Neuron counting in three dimensions; a proposal

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Abstract

The preparation of histological specimens for neuron cell counting is briefly reviewed. Problems related to the observation of biological sections are discussed and a proposal for neuron counting in three dimensions is made. A parallel algorithm for this purpose is described.

Introduction

For the last century people have investigated the nature and structure of the nervous system through its elementary units, the neurons. For this purpose they have developed different techniques that, with an improving technology, have successively furnished a better insight into the numerous problems existing in the field. We will point out the significant phases of the preparation of biological specimens as well as the difficulties that arise when using the conventional techniques. Our proposal is aimed at: a) the three-dimensional reconstruction of a specimen by means of a digital matrix and b) the use of a special algorithm that operates in three dimensions on all elements of the matrix simultaneously, to count the digitized neurons contained in the previous matrix.

In the preparations of histological specimens various substances are needed in order to obtain the slide that will be subsequently analyzed. One of these substances is the fixative, employed to stain the relevant elements to be observed either by optical or electronic means. For neurons the Nissl method with cresyl violet staining is commonly employed [1]. This method, as well as all others, produces large changes in tissue volume. Some authors rate this change up to about a 70% reduction [2] in the volume of the original sample. The embedding medium must satisfy the following requirements [3]: high resistivity for constant section thickness, discrete rigidity for sections of constant width, adequate elasticity to contain samples of any size. Two materials commonly employed are paraffin and celloidin. Paraffin allows fast embedding and thinner sections, while celloidin is more resistant. A good compromise is reached by using tissuemat and combining the advantages of paraffin with those of celloidin. As for the thickness of the section, the following considerations should be kept in mind. If the section is thick, then fewer sections for a given histological preparation are required, less time and space are needed, and fewer

cells, nuclei and nucleoli are split. This increases the probability for accurate counting. On the other hand, if we use thin sections less counting error will be introduced since fewer neurons will overlap. Neurons, as seen through an optical microscope, will be overlapping [4] and only certain parts within the section may be simultaneously in focus. Look at fig. 1a), b) and c). The thickness of the sections varies from 10 to 50 microns, usually about 15—20 microns.

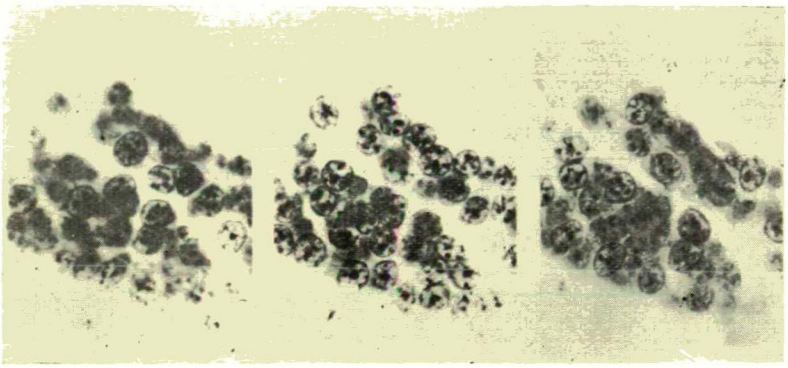

Fig. 1a), b), c)
Successive focal planes of habenular nucleus of frog (Rana esculenta).
Transverse section, x 1200

Although there are no absolute rules for the choice of a sectioning plane, one plane is chosen to show a specific structure on the basis of the interpretation of the biological material when cut along three orthogonal directions. If one could have a three-dimensional representation of the specimen then this one could suggest the best sectioning plane.

The problems most commonly met when counting neurons using the previously described specimens are: 1) contour definition of cells (the presence of neurons on different focal planes further complicates the picture); 2) the appearance of the same neurons in adjacent sections. To solve the first problem some authors have tried to outline, as precisely as possible, the contour of the nucleus with a very sharp pencil and Higgins green ink. To compensate for the poor discrimination between different planes some specific correction factors have been introduced, (e.g., Abercrombie, [5] 1946). The difficulty in assessing the exact number of neurons in a given specimen may be described by a figure ranging between 2% and 10% [2] of the total number of counts on the same specimen.

Three-dimensional representation

We have seen that when investigators use a specific technique for counting neurons with an optical microscope, different focal planes are inspected. We might ask ourselves how it could be possible to three-dimensionally reconstruct the sample so as to eliminate picture noise due to overlapping of cells and to the presence of non-focussed components. If this were possible then not only would we obtain a fully focussed representation but also a preservation of size and shape.

Under this assumption we propose a three-dimensional matrix in which biological information from the specimen will be stored. Let us consider a three-dimensional matrix of x, y parallel planes ordered along the z-axis. With each of these planes we may associate one focal plane, such that all histological sections will be stored in the matrix. From an operative standpoint, a focussed component of a picture has a contrast ratio above a specified threshold value. We must remark that before storing the information on a matrix plane, some processing of the picture must be performed in order to extract only those components which are focussed. This can be achieved by using techniques [6, 7] for eliminating spurious noise in digitized images. We then have a matrix which contains all relevant elements present in the specimen in digital form.

We may note that each element of the matrix has a grey scale value: this is due to the fact that the patterns involved are not black and white but rather continually varying in their intensity as a result of the use of staining techniques and of the complex structure of cells.

Three-dimensional matrices can also be considered as arrays for storing tactile sensory information from objects in space [8]. After making contact with the object a special sensor could, in principle, trace it, obtaining a quantized contour for every section along the z-axis. For this specific case we are involved in binary matrices and only information relevant to the surface will be stored. Once the three-dimensional matrix is obtained patterns stored in it can be processed according to the set of rules dictated by the task.

Problems existing in two dimensions regarding connectivity, adjacency, geometrical operations, should be reconsidered for three dimensions.

For this reason, for example, let us compare processing of two-dimensional patterns with three-dimensional ones. The memory occupation will obviously be larger since more data are needed because of the presence of an extra dimension but we must also note that more operations will be required to test certain properties in this space. As an example, when we define two elements $a(i, j, g)$; $b(h, k, m)$ to be d_2-adjacent if

$$d_2\{a(i, j, g); b(h, k, m)\} = \max(|i-h|, |j-k|, |g-m|) = 1$$

then, an isolated element test will require 26 check operations while only 8 are necessary in two dimensions.

If, instead of using sequential algorithms we are interested in performing parallel processing, further memory must be employed as a buffer unit for storage.

Neuron counting

For the problem of neuronal counts we propose an algorithm developed for counting objects in three dimensions [9]. This algorithm operates in parallel, first shrinking all objects and then normalizing them to single isolated elements. This procedure was obtained from the superposition of a two-dimensional algorithm acting along three orthogonal planes. It may operate on d_1- and d_2-connected objects where d_1 connectivity in three dimensions can be defined as follows: a set S of elements is d_1-connected if, any two elements in S having been considered, a path

exists joining them through successive elements $(a(i, j, g); b(h, k, m))$ all in S such that

$$d_1\{a(i, j, g); b(h, k, m)\} = |i-h|+|j-k|+|g-m| = 1.$$

The d_2 connectivity definition can be obtained from the d_2 adjacency formula. The algorithm is a parallel one since every element is processed simultaneously with all others and independently. The transformed state b^* at every step depends on the state of elements belonging to a $2\times2\times2$ window. In fig. 2 we can see that one vertex corresponds to element b. The small letters represent the 0, 1 states of each element.

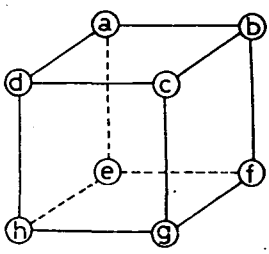

Fig. 2
$2\times2\times2$ window, b^* (transformed state of b) will depend on the state of elements contained in this window

The following relations hold for d_1- and d_2-connected objects respectively, if

$$u(t)=0, \quad t<0 \quad \text{and} \quad u(t)=1, \quad t\geqq0,$$

$$b^* = u[u(a+b-2)+u(c+b-2)+u(f+b-2)+u(a+d+ +c-3)+u(a+e+f-3)+u(c+g+f-3)],$$

$$b^* = u[u(a+b+g-2)+u(b+c+e-2)+u(a+c+f-2)+ +u(b+h-2)+u(b+f+d-2)].$$

Our algorithm is direction oriented since symmetrical configurations, e.g., eight elements forming a cube, must not be completely erased. For example, if we consider a parallelepiped circumscribing the object, the process of shrinking compels every element to move towards one vertex and, precisely, for the chosen disposition of elements in the formula, towards the top, right, backward vertex (vertex b, fig. 2). After a finite number of steps all objects will be shrunk to single elements (vertex elements), then extracted and counted. For a correct counting to be performed two conditions must be satisfied: no object must disconnect itself during the process of shrinking, and no two or more objects must merge during the same process. The first condition is always satisfied while the second is verified only if the parallelepipeds circumscribing the objects are not adjacent, i.e., the distance between them $d_2\geqq2$. Thus, the number of steps necessary to process all objects placed in a matrix depends only on the dimensions of the largest parallelepiped circumscribing the object.

Conclusion

A procedure is introduced to represent a biological specimen in digital form which preserves the spatial organization of its components. Every histological section is stored in a three-dimensional array in which every x, y plane corresponds to a single focal plane as seen by optical inspection. In this way, only strictly focussed components are preserved. Our processing takes into account the three-dimensional nature of the chosen description of the world and should not be seen as a set of successive differing processes in two dimensions. From this point of view picture processing methods should be reconsidered with respect to space geometry. As an example, a parallel shrinking algorithm which could perform counting of cells has been proposed.

Подсчёт нейроновых клеток в трёх димензиях

Количественные анализы в нейронной анатомии требуют метода автоматизации из-за необходимости исследования большого количества гистологических материалов. Первые шаги в этом направлении были сконцентрированы на расширение каналов передачи данных от микроскопа к вычислительной машине для того, чтобы хранить и как можно лучше обрабатывать данные, биологическую информацию, для решения задачи.

Коротко даётся обзор специфичных методов окраски и подготовки нейроновых клеток. Освещаются некоторые проблемы исследования человеком морфологической структуры клеток. Даются предложения к методу обработки, исследований биологических образцов, в котором вся информация трёхмерной реальной структуры клеток хранится и обрабатывается в памяти вычислительной машины.

Далее рассматривается алгоритм для специфичной задачи подсчёта нейроновых клеток, который симультанно и независимо друг от друга оперирует над всеми элементами трёхмерного изображения образца.

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