Fast-Scan EM With Digital Image Processing For Dynamic Experiments

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ABSTRACT. The recent introduction of accessory instrumentation capable of display at television scan rates suggests a broadened application for the scanning electron microscope—the direct observation of motion (dynamic events) at magnifications otherwise unattainable. In one illustrative experiment, the transverse surface of southern pine was observed when subjected to large compressive forces perpendicular to the grain. Tracheid walls were seen to bend or distort sideways and ultimately rupture. In a second example, a single tracheid of southern pine was shown to fail while under torsional stress and unwind into a ribbonlike element—a structure that provides the coherence necessary for strength development in refiner groundwood pulp. Complementing the dynamic applications of the fast-scan EM are newly developed techniques by which pictures can be stored, processed, and displayed by a digital computer system. Digital data processing can sharpen picture contrast, detect and display differences between pictures, and perform computational analysis.

One of the outstanding characteristics of the SEM image is its three-dimensional quality. Since the introduction of commercial instruments in 1965, microscopists have eagerly depicted the structure of wood with a clarity usually lacking in micrographs made by other means.

Observations at slow scan rates are largely limited to stationary surfaces, but recently introduced instrumentation provides the capability of display at television scan rates. This innovation allows the direct observation of motion (dynamic events) at magnifications otherwise unattainable.

Techniques have recently been developed by which television pictures can be stored, processed, and displayed by a digital computer system. Although the technology has principally been applied to aerospace video-data, it may also prove applicable in fast-scan EM. For example, TV-rate SEM images have relatively poor resolution, and computer processing can sharpen the picture and emphasize details. In addition, digital SEM data may be manipulated to reveal differences between pictures and to perform computational analysis.

The purpose of this paper was to illustrate typical dynamic experiments that can be accomplished with fast-scan EM, to discuss some details of the system design, and finally to indicate a few possibilities for digital image processing.

Dynamic Experiments

Figure 1 is a block diagram of system modifications that permit visual observation, video recording, and photography of dynamic events. The standard specimen stage (A) was modified with a variety of manipulative substages; a few examples are given later in the text. Output of the secondary electron detector photomultiplier (B) was fed through a preamplifier (C) to a video distribution amplifier (D). A scan generator (E) deflected the electron beam in synchronization with the

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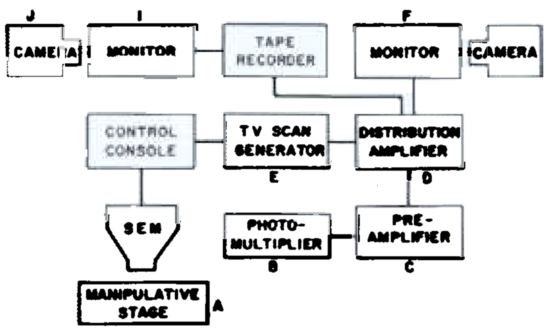

Figure 1. — Block diagram of fast-scan video display system.

video monitors at a standard rate of 30 frames per second, 525 lines per frame. Video output from the photomultiplier was viewed on an 11-inch monitor (F), and hard-copy micrographs (G) could be produced by photographing the monitor. It was also possible to make video tape (H) for subsequent display on a second monitor (I). Sequential photographs or photographs of single video frames could be made in this operational mode (J).

The video image on the monitors contained considerable noise, but the effect was minimized by using film exposure times of about 0.5 second. For reasons to be discussed later, the image was defocused at the monitor so that individual scan lines blended and disappeared.

While numerous refinements are possible, the equipment proved satisfactory for a variety of dynamic experiments. Two examples will be given here.

When wood is subjected to large compressive forces perpendicular to the grain direction, tracheid walls bend or distort sideways and ultimately rupture. Figure 2 shows a substage for observation of such inelastic failure. The stage consists of a fixed head (A) attached to a base plate (B) and a movable head (C) actuated by a pair of precision ball screws (D). The screws are driven by a worm-gear reduction unit (E) connected by shaft (F) to a slow-speed DC motor outside the specimen chamber. Gear ratios were such that one revolution of the external shaft advanced the movable head 0.0033 inch. With the unit in place, the normal X,Y,Z, and tilt motions of the stage could be operated in the usual manner. The transverse surface of a 6-mm cube of southern pine (G) was exposed by hand sectioning and positioned between the loading heads so that compression was applied to the radial walls of cells.

Figure 3 is a sequence of four micrographs of inelastic compression failure. The compressive force is in a direction from top to bottom of each micrograph. In the undeformed transverse surface (A), cell walls and a single ray are clearly visible. Details on the cell wall surface within the lumen are difficult to distinguish. The lower row of cells was used as a reference plane in each micrograph. The upper surface of the specimen (that adjacent to the movable loading head) was eight cell rows above the top shown in A. Micrograph B shows the sample after a moderate degree of compression; the ray cell has collapsed, and individual tracheids have distorted. The tracheid in the upper center has buckled and partially collapsed into the adjacent cell. Micrographs C and D show additional distortion of tracheids and progressive buckling and collapse as compression increases. Many fractures can be seen across cell walls as well as separations between cells in the area of the middle lamella.

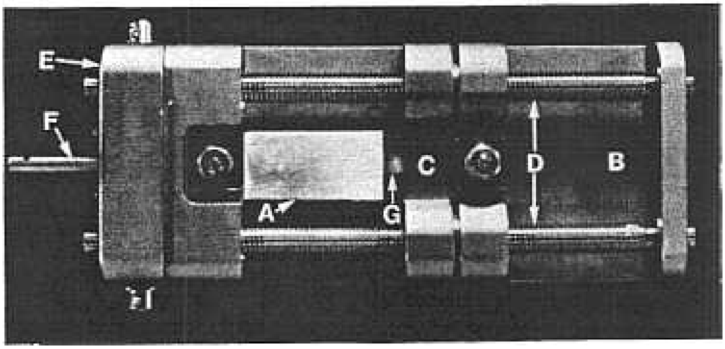

Figure 2. — Substage to observe inelastic wood failure in compression.

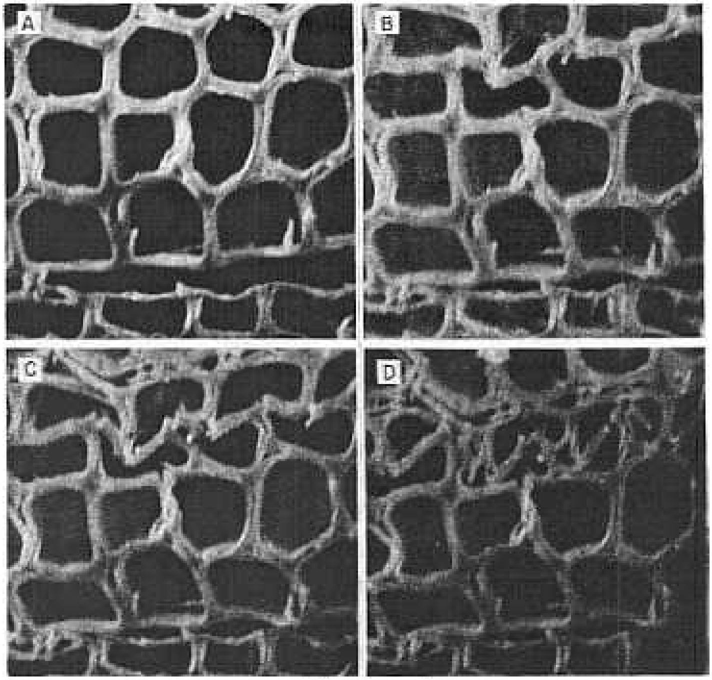

Figure 3. — Transverse surface of southern pine progressively stressed in inelastic compression perpendicular to the grain.

As a second example, previous research on the process for making groundwood in a double-disk refiner (McMillin 1969) had indicated that individual tracheids of southern pine may fail while under torsional stress and unwind into ribbonlike elements. Such elements provide the coherence necessary for strength in groundwood pulps (Forgacs 1963).

Figure 4 is a detailed view of the micromanipulative substage designed to apply torsional stresses to single fibers. Rotary motion is provided by a miniature 50-to-1 speed reducer (A) with an anvil attached to the output shaft (B). The reducer is powered by a shaft (C) attached to a variable-speed DC motor outside the specimen chamber. The surface of the anvil forms a plane passing through the axis of rotation. A stationary anvil is provided (D); its surface also forms a plane passing through the axis of rotation. The axial separation is varied by adjusting the micrometer (E). Fibers are attached to the anvils with silver paint and rotated in the

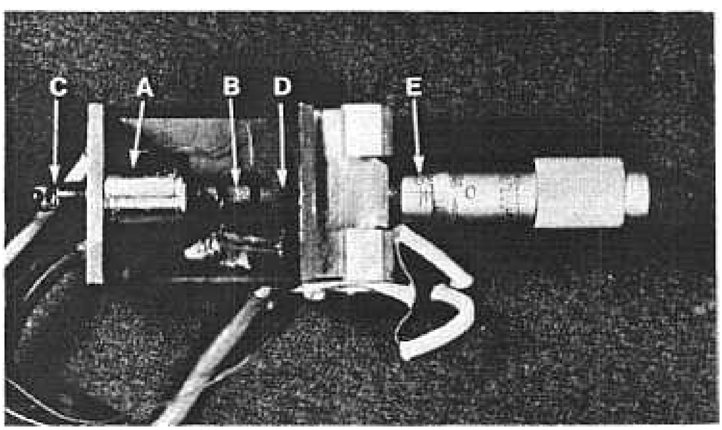

Figure 4. — Micromanipulative substage for stressing individual tracheids in torsion.

clockwise direction at a rate of 40 degrees per minute.

Figure 5 illustrates the progressive formation of the ribbonlike element. Micrograph A shows the undeformed fiber prior to application of torsional stress. As the fiber is rotated, stresses in the cell wall increase. A critical point is eventually reached where the strength of the fiber is exceeded and a crack abruptly forms at the zone of weakness delineated by the fibril helix (point 1 of micrograph B). With additional rotation, further unwinding occurs by a tearing process in a direction generally following the fibril angle. The areas between points 1 and 2 of micrograph C and between points 2 and 3 of micrograph D illustrate two steps of the process. The original crack remains visible at point 1 in all micrographs of the series.

Digital Image Processing

Signal Digitizing

Before the image-processing capabilities of the digital computer can be applied to fast-scan microscopy, it is necessary to convert the signal (or displayed image) to digital form. This can be done by various means. Assume

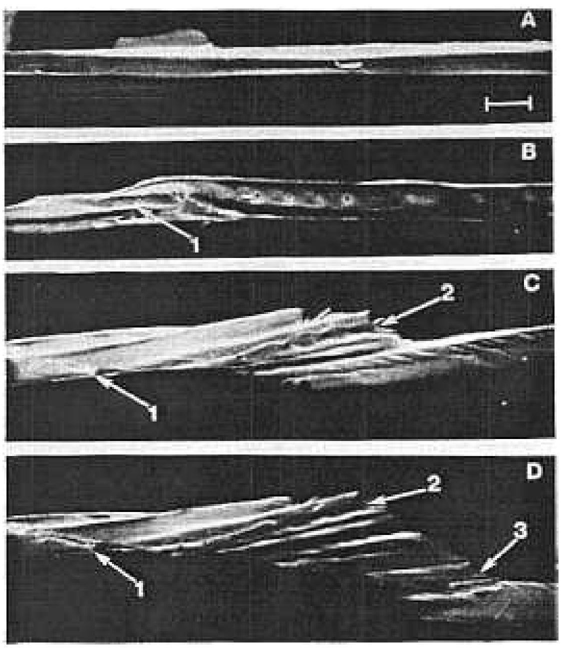

Figure 5. — Development of a ribbonlike element in an earlywood tracheid of southern pine. The scale mark in *A* shows 50μm. and is also applicable to *B, C, and D.*

that the sample is being scanned at standard television rates and that the image is displayed on a monitor. The problem then is to convert this signal to the slower rates required for digital recording. The three most likely possibilities are: 1) photograph the monitor with a film camera and scan the film with some form of microdensitometer; 2) record the signal on video tape for subsequent playback and digitizing; and 3) digitize the video signal directly from the photomultiplier.

In the monitor-film-densitometer method, television monitors (unless specially modified) produce a brightness which is nonlinear with respect to the incoming signal. The relationship between film density and log exposure also is nonlinear. These two effects make it difficult to estimate the original signal level from density measurements of the film.

Sharpness of image edges in the vertical direction is optimum if the monitor is adjusted so that the scan lines just blend and disappear. Any residual scan lines will produce a severe beat pattern with the digitizing raster unless the latter can be exactly superimposed on the former, a practical impossibility. On the other hand, if the scan lines are made to overlap excessively, the image sharpness will be degraded. In spite of its deficiencies, this system is the principal one in use. It requires a minimum of special equipment, and is low in cost and simple to operate.

The second approach, recording the video signal on tape, allows replay for digitizing but adds considerable noise. Playback introduces further problems, since the normal video bandwidth necessitates a digitizing rate far in excess of that which can be handled by conventional digital systems. Any additional processing to obtain slower speeds (i.e., transcription to a longitudinal recorder) adds even more noise and may create time-base instabilities and jitter in the picture.

Memories capable of digitizing the full bandwidth and reading out the signal at digital recording rates are available but very expensive. Further, they generally can be used to record only isolated frames so that extensive tape rewinding and frame finding would be required to digitize a series of frames. Rotating-disk digital memories are becoming available and will provide multiple frame storage at more intermediate cost.

The third suggested method, direct digitizing from the video signal, is feasible only when the image is repetitive. The technique normally is to digitize one column of picture elements of each video frame, so that the time to record a complete picture requires a number of frames equal to the number of columns desired. If the dynamics of the situation permit, this method will produce the best picture quality. For standard television display approximately 17 seconds would be needed for every complete picture. When the sample is moving at faster speeds, the repetitive image can be obtained via a "frame grabber," a device (normally a video disk recorder) such as is used in commercial TV for stop-motion display. However, signal-to-noise ratio is again a problem.

Further considerations for hardware systems applicable to the scanning electron microscope are given by Billingsley (1971).

Image Processing

Once the image is obtained in digital form, numerous types of processing are possible. Some applications, primarily in the aerospace field, have been described by Nathan (1971) and Billingsley (1970). In wood science, digital processing of fast-scan pictures can be illustrated with the micrographs of Figure 3.

Figure 3A was digitized via the monitor-film-densitometer route; the result is seen in Figure 6A. Some contrast was lost in scanning—an effect that would not have occurred had the signal been digitized directly from the photomultiplier. The histogram displays the range of digital numbers representing the gray scale from black to white. The height of each bar indicates the quantity of picture elements at each digital number.

This image has large light and dark areas, each of which contains detail whose local contrast is small (by virtue of the restricted digital range). Contrast stretching would improve visibility of the details. While photographic stretching would be useful in the linear portion of the film-response curve, it would cause bright areas to lose local contrast at the curve toe. The image may be stretched by computer, but the saturation problem reappears when the output image is photographically displayed. Therefore, a filtering method is used to remove the large difference in brightness between the light and dark areas. The result of such filtering is shown in Figure 6B. Again because of the limited digital range, the contrast appears low. Now, however, it may be stretched without running into the toe and shoulder of the film-response curve when the output is displayed. The stretched micrograph, Figure 6C, reveals considerable fine detail on the cell walls and within the lumen cavities. Further, edge sharpness at the juncture of the cell wall and lumen is improved.

Digitized micrographs can also provide quantitative data. As one illustration, Figure 6A was partitioned at about 130 in the digital-number histogram. The effect was to produce an image in which the lumen cavities and other voids appeared black, while cell walls were

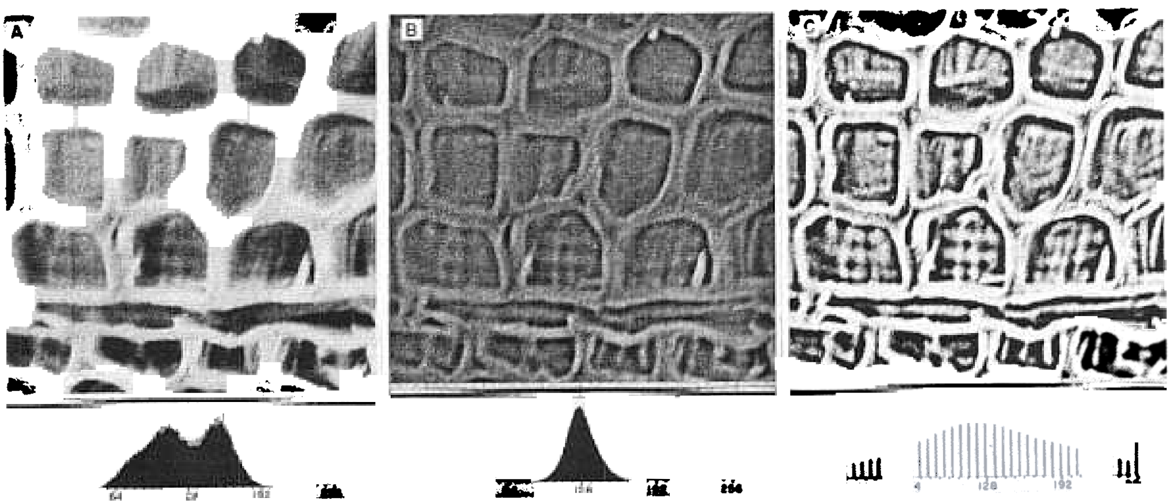

Figure 6. — *A*, Digitized version of Figure 3A. *B*, Digitized version after medium-size shading areas were removed with a 25-element filter. *C*, Computer-stretched version to enhance contrast.

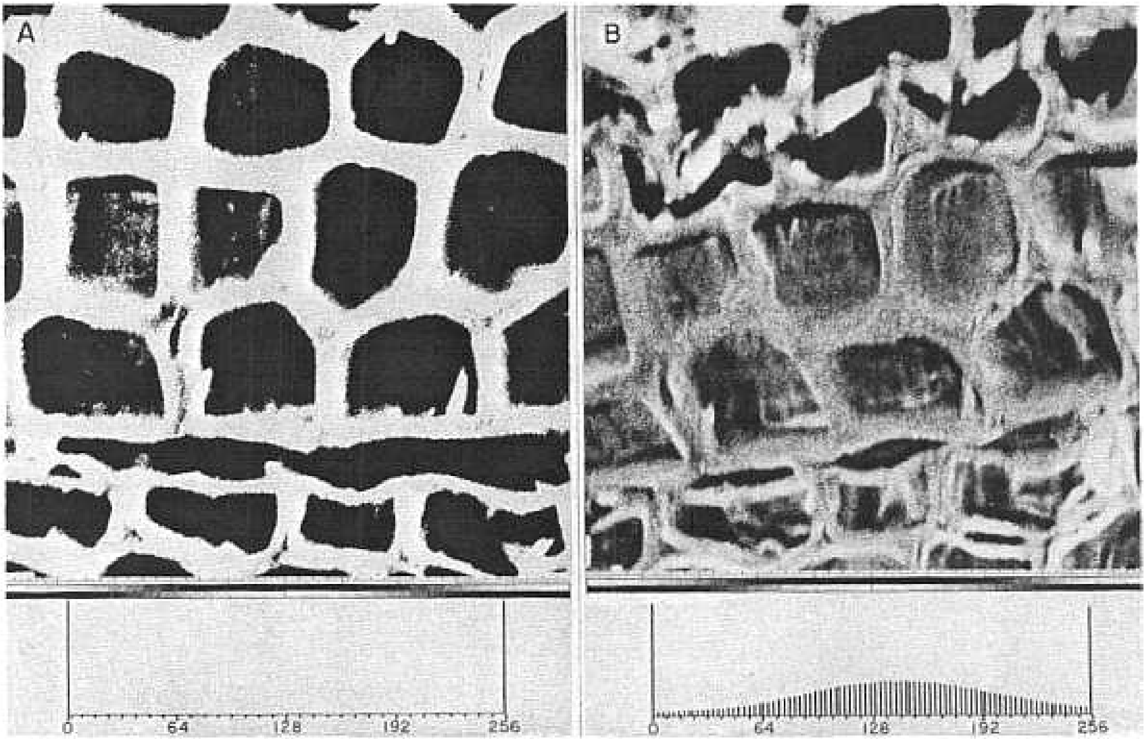

Figure 7. — *A*, Image resulting from partitioning Figure 6A at mid-gray. *B*, Difference picture resulting by subtracting digitized versions of Figures 3 C and 3 D.

white (Fig. 7A). A correlative computer listing of the partitioned digital numbers indicated that 47 percent of the micrograph was cell wall and 53 percent was lumen cavity. Essentially identical results were obtained when the areas were planimetered.

In dynamic experiments, the motion which occurs between micrographs can be displayed by the digital computer through a process of image subtraction (Billingsley 1970). Micrographs 3A and 3B of the original compression sequence were digitized, manually registered at the center row of cells, subtracted, and contrast-stretched. The result appears in Figure 7B. The major deformation appears in the upper rows of cells as areas of very dark and very light tones, while areas of little or no change are mid-gray.

Conclusion

This paper has merely attempted to sketch a few fast-scan EM techniques that offer promise in wood science. While digital image processing can augment fast-scan microscopy, it is also applicable to light and transmission microscopy. Additional developments may be anticipated. It is the opinion of the authors that the microscopist of the near future will be provided with a computer-driven display system and will be capable of selecting, by keyboard entry, a great variety of processing techniques that will vastly increase analytical capabilities and visual image modes.

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