

FIBER LENGTH MEASUREMENT SYSTEMS: A REVIEW AND MODIFICATION OF AN EXISTING METHOD

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ABSTRACT

This paper describes various methods of fiber length measurement used in fundamental studies of wood properties as well as those used in the more applied area of pulp quality control. Methods described include direct measurement of cells from microscopic images (from either tangential longitudinal sections or macerated tissue), through measurement of projected images, to fiber classification using classical mechanical fractionation techniques through to the most recent optical and laser scanning methods. A semi-automatic measurement system using a 35-mm slide projector and measuring wheel linked to a data processor designed for use in the Forest Products Laboratory of the University of California is described.

Keywords: Fiber length, measurement, classification, optical methods, projection systems, continuous monitoring, automation, digitizing.

INTRODUCTION

In the field of wood quality, improvements in mensuration techniques are of great importance to permit the measurement of large numbers of anatomical characters, while at the same time minimizing the problems of eye strain associated with data collection using a microscope. Possibly the single most frequently measured anatomical character is fiber or tracheid length (fiber length). Information on fiber length is important both in fundamental studies of wood structure as well as the more applied area of quality control of pulp furnish. As a result of the importance of information on fiber length, numerous methods have been devised for its more efficient measurement. Since these methods are generally tailor-designed for specific applications, they may not be universally suitable, and modifications may be required to meet a particular project's needs.

At the Forest Products Laboratory (FPL) of the University of California, a new project in the area of Tree Growth and Wood Formation has been initiated. This project group will be looking at fundamental causes in anatomical variation within trees, and at the application of breeding and stand management to improving the quality of harvested wood. Since this area of research will require accurate measurement of considerable numbers of anatomical dimensions, it was desirable to design an improved system for measuring and analyzing fiber lengths. Two constraints set by the laboratory administration significantly affected the design; these were financial and the fact that the system had to be designed and constructed by the laboratory's support staff. So, after examining the various methods currently in use, it was decided to modify and extend a system previously described by Sugden (1968). This updated system included a 35-mm slide projector, a measuring wheel, and a data processor.

The purpose of this paper is first to review the methods of fiber length men-

suration, and second, to provide a detailed description of the fiber mensuration system chosen here at FPL.

REVIEW OF MENSURATION METHODS

Sample preparation

Since most length measurements are made on whole, isolated cells where solid wood is under study, the wood sample is first macerated by a combination of chemical and mechanical separation of cell walls. Berlyn and Miksche (1976) describe several chemical processes for macerating woody tissue. Perhaps the two most popular are Jeffrey's method using equal volumes of 10% chromic acid and 10% nitric acid, and Franklin's method using equal parts of glacial acetic acid and 30% hydrogen peroxide. Various modifications of the original methods have been used. For example more rapid maceration may be achieved by using the modification of Franklin's method described by Berlyn and Miksche (1976) in which equal parts of glacial acetic acid and 30% hydrogen peroxide are replaced by one part of glacial acetic acid and two parts of 30% hydrogen peroxide. For macerating more refractory specimens, Burkart (1966) suggested using triethylene glycol in the presence of an organic acid catalyst such as phenosulfonic acid or paratoluenesulfonic acid. In each of the macerating methods described above, gentle heating of the macerating fluid will speed up the process, although care is needed to avoid over-macerating the wood samples.

After maceration the separated fibers are usually well washed in distilled water, and then transferred to open dishes for staining. For simple processing of macerated fibers, Taylor (1975) suggested pouring the slurry of washed fibers onto absorbent tissue to concentrate the fibers into a mat. This mat of fibers was then dried on fresh paper towels, and stained before mounting onto glass slides. However transfer of wet fibers onto paper tissue may cause the tissue to disintegrate, and result in contamination of the material under study with cells from the paper tissue. To overcome this, Jagels et al. (1982) recommended 70-mesh fourdrinier wire suspended over a glass jar to be an ideal material on which to collect, stain, and dehydrate cells prior to mounting.

Staining is intended to improve the definition of cell walls, in particular the tips of cells, to help identify whole from broken fibers. Numerous stains have been used including acridine orange (Taylor 1975) basic fuchsin (Burley et al. 1970), chlorazol black (Jagels et al. 1982; McMillin 1982), methylene blue (Fukazawa and Ohtani 1982) and safranin O (Randel and Winstead 1976; Winstead 1978). However, staining is not essential; Burkart (1977) and Sugden (1968) both report taking measurements of cell length without having stained the macerate.

Because of the large numbers of slides that frequently have to be prepared, it is usually convenient to make temporary mounts of macerations in water or glycerine. Echols (1959) recommended the use of a water-soluble mounting medium to avoid distortion and shrinkage of cells that would result if they had to undergo a dehydration process. Sugden (1968) suggested mounting macerates on glass slides without mounting medium or cover glass. By this method the wet mat of fibers was placed on a glass slide which was then gently heated to dryness resulting in bonding of the fibers to the slide.

Although most measurements of fiber length have been made from macerated

samples, Bailey (1920) used tangential longitudinal sections for his studies of cell length variation outwards from the pith in gymnosperms and angiosperms. Ladell (1959), also using tangential longitudinal sections, devised a rapid method of estimating mean tracheid length from counts of cell tips falling within specified sampling bands. In this method an image of the tangential section was projected onto a screen that had two horizontal lines at a known distance apart. A predetermined number of tangentially adjacent cells was selected, and, within the two lines, the number of tips of cells that had crossed the upper line was recorded. Mean tracheid length was estimated as the product of the number of tangentially adjacent cells and the distance between lines, divided by the count of cell tips.

Measurement systems

Methods of fiber length mensuration have been reviewed by Graff and Miller (1939) and Wilson (1954). For macerated tissues, Wilson (1954) recognized three groups of mensuration methods: those using only a microscope, those using optical projection techniques, and those using mechanical evaluation procedures. Since Wilson's review was written advances in technology, in particular in the field of electronics, have made possible improvements in both the collection and the processing of data.

Since most laboratories have a microscope available, this remains the cheapest (in terms of equipment expenditure) method of fiber length measurement. Necessary additional items include an eyepiece micrometer disk, which is calibrated against a stage micrometer, or a filar micrometer eyepiece. In the latter, a micrometer screw on the outside of the eyepiece is turned to move a wire slowly across the field of view. Graff and Miller (1939) suggested that an eyepiece micrometer was adequate for fiber length measurement, but more accurate measurement using the filar micrometer was desirable for fiber width measurement. The major disadvantage of direct microscopic measurement of fiber length is the tedious work of aligning fibers with the scale used in the eyepiece. This may often mean eliminating numerous cells because they are folded. In view of the problem of folded fibers, Bergman and Backman (1929), Schulze (1931), and Brecht and Mory (1934, 1935) found that considerable experience was needed to make accurate length measurements using an eyepiece micrometer, and they preferred the use of a projection system. Projection methods have the added advantage of reducing the problem of eyestrain associated with prolonged microscope work.

In the early projection systems, the image from a compound microscope or microprojector was focused onto a suitable surface from which measurements could be taken using a calibrated scale (Desch 1932; Bergman 1949; Burkhart 1977). As an alternative to linear scales, various forms of calibrated screen were used including a series of concentric circles at calibrated distances apart (Fyfe 1951). Wilson (1954) also adopted a projection screen with calibrated concentric circles. He claimed that the method was 20–40% more productive than standard eyepiece micrometer methods; fibers had to be aligned along a radius of the circles, but fibers with a single bend could be measured by positioning them with the apex of their bend at the center of the circles. A series of concentric circles was adapted by Taylor (1975) for use as an eyepiece reticle in a compound microscope. Length measurements were made by placing the center of the circles on the fiber to be measured, and counting the number of circles crossed by the fiber.

One of the problems of using portable microprojectors or microscopes to project images onto surfaces is that any change in the distance between the projector and the surface alters the magnification of the image, and so the system requires recalibration. This problem was overcome by incorporating both the microprojector and the screen in a single unit. Echols (1959) designed a projection system in which a lens mounted in a box frame directed the image of a standard slide preparation onto an inclined mirror. The mirror then reflected the image up onto a sloping opal glass screen positioned at about desk height. All parts were housed together in a unit called an ampliscope, and since the distance between projection lenses and projection surfaces was constant, the magnification was also constant, in this case at $25.4\times$. Clark (1961) suggested using a standard 35-mm photographic enlarger with its rays turned by a prism into a dark room where a chromium-plated, rolled brass plate directed the rays to the underside of a 12×12 inch frosted glass screen mounted in a bench. This method was adopted as a TAPPI suggested method (TAPPI 1968), and either a standard 35-mm slide projector or photographic enlarger was recommended to provide the image. The TAPPI method suggested creating a grid on the glass screen, for length measurement, by attaching a number of threads to the undersurface of the glass. Sugden (1968) incorporated the 35-mm projector in the same bench as the projection surface, and although space limited the final magnification to $40\times$ this was greater than the $25.4\times$ magnification suggested by TAPPI (1968).

The search for more rapid measurement techniques led to the development of various semi-automated measuring instruments. Map measuring wheels (often referred to as curvimeters in the literature) proved to be both inexpensive, and more rapid measuring instruments particularly for measuring bent fibers (Burley et al. 1970; Harris 1966). A simple curvimeter, or rule, still required that the data be recorded by hand and subsequently processed, which was both time-consuming and a potential source of transcription error. By linking the measuring device to a data storage machine, the speed of data collection was increased and the risk of incorrectly transcribing data was reduced. Ilvessalo-Pfäffli and Althan (1957) designed a system in which a curvimeter, used to trace the fiber image, when lifted from the projection surface activated a microswitch causing an electrical impulse to be sent to a counting relay. There was a total of 29 counting relays; one for each of 28 fiber length classes, and one for tallying the total length and number of fibers. This system could record, not only the mean fiber length of the sample, but also a weighted average by fiber length class, which according to Ilvessalo-Pfäffli (1956) provided a more meaningful measure of fiber length of a pulp sample. Sugden (1968) used a curvimeter with a measuring wheel having a circumference proportional to the projection magnification, so that a fiber image length corresponding to one millimeter of true fiber length was covered with each revolution of the wheel. A sub-miniature switch, located in the head of the probe, was closed twice during a complete revolution of the wheel, which corresponded with each 0.5 mm of actual fiber length. The microswitch activated an electro-mechanical stepping switch, which had contacts wired to each of 10 fiber length classes, electromechanical counters, and an overall total counter. Tallies in the counters were then transferred to a report form for analysis. Most curvimeters had relatively large probe heads, which tended to obscure the image being traced, and so rendered accurate measurement difficult. Ohn (1969) designed a probe in

which the head was kept as small as possible by keeping to a minimum the functions performed there. The movement of the wheel was transferred to the upper end of the probe shaft by means of miter gears and a spindle extending the length of the shaft. In this system, the length of fiber traced by the wheel was transformed into pulses photoelectrically. The spindle was attached to a rotating drum enclosing a small light bulb. As the drum rotated slits in its wall allowed light through, which was received by a photocell housed on the outside of the probe.

These measurement systems provided counts of numbers of fibers in each fiber length class size, but the data still had to be transcribed from the machine for further analysis. With the advances in microelectronic technology came the possibility of directly linking the measuring probe to a data processor, either by remote access to a main frame computer, or directly to a microcomputer. The large storage capacity and rapid processing capability of computers made it possible to move from continuous measurement of fiber length by map wheels or linear scales, to digitizing discrete x,y coordinates along the length of the fiber. The latter system has the advantage of being more generally applicable to measurement of a variety of nonlinear anatomical forms. Hiller et al. (1971) described a digitizing system, in which fibers were projected onto a formica-topped screen, and measured using a cursor wired to two potentiometric displacement transducers. An alternative method of digitizing points along a fiber image uses sonic impulses created by a cursor, and received by microphone sensors along the x and y axes (Quirk 1981). In this system data are automatically transferred to magnetic tape for subsequent computer analysis. Crosby et al. (1981) described the applicability of a computer interfaced digitizer for paper sheet measurements other than fiber length, such as fiber orientation, internal geometry of fiber bonds, paper thickness and mass distribution. One of the advantages of a computer-interfaced digitizer is the capability of checking for errors by calling for a visual display of the tracings (Jagels et al. 1982). However, although straight fibers are rapidly measured by digitizing their two ends, for bent fibers intermediate positions need to be digitized since the length of the fiber is computed as the linear distance between two sets of x,y coordinates.

The most recent advance towards automatic quantitative measurement of projected images is the particle analyzer or image analyzer. This technique makes use of a photosensitive scanner, which converts the image of a specimen into video signals according to differences in optical density. Although the system has not yet been extensively used in studies of wood or paper structure, McMillin (1982) suggested that it should prove useful in wood scientific research, and suggested various specific applications including fiber length measurement.

For the purposes of quality control, the pulp and paper industry has been interested in classifying pulp according to the different fiber length fractions in the mix. A form of mechanical separation of the pulp is probably the most commonly used method of obtaining the different fiber length fractions in the mill. The history of mechanical evaluation of pulp has been reviewed in detail by Wilson (1954), and will be mentioned only briefly here. The different fiber length fractions are estimated by measuring the weight of fibers retained by screens having different perforation sizes. The TAPPI standard method (TAPPI 1975) recommended using a classifier with four or more screens such as the Clark classifier or Bauer-McNett

classifier. Valley and Morse (1965) considered that although screen retention methods were generally precise, the relation between retention and fiber length could be confounded by differences in type of fiber and the history of the pulp. They recommended measuring cell length from the displacement of electrolyte in a modified Coulter particle analyzer. The Coulter particle analyzer measures the drop in voltage caused by an impedance of ion flow when a particle passes through an aperture. The size of the particle can be estimated because the voltage drop is determined by the relation between particle volume and aperture size. Valley and Morse (1965) modified the standard Coulter particle analyzer to estimate the time taken for a fiber to pass through the aperture, by measuring the length of a pulse caused by the voltage drop. By varying a threshold pulse length, the analyzer could be used to successively measure the different fiber length fractions.

An alternative approach to the measurement of fiber length using a fibrograph was developed by the textile industry (Hertel 1940; Tallant 1952). The fibrograph used a light beam and a photovoltaic cell to scan samples of parallel fibers that were held on a comb. As the comb of fibers was moved across the light source, a change in current was registered across the photovoltaic cell. An iris placed in front of a second photovoltaic cell was moved to balance the current across the two cells. A pen was attached to the comb of fibers, and a card was attached to the iris such that during an analysis, movement of the comb, and the balancing movement of the iris enabled a frequency plot of fiber length to be automatically drawn on the card. Recently Cook (1983) described an optical fiber length measuring instrument that was able to classify fibers into 35 different length fractions, and also compute the arithmetic and length weighted mean fiber lengths. The system included an analyzer, a keyboard, and a printer with the option of a plotter. Samples of the pulp suspension were poured into a measuring chamber from which the suspension was sucked past the optical measuring instrument. The total time taken to prepare and measure a sample of perhaps 5,000–10,000 fibers was about 15 minutes, of which the measurement time was about 3–5 minutes.

These methods of classification estimate fiber length from samples of the pulp suspension. To provide more effective means of controlling pulp quality, continuous monitoring of critical pulp characteristics is necessary. Karnis et al. (1972) described a system of continuous fiber length measurement using a Celleco fractionator. Within the fractionator, the pulp suspension was sprayed against a vertically mounted nylon screen which retained a long fiber fraction, and allowed a short fiber fraction to pass through. Both the long and short fiber fractions then drained separately out of the fractionator. The weight of pulp retained by the screen in unit time was found to be a function of the flow rate and consistency, both of the pulp being fed to the fractionator and of the pulp draining from the vessel on the retained side of the screen, together with a weight-average fiber length factor. By controlling the flow rate and consistency of the feed, and measuring the flow rate and consistency of the drained suspension, the weight-average fiber length was estimated. Further developments in the continual monitoring of pulp quality have made use of optical scanning of fibers in parallel orientation (Hill and Eriksson 1976; Hill et al. 1977; Hill 1981). The pulp suspension is fed through an appropriately designed flow cell so that the fibers orientate themselves into parallel alignment. As the fibers flow past a light beam directed at right angles

to their long axis, the light received by a photodetector is interrupted, resulting in production of a pulse that is proportional in length to the length of the fiber. Eriksson and Hill (1973) reported the possibility of using a beam from a laser in place of the conventional light source.

These systems of optical scanning require a specialized flow cell that could become blocked by the particles in the pulp suspension. Dion et al. (1982) designed a system using acoustic signals, which avoided the need to have narrow or constricted tubes that could become blocked. They used the acoustic signal to cause the fibers to orientate in parallel alignment, and, at the same time, to migrate into groups. The speed of orientation and migration was a function of the length of the cells, and was more rapid for shorter than for longer fibers. A beam of light was directed across the flow of pulp, and the speed of orientation and migration (and so fiber length) were estimated from the level of scattered light and transmitted light detected. According to Dion et al. (1982), this technique could permit very fast on-line differentiation of pulp suspensions containing fibers of differing average length. Their analysis of a 10-ml sample of pulp suspension took only 5 s to complete.

A semi-automatic fiber length measuring system

Because of the need to identify different cell types, and to distinguish whole from broken fibers, it was decided that a semi-automatic system of fiber length measurement would best serve the needs of FPL. As noted earlier cost was a major constraint, as was the administrative decision to select a system that, as far as possible, could be designed and constructed by our own technical staff. For this reason the basic design of a projection system described by Sugden (1968) was selected, using a standard 35-mm slide projector and a measuring wheel to trace the fiber images. However, this design was extended to include a data processor to avoid the necessity of transcribing data. Since some additional modifications to Sugden's design were also incorporated, a detailed description of our system will be given here. The system included a 35-mm slide projector, a measuring wheel, and a data processor.

The projection system.—A standard 35-mm carousel slide projector with a 4-inch focal length lens, and remote control for focussing and slide advancement, was chosen for the projection system. No modification of the projector was necessary as standard 2- × 2-inch thin slide cover glasses with metal binders were used for mounting the fibers, rather than standard microscope slides. This permitted 80 specimen slides to be loaded into a common universal slide tray (Fig. 2a). The projector was mounted on a moveable sledge, just above ground level, at one end of a 15-ft-long projection cabinet (Fig. 1). Images from the projector were focussed onto a 26- × 26-inch chrome surfaced mirror placed at the other end of the cabinet, and angled at 45° to turn the image onto an 18- × 24-inch frosted glass screen that was mounted flush on the top of the cabinet. The distance between the projector and the glass screen was selected so that the image of a 1-mm-long fiber would appear as 40 mm on the screen. To reduce the risk of bias in selecting fibers for measurement, a sampling area was designated so that fibers falling wholly within the sampling area would be measured twice, those with one end of the fiber within the sampling area would be measured once, and those with both ends falling outside the area would not be measured (Hart and Swindel

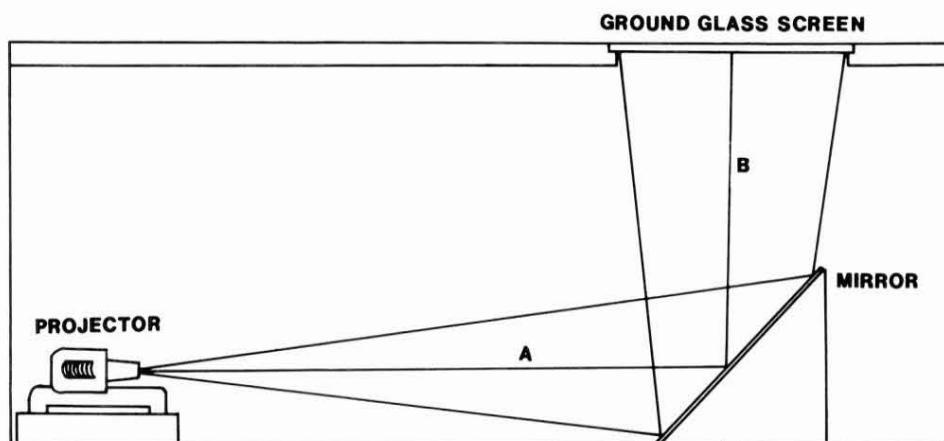


FIG. 1. General view of fiber projection system ($A + B = 40 \times \text{focal length of projection lens}$).

1967). The sampling area was defined by attaching two lengths of 1/64-inch graphic art tape in parallel bands 150 mm apart on the back of the glass screen (Fig. 4).

The projector, cover glasses, metal binders and frosted glass screen used for this system were all purchased from a photographic supply company. The chrome-surfaced mirror was purchased from a glass company, and the cabinet was constructed from plywood.

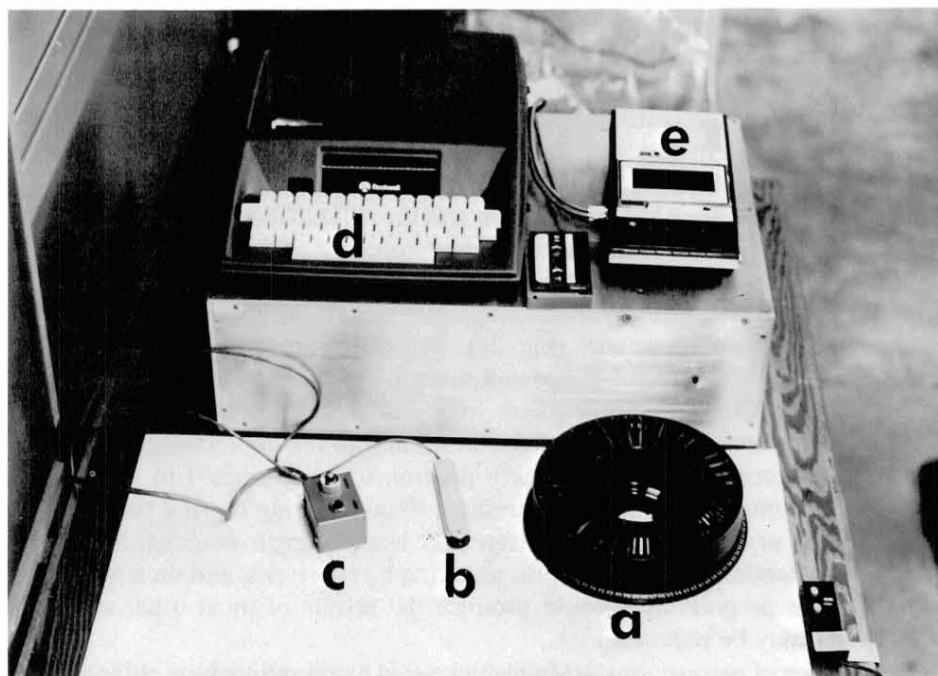


FIG. 2. The fiber length measurement system. (a) Slide tray. (b) Measuring wheel. (c) Control box. (d) Microcomputer data processor, (e) Audio cassette recorder.

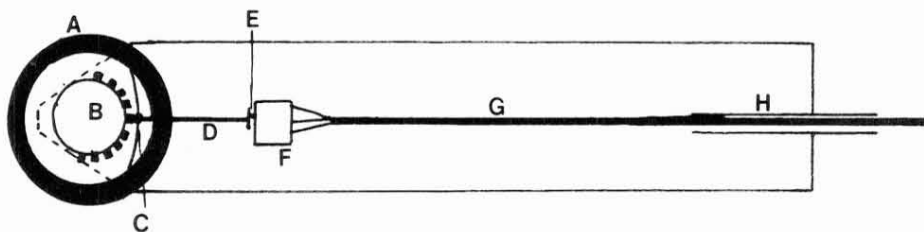


FIG. 3. Detailed plan of the measuring wheel. A. Rubber coated wheel 1 in. in circumference. B. Wheel with 20 cams. C. Ball bearing depressed by cams. D. Metal shaft. E. Activation point on microswitch. F. Microswitch. G. Electric cable to control box. H. Plastic sleeve to protect cable.

The measuring wheel.—To determine the length of the projected fiber images, a measurement probe was constructed (Figs. 2b and 3). It consisted of a measurement wheel mounted on a shaft. The wheel was 80 mm in circumference, with its outer edge covered by a hard rubber ring to provide friction when tracing the fibers on the screen. Attached to the wheel was a 20 tooth cam, which activated a subminiature microswitch located in the shaft of the probe. With the $40\times$ enlargement of the projection system, one complete revolution of the wheel when tracing a fiber, corresponded to a fiber length of 2 mm. During the course of that revolution, the cam activated the microswitch 20 times, with each activation representing 0.1 mm in fiber length. The microswitch was connected to a control box (Fig. 2c) containing two single pole, double throw switches, which in turn were connected to an electronic counter. Each time the probe microswitch was activated by the cam, an electrical impulse was sent to the counter, which stored a cumulative impulse count. When the measuring wheel was brought to the end of a fiber one of the control box switches (labelled read) was pushed, transferring the cumulative count (which represented fiber length) to the data processor. The second switch on the control box (labelled reset) was then pushed to clear the electronic counter. In this way the lengths of individual fibers could be entered into the memory of the data processor with an accuracy of 0.1 mm.

The data processor.—A data processor was incorporated into the system so that data obtained from the measurement wheel could be processed without further data transcription. The microcomputer that was chosen included a 20-column-wide thermal printer, an alpha-numeric display, and full-sized keyboard (Fig. 2d), together with an audio cassette (Fig. 2e). The electronic counter was connected to the memory banks of the microcomputer to complete the measurement and storage system. Simple programs were then written to analyze the data, and the programs were stored on cassette disks for input into the microcomputer through the cassette recorder. Our preliminary programs were designed to retrieve the data from memory, print the measurement data on a ring by ring basis, and to compute and print the arithmetic mean fiber length, length weighted mean fiber length, and standard deviation, both, on a ring by ring basis, and on a whole tree basis. Simple programming could produce the results of most other statistical tests which may be required.

Preparation of macerations.—Matchstick-sized wood splints were chipped from the sample material and macerated using standard Jeffrey's solution for 24 hours. The macerated tissue was then rinsed several times in distilled water, and samples

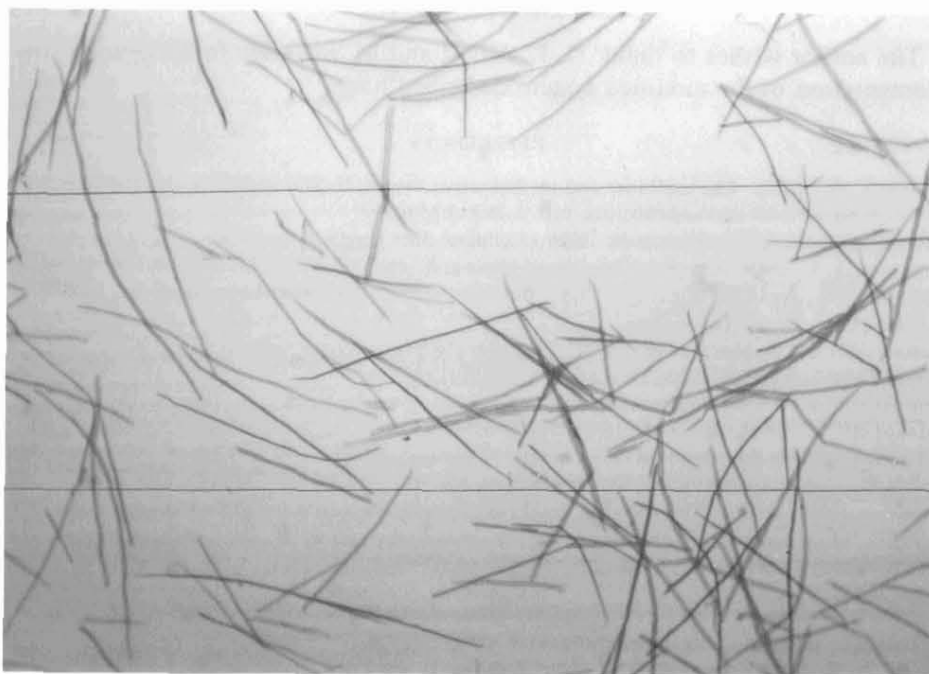


FIG. 4. Projected image of macerated fibers on ground glass screen, showing boundaries of acceptance area.

of separated fibers were transferred to porcelain spotting plates for staining with 1% safranin O in 60% ethanol. The fibers were then lightly rinsed in ethanol and mounted in glycerine between 2- × 2-inch glass slides held together by metal binders. A well-rinsed pipette was used to suck a sample of fibers, suspended in ethanol, out of the spotting plate, and transfer it to the glass slide. This method was found to give a good distribution of fibers as shown in Fig. 4.

Evaluation of the system.—In order to determine the accuracy of the measurement system, a stage micrometer with a 10-mm linear scale was placed in the slide projector. The resulting linear image was traced with the measuring wheel. If the reading was above or below 10 mm, the projection system was adjusted by moving the projector forwards or backwards respectively on its sledge until the reading was 10 mm. The image of the scale was then traced 20 times, and on no occasions did the reading differ by more than 0.1 mm from 10 mm.

As part of the system evaluation, a time study was conducted to determine the speed of measurement. It was computed from this study that an average of 500 fibers could be measured in one hour. This did not include the time needed for specimen preparation, but did include time taken to advance through several slides in the projector.

In conclusion, the semi-automatic fiber length measurement system designed at FPL has proved to be both an accurate and a rapid method of measuring fiber lengths. The system is easy to use, and the procedures are well documented so that inexperienced personnel can quickly become proficient in using the method. The system also has the advantage of direct data analysis, thereby reducing both the time taken to analyze samples and the risk of wrongly transcribing data to a separate computer.

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