Abstract

Contemporary analysis of human or experimental muscle biopsies includes documentation of muscle fiber size for each fiber type. A semi-automated system has been devised to classify fibers, measure diameters, and display results. Special stains convert different fiber types to specific shades of gray. The microscope image is transmitted by a TV camera to a picture-processing computer. Muscle cells are recognized, classified, and measured for their minimum diameter. Data on consecutive fibers are summed and displayed as lists, histograms, or graphs according to each fiber type. Interpretation of the results is provided by the neuropathologist based on an awareness of probabilities associated with prior comparable results. Data on all patients is accumulated in categories according to disease, sex, age, and site.

Methods

Input of the muscle image to the computer system is through a Cohu 512 line by 512 pixel plumbicon television camera mounted on the phototube of the microscope (Fig. 1). The microscope has an automated stage that permits recording of X, Y coordinates and return to points of interest with a 1-μm resolution. The camera input is carried by cable to the TEXAC Image Analyzer where the signal is shown on a 9-inch TV monitor screen and enters the image analysis system (Fig. 2). The analog image is converted to digital signals based on the intensity of light at each of the 262,144 pixels. Use of the standard eight bits in one byte permits 256 gray levels per pixel per byte.

Fig. 1. A high-resolution TV camera can be seen atop the research microscope. The stage is motorized and can be driven in the X and Y directions either by the operator (control at lower center) or by the computer in 1-μm increments. A joystick for guiding the cursors across the TV screen image is visible at the lower left [6].
Fig. 2. Two computer racks house the components of the TEXAC Image Analyzer. The 23-inch color TV screen is in the upper left. Descending, we see a DEC PDP-11/34A computer containing fixed and removable 2.5 megabyte discs, the dedicated image analysis computer, and power supply. The rack on the right has at the top four 9-inch TV screens. The upper left is the camera monitor; the other three reflect the memory channels in the image analyzer. Next comes the automated microscope stage driver and then the 160-megabyte Winchester disc [6].

The hub of the TEXAC Image Analyzer is a host or executive PDP 11/34A computer with a memory of 128K bytes (Fig. 3). It is linked to a dedicated image analysis computer, the microscope stage, and disc storage which are controlled by its commands. Only half of the image analyzer capacity is currently employed, thereby permitting additional applications without stress on the image processor. The fixed, random access disc has a 2.5-megabyte capacity and contains the software shared by all projects. The 2.5-megabyte removable disc contains software to compare consecutive images, assist in reduction of the image to only the vital components, and ultimately store the cumulative data for a dedicated project. A random access, high speed Winchester disc with a capacity of 160 megabytes (1.28 billion bits) can store 610 full images from a television camera system with 512 data points (pixels) per line, each having a resolving power of 256 gray levels.

The digital images can be placed into any of three memory channels and, in turn, will be projected to the black-and-white TV monitor of that memory channel. Each of the three memory channels projects a single color (red, blue, or green) to the composite 23-inch color TV screen in Figure 2. This permits superimposition of editing procedures on the basic image using the large screen with high resolution. Memory channel images can be added or subtracted from each other. By this means a blank microscope field can be subtracted from the muscle image next projected onto channel two. This removes light artifacts inherent in the microscope and yields in channel three an image superior to that visible through the scope itself.

Through the system console terminal, the operator initiates the muscle analysis program. The control unit starts the picture processing through a border enhancement routine that highlights the edges of the muscle fibers and helps separate some of the touching fibers (Fig. 4A). A joystick-controlled pair of cursors is then used to fill in, separate, or delete components of the memory image (Fig. 4B). The joystick signal is directed to the picture processor, but is visible to the operator on different memory channels and the color composite.

The operator indicates when editing is complete; supplies the minimum size of objects to be measured (eliminating capillaries), the gray level thresholds (type I and II fibers), the magnification of the objective on the microscope, the normal range of fiber diameters for the muscle being measured; and indicates whether the patient is a child or adult (to set scale for fiber diameters). The image analyzer then determines the minor diameter of the muscle fibers (see Fig. 4C) using whole picture operations which rotate one image in increasing circles while comparing it with a fixed image [5].

The results are printed as a list showing the diameter of each object measured; each object on the TV screen is identified with a number (Fig. 4D). With the original image still on the TV
Fig. 4. (A) Muscle fibers show border enhancement and reasonably definite separation from their neighbors. (B) Editing options include cutting (separating), filling, and erasing (central white spot, formerly a capillary). (C) The minor diameter of muscle fibers is determined by rotating the original image in one memory (gray) while comparing it with the image in another memory (black). The outer periphery of the cells is gradually removed from the black image as processing progresses. (D) Identity numbers permit operator to compare results with original image and to delete improper values (e.g. capillary at No. 48) [6].

A composite histogram indicating patient name, history number, surgical accession number, normal range for muscle, sex, and age, and frequency distribution of actual measurements by fiber type is printed. Results are plotted in 5-um increments for children and 10-um for adults. Atrophy and hypertrophy factors, the mean (±SD) diameter, and number of fibers measured for each fiber type are recorded. The patient's results are added to cumulative data from previous patients by disease, sex, age, and site. Photoprints of the muscle are obtained on the screen printer for proof for the physician. The histogram can be superimposed on the muscle photo, but is usually printed separately along with the statistics.

Results

Review of over 1,000 histograms shows several fingerprint patterns, each highly suggestive but not pathognomonic. Double disorders, unusual presentations of standard disorders, malnutrition, and carcinomatous effects have a tendency to mimic other established patterns. Nonetheless, fingerprints strongly suggestive for several disorders have emerged.

Fiber type disproportion is one of many causes of the floppy infant syndrome. A biopsy at age one month shows almost all fibers to be round and small, as in Werdnig-Hoffmann neuromyopathy, but the few fibers that are large are all type II. Rather than random anterior horn cell loss, this pattern (Fig. 5) suggests slow development of a maturation factor and the potential for recovery. Indeed, repeat biopsy later in childhood shows a shift to the right or maturation with some compensatory hypertrophy of both fiber types, but type II is still ahead of type I. Change from type II to type I dominance in Figure 5 is most likely a sampling error due to changing biopsy site from quadriceps to deltoid.

Duchenne's muscular dystrophy at about age 10 years shows considerable variation of fiber size including individual fibers of great dimension that are generally round and appear to be splitting. Some large fibers show coagulative
Discussion

Automation of muscle fiber measurements was originally intended to be a labor-saving device, which it is. However, there has also been a greater reproducibility of more accurate results with simultaneous subclassification and statistical analysis. The output is a pleasing, graphic display of the patient’s (or animal’s) status that is camera-ready and awaits analysis by the neuropathologist. A photoprint of the biopsy image on the TV screen is also generated electronically for transmission to the referring physician. User satisfaction has been very high with both the pathologist interpreter and the patient’s physician.

REFERENCES