the neuron soma.

Acknowledgements. The author thanks Prof. T. Maruyasu, Institute of Industrial Science, University of Tokyo, for his help and advice. Dr. T. Oshima, Institute of Industrial Science, University of Tokyo, Dr. K. Nakamura, Chief of the Laboratory, Kokusai-Kogyo Co., Tokyo, and Dr. T. Ueno, Chief of the Laboratory, Nippon-Kogaku, Co., Tokyo, have given me the benefit of their unrivaled knowledge of photogrammetry. I am grateful to Mr. Y. Suzuki for his technical assistance with the photogrammetry. The thin tin foil on which the scale of depth for photogrammetry was based was provided through the courtesy of Mr. T. Tasaki, Olympus Optical Co., Tokyo.

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