Optical sectioning of pulp fibers using confocal scanning laser microscopy

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The morphological properties of pulp fibers—particularly the transverse dimensions such as wall thickness and cross-sectional area—influence how fibers respond to processing and strongly affect the properties of the end product (*1*). However, the transverse dimensions of fibers are difficult to measure directly.

Fiber coarseness and cross-sectional area are related properties. While fiber coarseness can be measured easily as a population average (*2*), there is no rapid technique to obtain the distribution of fiber coarseness within a population. Moreover, fiber coarseness alone is not an adequate predictor of transverse dimensions, since wall thickness for fibers with the same coarseness can vary greatly.

The distribution of fiber wall thickness may well be an important pulp quality parameter. However, a rapid method for measuring fiber wall thickness and its distribution has not yet been developed. As a result, this parameter has not been sufficiently explored or understood. Kibblewhite and Bailey (*3*) recently measured fiber cross-sectional dimensions using image analysis, but their technique of specimen preparation is tedious, requiring aligning, embedding, and microtome sectioning of fibers.

In this technical brief, we describe a new technique for rapidly producing cross-sectional images of wood pulp fibers. These images, in turn, can be used to determine several transverse dimensions and features. The method is based on optical sectioning using confocal scanning laser microscopy (CSLM). Image analysis is then used to calculate such fiber properties as area of cross-section, perimeter, and wall thickness. Simultaneous information on fiber structure, such as cross-sectional shape and the degree of collapse, also is available. The technique can be used to determine variation of cross-sectional fiber properties along an individual fiber or within a population of fibers. The prospect of extending the range of applications for this technique appears promising.

The confocal scanning laser microscope

A confocal scanning laser microscope differs from a conventional optical microscope in that the image is built up pixel by pixel by a scanning laser beam (*4*). The term "confocal" indicates that the condenser and the objective lenses are both focused at the same point on the object. A key feature of CSLM is its ability to produce a clear image of a focal plane without interference from adjacent layers above or below. This is accomplished by placing a pinhole in front of the photodetector, which prevents light originating outside the focal plane from reaching the detector. A two-dimensional cross-sectional image of a fiber is obtained by stacking a series of line scans acquired over the depth of the fiber. A three-dimensional image of an object can be reconstructed by stacking the acquired two-dimensional images.

Applications

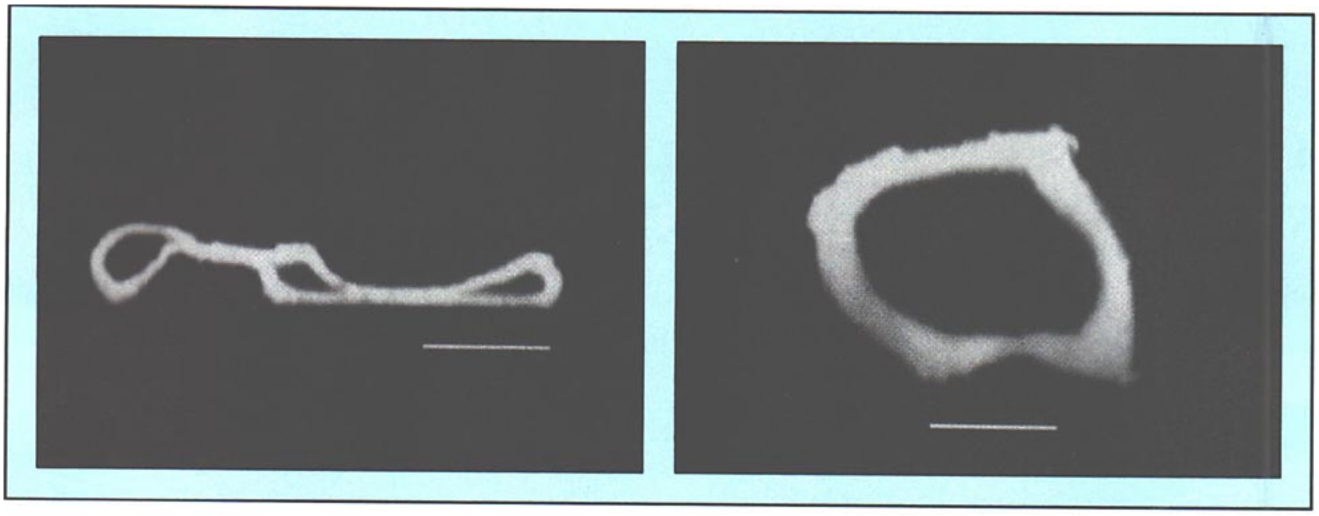
In this study, we used a BIO-RAD MRC-500 confocal scanning laser microscope attached to a Zeiss Axiophot microscope. All observations were made with a 100 × oil-immersion Plan-Apochromat objective with a numerical aperture of 1.3. The system's lateral resolution was approximately 0.25 μm.

Images of unbleached softwood kraft pulp fibers were acquired in epi-fluorescence confocal mode. Since unbleached fibers have low autofluorescence, additional fluorescence was induced by dyeing fibers with a fluorochrome dye. Dyed fibers were air dried on microscope slides. Fiber cross-sectional images were generated from a series of horizontal line scans, which were taken perpendicular to the fiber axis while the sample stage was stepped vertically. The smallest available step size (0.16 μm) was used.

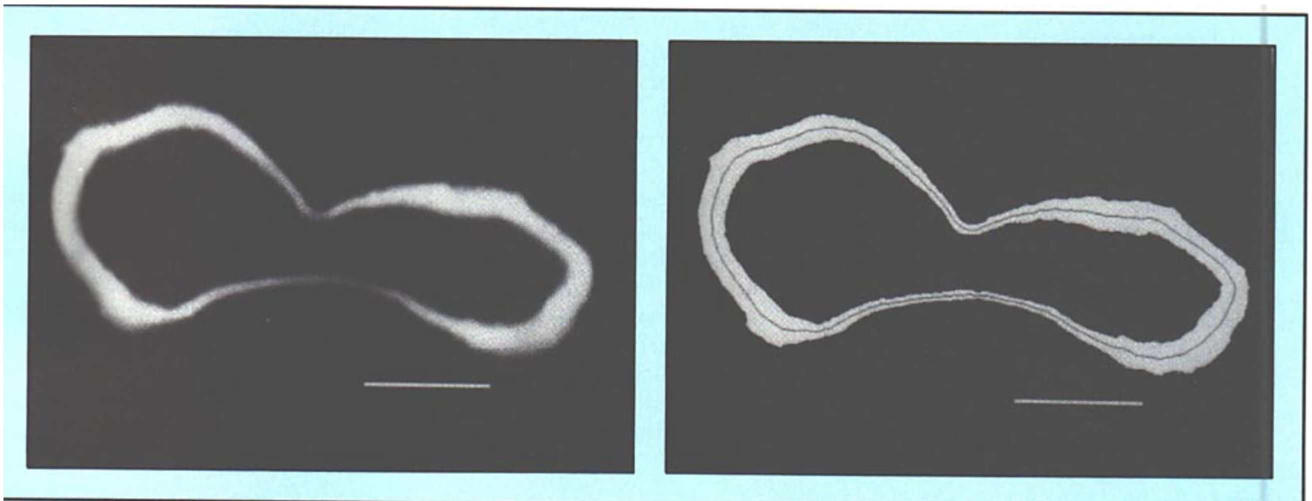
Figure 1 shows typical cross-sections of fibers, indicating their shape and degree of collapse.

Fiber cross-sectional areas and center-line perimeters were obtained using a Kontron IBAS image analyzer. Segmentation was based on the maximum-gradient method for edge detection, which was verified on a series of calibrated microspheres (*5*). **Figure 2** shows the original and the segmented image of a fiber cross section. The cross-sectional area was 177 μm^2, and the center-line perimeter was 103 μm. The average wall

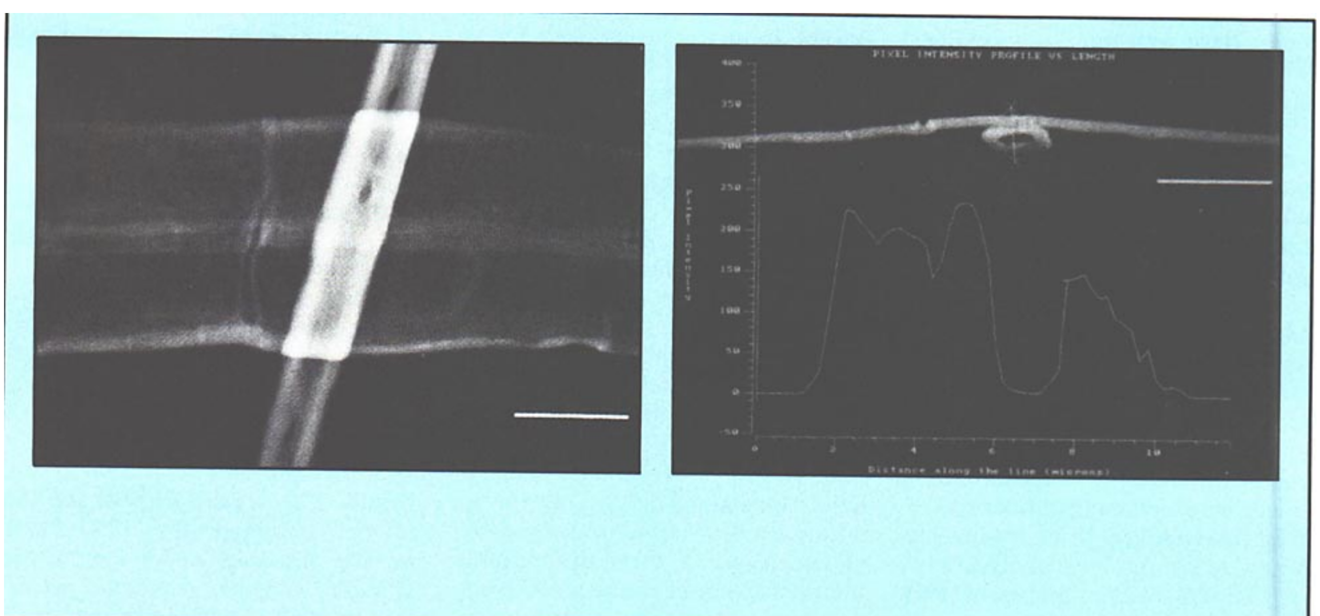
1. Cross-sectional images of lightly fluorochrome-dyed fibers. Scale bar = 10 μm.



2. Original CSLM image (left) and processed image (right). Scale bar = 10 μm.



3. Two crossed fibers: top view (left) and cross-sectional view (right). The grey-level profile corresponds to the vertical section x - x. Scale bar = 25 μm.



thickness—defined as the cross-sectional area divided by the length of the center-line perimeter (*3*)—was 1.72 μm.

Figure 3, another example of optical cross-sectioning via CSLM, shows simultaneously obtained lateral and cross-sectional views of two crossed fibers. The extent of contact between the two fibers is apparent from the cross-sectional view.

Conclusion

Optical sectioning with CSLM is a powerful new tool for rapidly acquiring images of fiber cross-sections. The primary advantage of the technique is the simple method of specimen preparation. The technique facilitates measurement of several transverse physical features and dimensions of fibers. Such measurements were difficult to obtain using previous methods.

We are using this approach to determine fiber wall thickness and its distribution in pulps. We also are exploring the use of this technique to determine changes in fiber shape and conformability with pulp processing (pressing, refining, recycling).□

Literature cited

1. Seth, R. S., MRS Symposium Proceedings 197, Materials Research Society Pittsburgh, Pa., 1990, pp. 143–61.
2. Piirainen, R., and Paavilainen, L., 1986 International Process and Materials Quality Evaluation Conference Proceedings, TAPPI PRESS, Atlanta, p. 67.
3. Kibblewhite, R. P., and Bailey, D. G., Appita 41(4): 297(1988).
4. Handbook of Biological Confocal Microscopy (J. B. Pawley, Ed.), Plenum, New York, 1990.
5. Jang, H. F., Robertson, A. G., and Seth, R. S., Transverse dimensions of wood pulp fibers by confocal laser scanning microscopy and image analysis, unpublished work.

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