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SEMIAUTOMATIC PROCEDURES IN PERIPHERAL NERVE MORPHOMETRY: BASIC PROGRAMS FOR A PERSONAL COMPUTER

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ABSTRACT

The authors provide software for the morphometric evaluation of peripheral nerves for a table-top personal computer. After slight modifications these programs can be used for more general morphometric problems.

INTRODUCTION

Computer-assisted analog-digital converters (digitizers) are today much used in morphometry (Haug H, 1980, Gerdes A, 1982, Spagnoli LG et al., 1982, Gamel JW et al., 1982). Several such devices with all-purpose oriented software are available, but they are often expensive and not flexible for research.

Micro- and personal computers represent a true revolution in many professional fields; an enormous calculation power is now easily accessible; indeed, it is very easy to program such machines for one's own particular purpose (Puittinen J, 1982).

Among many microcomputer-assisted digitizers we chose the Apple computer Graphics Tablet, whose programs are written in BASIC, and we developed software oriented towards morphometrical applications.

METHODS

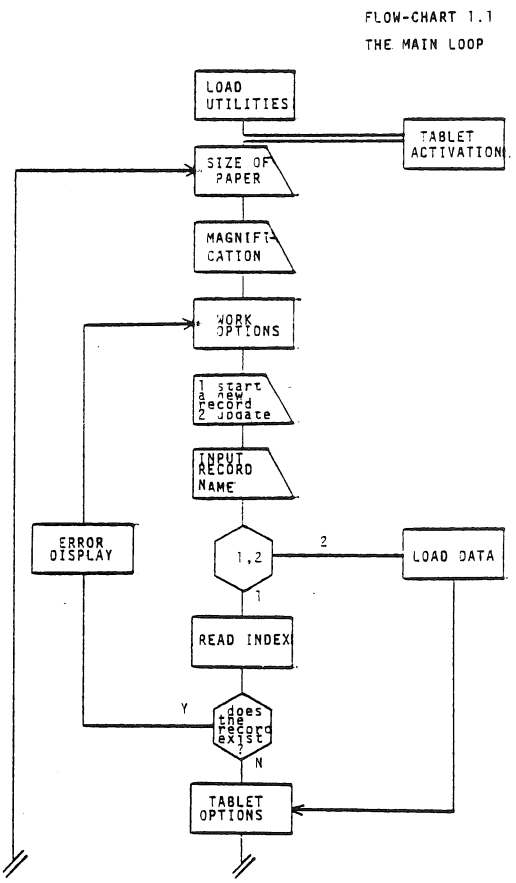
For technical information about the digitizer, we refer to the operation and reference manual (Graphics, 1979). Many

features of the program dealing with graphics applications have been erased to increase the Random Access Memory (RAM) available for storage of data. Three subroutines (Area, Distance and Dots) have been modified for morphometrical purposes.

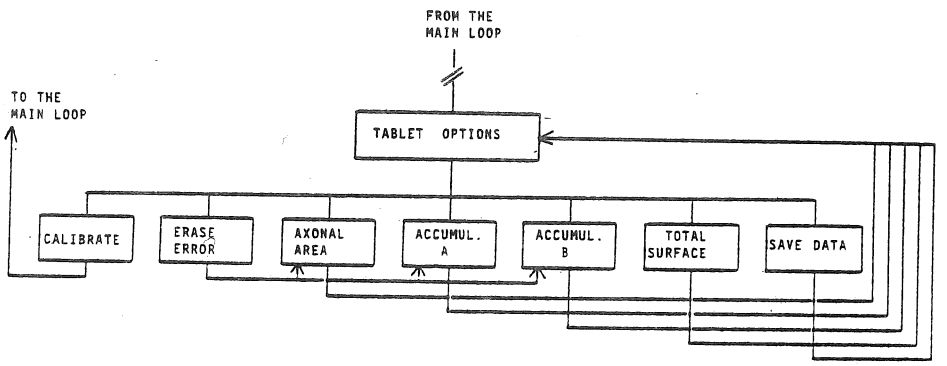
A routine of calibration has been introduced; it gives every measurement in micrometers, according to the magnification of the object. It is possible to work on photographic enlargements or on the image projected by a microscope; projections of negatives are also recommended if the sample to be evaluated is very large. Such calibration implies the calculation of total surface examined; a specific command adds the field measured to the total surface.

Flow-chart 1.1 shows the general configuration of the main loop.

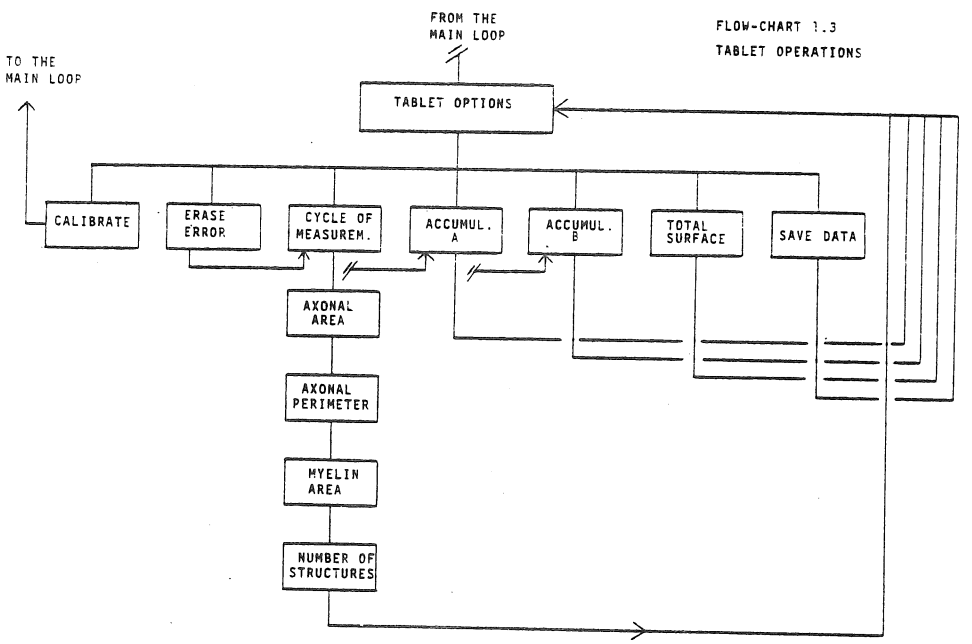
Flow-charts 1.2 and 1.3 show two possible applications of the routine of measurement; the first one fills a uni-dimensional array with up to 2,000 area measurements; this option

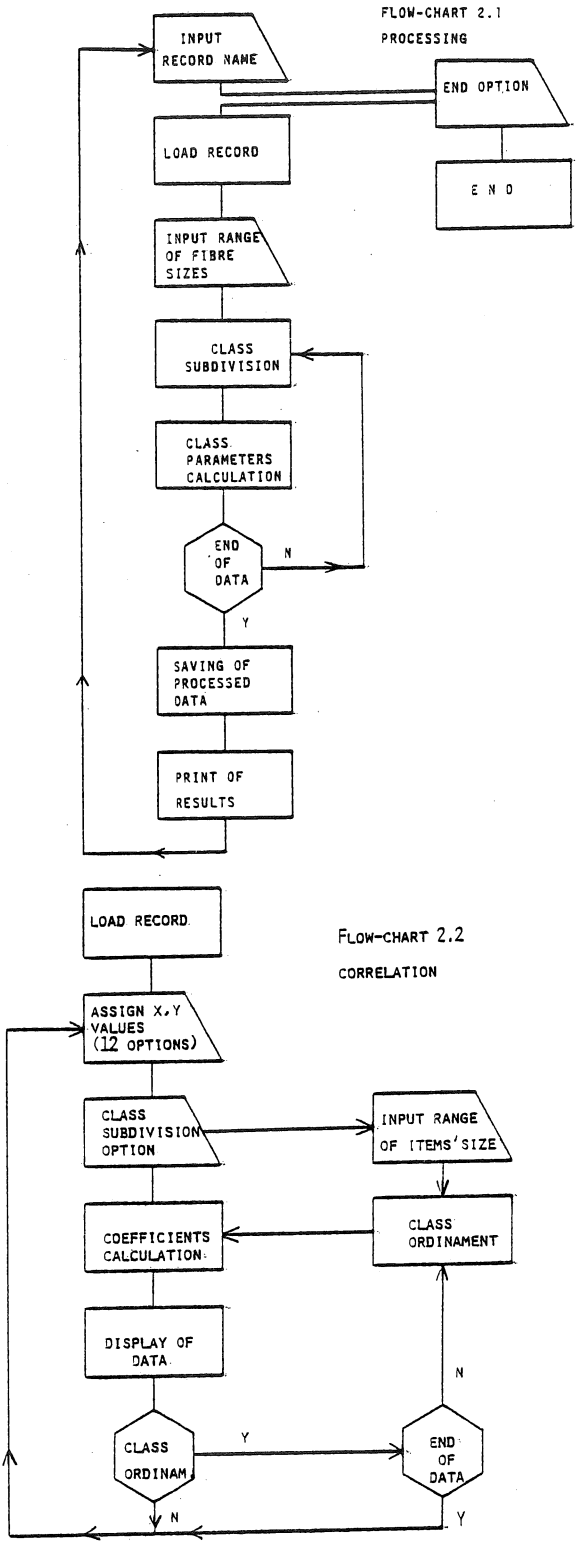


FLOW-CHART 1.2
TABLET OPERATIONS



FLOW-CHART 1.3
TABLET OPERATIONS




FLOW-CHART 2.1
PROCESSING
INPUT RECORD NAME
END OPTION
LOAD RECORD
E N D
INPUT RANGE OF FIBRE SIZES
CLASS SUBDIVISION
CLASS PARAMETERS CALCULATION
END OF DATA
N
Y
SAVING OF PROCESSED DATA
PRINT OF RESULTS
LOAD RECORD
FLOW-CHART 2.2
CORRELATION
ASSIGN X,Y VALUES (12 OPTIONS)
CLASS SUBDIVISION OPTION
INPUT RANGE OF ITEMS' SIZE
COEFFICIENTS CALCULATION
CLASS ORDINAMENT
DISPLAY OF DATA
N
CLASS ORDINAM.
Y
END OF DATA
N
Y
FLOW-CHART 2.3
STATISTICS
INPUT RECORD NAMES
LOAD CLASS PARAMETERS
CALCULATION OF MEAN, VARIANCE,
CLASS COUNTER
E N D OF DATA
N
Y
DISPLAY AND PRINT RESULTS
GRAPHIC REPRESENTATION
E N D

is useful for the construction of size frequency histograms, and has been applied by our group to peripheral nerve fibres. Additional information given by the total surface examined, number of structures and the possibility of accumulating two different other surface groups, make this option a powerful instrument in assessing tissue pattern in real time.

The second option (diag. 1.3) performs the sequential measurement of two different areas, one distance (i.e. a perimeter), and counts the number of structures. In our example, axonal area, myelin area, axonal perimeter and number of neurofilaments have been assessed for each nerve fibre. Up to 500 bytes of information for each group of measurements can be stored in the disk memory for each case. This option provides two independent accumulators to save areas of items whose total amount only needs to be known, i.e. cytoplasmic inclusions or cytoplasmic pseudopods.

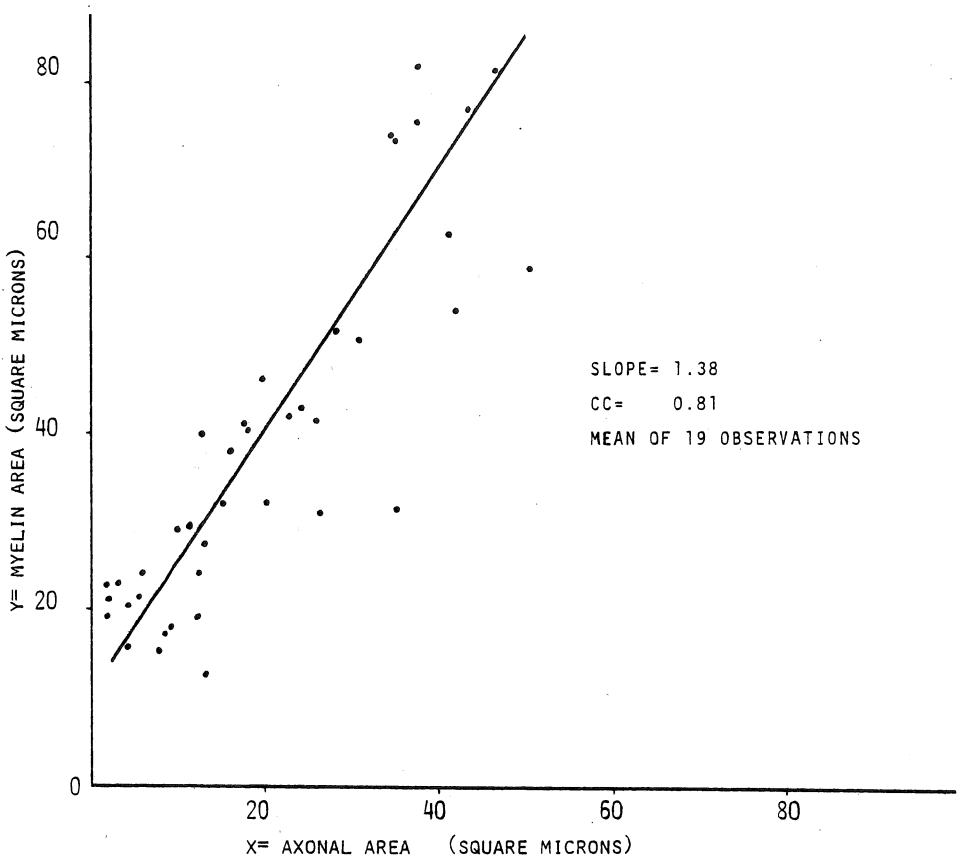
A "processing" program (diag. 2.1) carries out the class subdivision of items according to their size; the relative ratio between correlated measurements (diag. 1.3) can be also assessed for each class. In our example, myelin-axonal ratio has been obtained. Values of N,NV,A,SV and LV are calculated by this routine. Processed data occupy only 500 bytes in the memory; therefore, it is possible to place "in line" up to 300 cases in each 5 1/4" floppy disk, thus making them easily available for statistics.

Diagrams 2.2 and 2.3 show two simple subroutines for statistical calculation; the analysis of correlation (diag. 2.2) of parameters known to have a linear relationship can supply useful information in pathology (see example).

DISCUSSION

The programs described here have been adapted to peripheral nerve pathology; their application to more general morphometric investigations is possible. Several problem-oriented modifications can be developed but correction subroutines for the spatial pattern of the specimen must be added when a correct orientation is not possible. This problem is easily solved in plastic-embedded peripheral nerves, but does exist when more randomly-arranged patterns have to be evaluated.

The use of microcomputer-assisted digitizers make the morphometric approach in diagnostic pathology easy and quick at a reasonable cost. We feel that in the near future these devices should perform standardized algorithms and should give standardized results. Under such conditions international exchange of information and experience will be possible which supports our efforts to make pathology a science.



EXAMPLE

Myelin-axonal correlation has been evaluated in 19 patients with cancer of the larynx. Over 50 nerve fibres have been measured in each nerve (branches of the IX cranial nerve) on standard electron microscopic fields. The value of correlation coefficient CC is significantly lower than in subjects without tumor; a general trend towards relative increase of the myelin is also present (axonal hypotrophy?).

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