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# Application of Computer System to Analysis of Muscle Morphology : Statistical Analysis of Spatial Pattern of Muscle Fibers*)

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## ABSTRACT

The spatial pattern of histochemically stained normal muscle fibers and reinnervated muscle fibers is called mosaic pattern or type grouping, respectively only from appearance. In this study we classified the spatial distribution of rats' anterior tibial muscle fibers into homogeneous, clustered and random distribution by the distance method of Hopkins and Skellam and quantitatively expressed the spatial distribution of each muscle fiber type using our newly developed computer system. The results showed that normal red and white muscle fibers are distributed homogeneously or at random, whereas reinnervated muscle fibers are in a clustered pattern and the degree of clustered pattern becomes larger with the lapse of time after reinnervation. These results suggest the possibility of quantitatively indicating the degree of muscle fiber recovery after reinnervation.

## INTRODUCTION

The mosaic pattern or checkerboard-like appearance usually seen in the histochemically stained normal muscle fibers is caused by the intermingling of fibers of different motor units. Therefore various kinds of mosaic pattern are observed in muscles according to the degree of this intermingling. But they have not been stedied in detail, particularly quantitatively. Only Jennekens[5)] has attempted an evaluation of the degree of clustering of one muscle fiber type by hand. On the other hand Miyamoto et al.[6)] expressed the spatial pattern of axons quantitatively using a new computer assisted method.

If a spatial pattern of reinnervated muscle fibers could be expressed quantitatively, we would have an objective and numerical index to evaluate the degree of muscle recovery after reinnervation. On the basis of this line of thinking we tried to apply this computer system to the statistical analysis of spatial pattern of histochemically stained muscle fibers after peripheral nerve repair.

## METHODS

The right sciatic nerves of male Wistar albino rats were cut at the middle of the thigh and then immediately repaired with 8-0 nylon threads. Both sides of anterior tibial muscles were removed 3 and 5 months after neurorrhaphy to prepare 10 $\mu$m thick cross sections. These sections were stained for succinic dehydrogenase (SDH)[7)]. Photomicrographs of 184 magnification were prepared for computer analysis. A computer system with the necessary hardware and program permitted the input, editing and analysis of the photomicrographs. The input device was a Graf/Pen Model GP 3 binary digitizer (Scientific Accessories Corporation) having

*) 畑野栄治，宮本義洋，有田清三郎，堀　義巳：筋線維の空間パターン解析にコンピューターを利用する試み

an effective tablet area of 350×350 mm. By placing the photomicrographs on this tablet, the X-Y coordinates of each short diameter of muscle fibers by means of two points and the circumference of muscle fibers were entered into a minicomputer NOVA-01 manufactured by DATA GENERAC. Computer programs were developed by the authors exclusively. The X-Y coordinates were employed to determine the position of the muscle fibers for the display process and the circumference was used in calculating the cross sectional area. The stored information was displayed in digitized from during the data input and therefore prior to the final analysis it was possible to ensure that information on each muscle fiber was properly entered. The data thus obtained were analyzed with another computer (HP 2100S) to display the simulation of muscle fiber and the pattern of spatial distribution with the aid of an X-Y polotter.

The distance method of Hopkins and Skellam[3] was used to analyze the spatial distribution. This method involves the measurement of the squared distance (U) from a random point to the nearest neighboring muscle fiber and the squared distance (V) from a randomly selected muscle fiber to its nearest neighbor. When $A=\sum U/\sum V$ is reduced, if the pattern is a random distribution, $A=1$, the clustered distribution will be $A>1$ and the homogeneous distribution will be $A<1$ (Fig. 1).

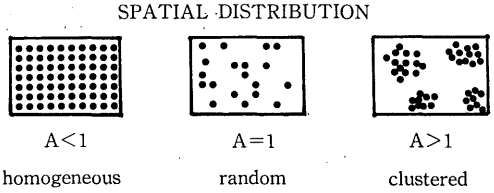
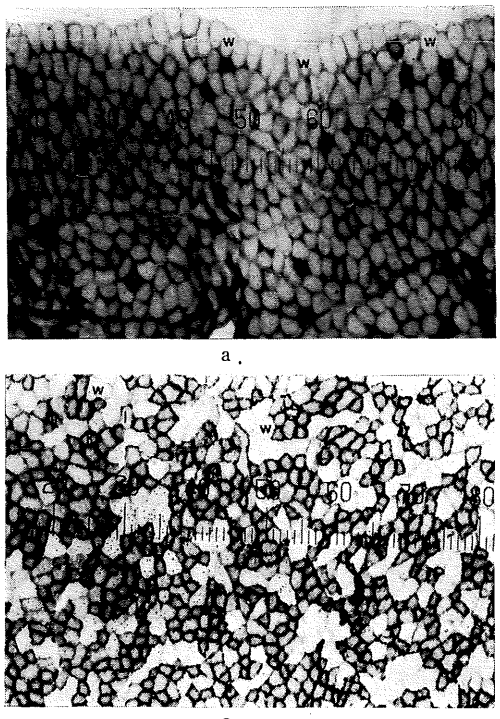



**Fig. 1.** The pattern of spatial distribution was divided into homogeneous, random and clustered distribution. According to Hopkins index, random distribution is $A=1$, clustered $A>1$ and homogeneous $A<1$.

The following variable is used to determine if this value A is significantly different from 1: $x=A/1+A$, at the level of significance of $P=0.05$

if $x<1/2-1/\sqrt{2n+1}$,
the spatial distribution is homogeneous;
if $1/2-1/\sqrt{2n+1}\leq x\leq 1/2+1/\sqrt{2n+1}$,
the distribution is random;
if $x>1/2+1/\sqrt{2n+1}$,
the distribution is clustered.



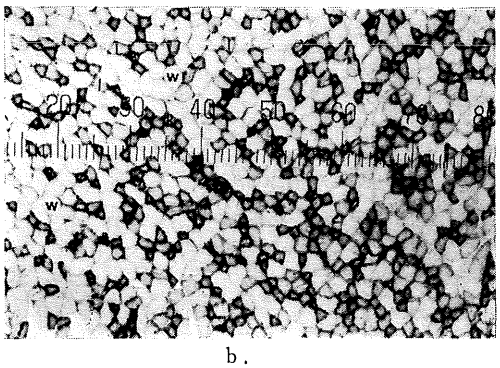




**Fig. 2.** Cross sections of the anterior tibial muscle of rat (40×).
a. superficial layer. b. intermediate layer. c. deep layer.
R, W and I stand for red, white and intermediate muscle fibers, respectively.

('n' is the number of muscle fibers.)

When this value of A is used to compare the patterns of spatial distribution, many muscle fibers should be examined to eliminate errors. In this study we examined from 50 to 400 muscle fibers of each muscle fiber type.

## RESULTS

Figs. 2a, b and c are photomicrographs of the superficial, intermediate and deep layers of the anterior tibial muscle, respectively. Red muscle fibers are small and dark, white fibers are large and pale and intermediate muscle fibers are between red and white muscle fibers in size and enzyme activity. In the superficial layer, white muscle fibers are predominat, but in the deep layer red fibers predominate among these different muscle fibers[1,2,4].

### 1. Pattern of spatial distribution of normal muscle fibers

The pattern of spatial distribution of each muscle fiber type show in Figs. 2c was analyzed as follows;

For red muscle fibers: $A=0.610$, $x=0.379$ and $0.379<1/2-1/\sqrt{2n+1}$

Thus the spatial distribution is homogeneous.

For white muscle fibers: $A=0.825$, $x=0.452$ and $1/2-1/\sqrt{2n+1}<0.452<1/2+1/\sqrt{2n+1}$

Thus the spatial distribution is random.

For intermediate fibers: $A=4.519$, $x=0.819$ and $0.819>1/2+1/\sqrt{2n+1}$

Thus the spatial distribution is clustered.

The number of intermediate fibers seems not to be enough to analyze the spatial distribution in the deep portion.

The spatial distribution of each muscle fiber type of the potomicrographs in Figs. 2a and 2b was analyzed in the same way. The results obtained are shown in Table 1.

**Table 1.** Findings of the spatial distribution of each muscle fiber type of the respective layer of the normal anterior tibial muscles.

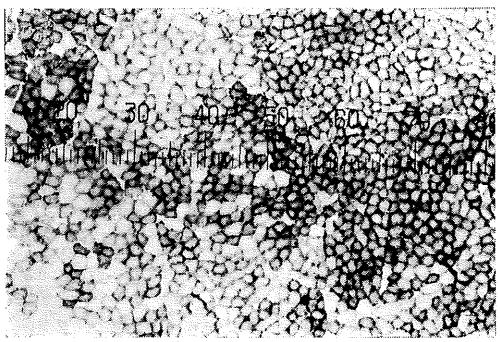
| | superficial layer | intermediate layer | deep layer |
|---|---|---|---|
| red fiber | random | random | homogeneous |
| white fiber | homogeneous | homogeneous | random |
| intermediate fiber | random | random | clustered |

### 2. Pattern of spatial distribution of reinnervated muscle fibers

The spatial distribution of reinnervated muscle fibers was analyzed in the same way. Figs. 3a and 4a are photomicrographs taken 3 and 5 months after nerve repair, respectively.

For red muscle fibers of the 3rd month (after nerve repair): $A=2.381$, $x=0.704$ and $1/2+1/\sqrt{2n+1}=0.5001$. Thus, the saptial distribution is considered to be clustered.

For red muscle fibers of the 5th month (after nerve repair): $A=6.336$, $x=0.864$ and $1/2+1/\sqrt{2n+1}=0.5001$. Thus, the spatial



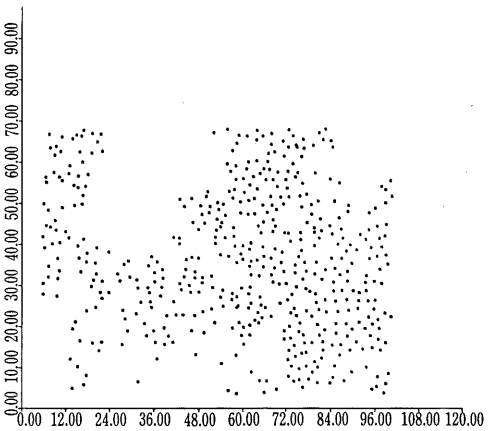
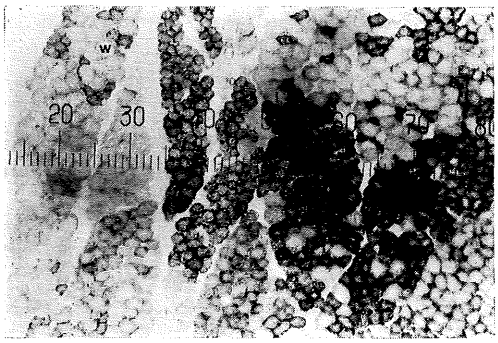



**Fig. 3.** Histochemical findings at the 3rd month after nerve repair.

a. The difference between each muscle fiber is not so distinct. Type grouping is small and not clear (40×).

b. Only the central points of red muscle fibers are plotted from Fig. 3a. $A=2,381$, $x=0,704$, $1/2+1/\sqrt{2n+1}=0,5001$: clustered distribution.



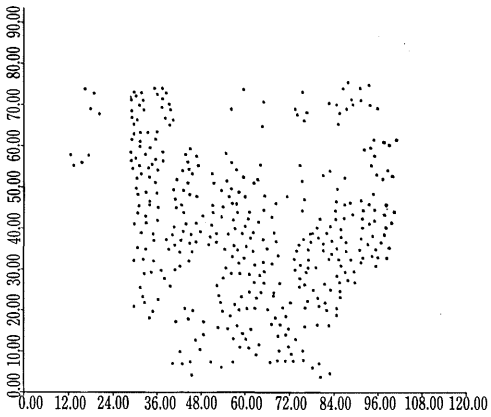



**Fig. 4.** Histochemical findings at the 5th month after nerve repair.
a. Red muscle fibers can be easily identified chiefly due to the highest SDH activity and subsarcolemmal aggregation (40×).
b. Only the central points of red muscle fibers are plotted from Fig. 4a. A=6.336, x=0.864, $1/2+1/\sqrt{2n+1}=0.5001$: clustered distribution.

distribution is considered to be clustered.
A in Fig. 4 is larger than A in Fig. 3. This suggests that the type grouping of red fibers is more remarkable in the 5th month than in the 3rd month (after nerve repair).

## DISCUSSION

Many investigators have shown that histochemical differentiation of muscle fiber type is governed by its own axon. Regenerative axons which have a strong tendency for collateral sprouting innervate neighboring muscle fibers, as such muscle fibers of the same type make grouping. This type grouping becomes more remarkable and larger with the lapse of time, because axon's sprouting extends around after nerve repair, but finally the development of grouping seems to stop. In this study we have quantitatively evaluated the spatial pattern of muscle fibers using the method of Hopkins and Skellam and found that red and white muscle fibers are distributed either homogeneously or at random, whereas the reinnervated muscle fibers are in a clustered pattern and the degree of clustered pattern has tendency to become larger with the lapse of time after nerve repair. Red muscle fibers were easiest to identify histochemically among three different muscle fibers, because they are characteristic of the highest SDH activity and aggregation of SDH activity just around the subsarcolemma (subsarcolemmal aggregation)[1,4]. And the number of red muscle fibers was predominantly higher in the reinnervated sections. Therefore we analyzed the spatial distribution of red muscle fibers to evaluate the degree of muscle recovery as shown in Figs. 3 and 4. In this specimen A was 2.381 in the 3rd month and 6.336 in the 5th month after nerve repair for red muscle fibers. These results strongly suggest that the value of A can be used as a quantitative indication of the degree of muscle recovery after nerve regeneration. However, when we evaluate the spatial distribution of muscle fibers using this computer system, we are confronted with the following problems[6]. 1. The analysis covered in this study is of only a small portion of the whole cross sectional muscle fibers because great effort had to be given during input. 2. As the effective area of the currently available tablet is limited, photomicrographs should be at magnification as low as possible to input a wide range of data. As the photograph is very small, the error will increase during input.

In this report, we introduced the fact that spatial analysis could be applied to the evaluation of muscle recovery after reinnervation.

The number of cases in this study is too small, therefore it is considered that this method should be extended to a larger number of muscles so that these points can be elucidated.

## REFERENCES


1. **Dahlbäck, L. O.** 1970. Effects of temporary tourniquet ischemia on striated muscle fibers and motor end-plates. Scand. J. Plast. & Reconstr. Surg. Suppl. 7 : 6-91.
2. **Hatano, E.** 1981. A Comparative Study on Priamry and Secondary Nerve Repair. Plast. Reconstr. Surg. 68 : 760-767.
3. **Hopkins, B. and Skellam, J. G.** 1954. A new method for determinating the type of distribution of plant individuals. Annals of Botany. 18 : 213-227.
4. **James, N. T.** 1979. Studies on the responses of different types of muscle fiber during surgically induced compensatory hypertrophy. J. Anat. 129 : 769-776.
5. **Jennekens, F. G. I.** 1971. Data on the distribution of fiber type in five human limb muscles. J. Neurol. Sci. 14 : 245-257.
6. **Miyamoto, Y., Arita. S., Hori. Y., Miyamoto. H., Hatano, E. and Tsuge. K.** 1982. A New Computer Assisted Method for Morphological Assessment of Peripheral Nerve Regeneration: Statistical Analysis of Spatial Patterns of Axons. Hiroshima J. Med. Sci. 31 : 211-218.
7. **Nachlas, M. M., Tsou, K. G., De Souza, E., Cheng, C. S. and Seligman, A. M.** 1957. Cytochemical demonstration of succinic dehydrogenase by the use of a new p-nitrophenyl substituted ditetrazole. J. Histochem. Cytochem. 5 : 420-426.