A flexible microprocessor system for the measurement of cell size

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SUMMARY   A flexible system for the measurement of length and area is described. The system consists of the Reichert Jung MOP-1 area measuring device interfaced with a Commodore PET computer. Its use is illustrated by the planimetric measurement of cross sectional areas in histochemical preparations of normal and diseased muscle. While measurements are being made data can be displayed on the computer screen either in numerical form or as a frequency histogram together with simple statistical analyses. Hard copy can be obtained from an attached printer. Mean values for fibre area in normal human skeletal muscle are reported.

An alternative, widely used method of calculating fibre area from the lesser diameter was found to give a consistent underestimate of approximately 30% when compared with our planimetric method. In diseased muscle with abnormally shaped fibres the discrepancy is even larger; such fibres can be identified using a "form factor" which relates the area of a cell to its perimeter. This rapid, accurate and flexible system is also suitable for the measurement of many different types of graphical record.

Numerous methods have been used to estimate the size of muscle fibres in transverse section.[1-4] Some methods, such as planimetry are accurate but time-consuming. Others—for example, measuring the lesser or greater fibre diameters are quicker but considerably less accurate and if used to calculate area lead to further errors as the cross section of a muscle fibre is rarely circular. The accurate measurement of fibre cross sectional area is important as it is directly related to muscle strength and in wasting diseases a selective atrophy of one fibre type may occur.

It is generally accepted that planimetry, if available provides the most accurate method of measuring areas[5] and with the development of microprocessors coupled to digitising tablets the ease with which planimetry can be performed is greatly increased. We have evaluated a convenient and rapid system which couples an area measuring device (Reichert-Jung MOP-1) with a bench top microcomputer (Commodore PET). A brief report of this system (MOPPET) has been published.[6]

Material and methods

NEEDLE BIOPSY AND HISTOCHEMISTRY
Samples of human muscle, usually from the quad-

riceps, are obtained by the needle biopsy technique.[7] The muscle samples are orientated under a dissecting microscope to give parallel fibre bundles and these are kept vertical on cork discs using OCT compound mounting medium (Lab Tek products, Miles Labs Inc, Illinois, USA). The specimen is frozen rapidly in isopentane which has been cooled to its freezing point in liquid nitrogen. The blocks are stored at $-60°C$. We do not stretch the specimens before freezing and electron micrographs taken at the same time have consistently shown the sarcomeres to be fully contracted. For area measurements cryostat sections are cut at 8 μm and stained for myosin ATPase activity at pH 9·4.[8]

MICROSCOPY
Sections are examined using a binocular microscope with drawing tube attachment which permits the image of the MOP drawing tablet and the image of the specimen to be superimposed in the same plane.

The microscope and side arm are calibrated using a Vernier slide with a × 25 objective and × 10 eyepieces. The side arm is adjusted so that 0·1 mm on the Vernier slide is equivalent to 20 mm on the tablet. The magnification factor (× 200) is stored in the MOP microprocessor and used to give a readout of true area (in μm²). This magnification gives a conveniently sized image for drawing, with normal

muscle a single field usually contains about 25-30 fibres.

AREA MEASUREMENTS

The fibres are outlined using the drawing pen. The passage of the pen over the tablet produces a stream of co-ordinates from which the MOP microprocessor calculates both the perimeter and area of each cell drawn. The measurements of length and area can then be directly printed out or passed through a serial interface (RS 232) to the PET computer (Fig. 1). Algorithms have been written to receive, display and store data. Machine code subroutines present on a programmable ROM (PROM) are used for the data transfer, displaying of stored data as a histogram, automatic scaling of the histogram and for printing out the contents of the PET screen to an attached printer. Data on area and perimeter for three different cell types can be simultaneously stored and up to 250 measurements made on each cell type. As the measurements are being made the data may be displayed as either a listing or a histogram together with a running mean and standard deviation. The histogram usually consists of 20 bars and the bin size can be determined by selecting the size of the largest bin. Any values greater than this are placed in the highest bar. An additional feature relates the area of the structure to its perimeter giving a "form factor" (FF). The FF indicates the shape of the cell by comparing the perimeter of the fibre with the perimeter of a circle of the same area, so that:

$$FF = \frac{4\pi A}{P^2} \quad (A = \text{area}; P = \text{perimeter of the cell})$$

If the cell is circular the FF will be 1·0, any deviation from the circular gives a smaller value. Most normal muscle cells cut in true cross section give an FF of 0·8 with a range of 0·85 to 0·75. Examples of different cell shapes and their appropriate FF are shown in Fig. 2.

All original data can be stored on a disk for future recall and analysis.

Results

ACCURACY AND PRECISION

Drawing around an area of exactly 200 mm² (on graph paper) 20 times gave a result which was

Fig. 1 *Microscope and microprocessor system for measuring cell areas. Front, left to right, microscope with drawing tube side arm, MOP drawing tablet, MOP microprocessor. Back row left to right, disk storage unit, PET computer, printer.*
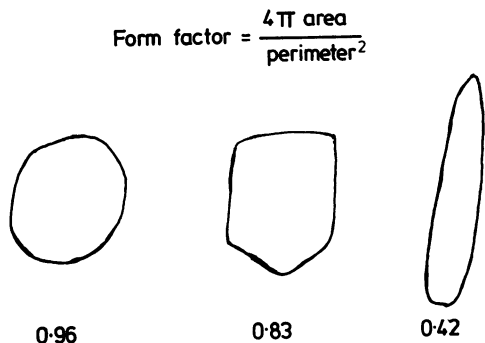
Form factor = $\dfrac{4\pi \text{ area}}{\text{perimeter}^2}$

0·96   0·83   0·42

Fig. 2   *Form factor (FF) relating cell perimeter to area. Three examples of cell shape are shown with their FF. Note that a circle would have an FF of 1·0.*

Fig. 3   *Comparison between muscle fibre areas measured by lesser diameter (ordinate) and by MOP planimetery (abscissa). A systematic underestimate occurs when calculating area from lesser diameter giving an answer for normal subjects approximately 70% of the true value.*

accurate to 0·5%; the coefficient of variation for this was 1·7%.

One preparation of quadriceps muscle taken from a normal male volunteer was used to evaluate the error by one operator when counting one cell 100 times. The coefficient of variation for this task was 1·9% for a mean cell area of 6900 μm². There was little "interoperator" variation in performance.

COMPARISON WITH DIAMETER METHOD OF MEASURING CELL AREA

We have previously calculated muscle fibre areas using the measurement of the lesser diameters of each fibre and making the assumption that the cells were circular. This method has the advantage that if the section is not fully transverse a falsely large area is not obtained.[4] More recently it has been shown that unless the section is very far from transverse (> 15°) the error involved is small.[5] If muscle preparations are carefully orientated under a dissecting microscope before freezing this problem seldom arises. Non-transversely cut cells are identifiable to the trained observer and their measurement can usually be avoided. A number of normal muscle fibre preparations have been measured by both methods (Fig. 3) and it is clear that a systematic underestimate occurs when using diameters to calculate area. Areas calculated from the lesser diameters give an answer which is approximately 70% of the true area. The extent of the underestimate is clearly related to the fact that most normal muscle fibres have an FF form factor of around 0·8. In diseased muscle abnormal fibre shapes—for example, small angular fibres, are frequently seen and this will increase the discrepancy between true area measurements and areas estimated from measurements of the lesser diameter. In a biopsy from a patient with a severe endocrine myopathy (see Fig. 4), the mean
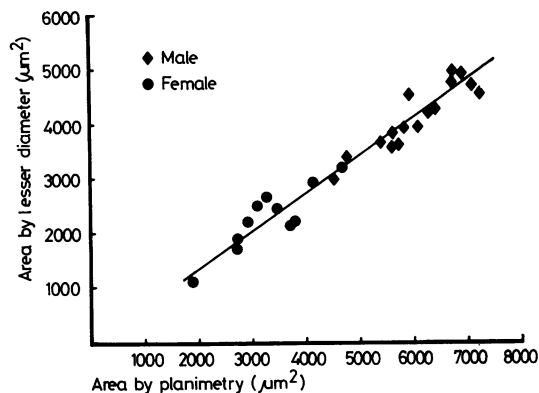
type II fibre area, obtained using the MOPPET system was 326 μm² as compared to a value of 82 μm² obtained by measuring the lesser diameter. The FF for these cells was 0·47. This finding was not due to the section being cut obliquely as the type I fibres in the same section had a mean area of 2692 μm² using the MOPPET system and 2226 μm²
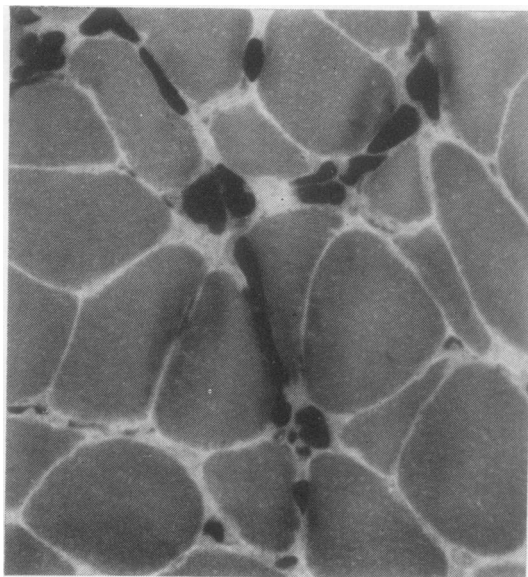

Fig. 4   *Transverse section of muscle biopsy from a patient with severe endocrine myopathy. Form factor for type I (light) fibres 0·85, the mean FF for type II (dark) fibres 0·47.*

SPECIMEN DYST/SOL/TYPE 1   AREA/FREQUENCY   SPECIMEN DYS/SOL/TYPE 2   AREA/FREQUENCY
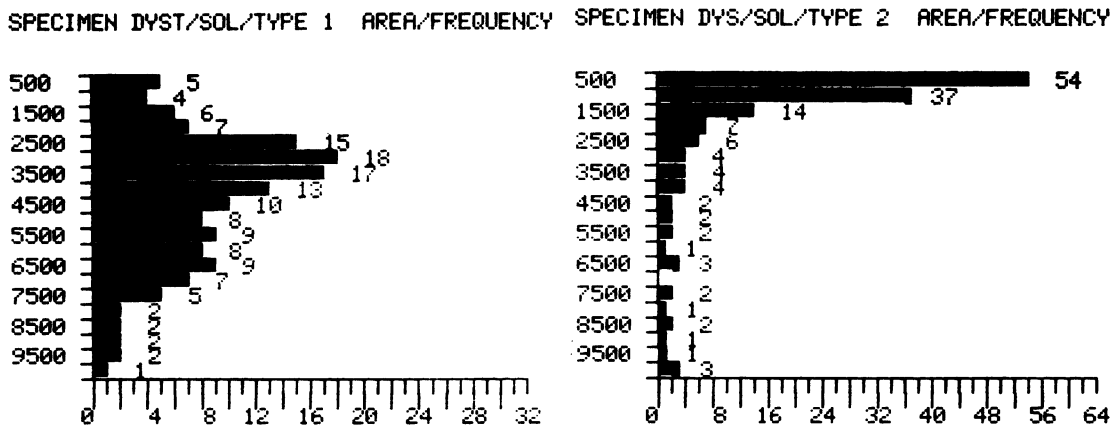
Fig. 5 *Histograms of fibre area of soleus muscle from a patient with muscular dystrophy. The distribution of the type II fibres shows marked skew. Vertical axis, fibre area in μm^2; horizontal axis, number of fibres.*

by the lesser diameter method. The FF for the type I fibres was 0·85.

In diseased muscle where the distribution of fibres is not Gaussian, simple statistics can be inappropriate and misleading. In these circumstances it is particularly valuable to see the histogram of fibre size distribution. Histograms for a sample of dystrophic soleus muscle are shown in Fig. 5. The distribution of the type I fibres is normal while that for the type II fibres is heavily skewed. In diseased muscle such as this more can be learned from a histogram of the areas than by manipulation of the data into a form from which means and standard deviations can be calculated.

Using the MOPPET system a single muscle biopsy can be quantified in about 10 minutes, measuring about 100-150 cells of each fibre type. This time includes plotting histograms, calculating statistics and FF and storing the data on disk. Similar measurements made by hand would take at least an hour. In our laboratory the mean range for fibre areas for men was from 3500-7500 μm^2 (n = 26) and for women 2000-4500 μm^2 (n = 18). These values were from subjects of average build and within the 10th-90th centile for height and agree well with previously reported areas measured by planimetry.[9][10] It was apparent that fibre area is related to stature and this may account for the differences between the men and women. No significant differences were seen in the mean areas of the type I or II fibres in the normal subjects nor was there any significant difference between the preparations stained with haematoxylin and eosin or for ATPase.

The methods described have the advantage of speed, together with a high degree of operator control and flexibility. The operator has full control of the fields measured and can reject damaged or obliquely cut cells as well as artifacts and other structures such as blood vessels and connective tissue. The data can be displayed in a number of different ways to suit the particular comparisons being made and can be stored and recalled at a later date.

The system is not dedicated to one task. The MOP can be used to send co-ordinates to the PET and programmes have been written which use these to quantify lung flow volume loops plotted on an XY recorder in a respiratory function laboratory, ultrasound records of aortic blood flow, the density of particles on freeze fracture faces of cell membranes and the distribution of bone erosions seen on x-ray of the hand. In addition the microcomputer is available as a general laboratory instrument for interfacing with other instruments, for data and reference storage and for word processing.

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