Journal of Korea TAPPI
Vol. 31. No. 3, 1999
Printed in Korea

Application of Confocal Laser Scanning Microscopy and Fiber Distribution Index to Study Kenaf Handsheet Properties

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ABSTRACT

This study was to quantify fiber distributions in thickness direction of kenaf handsheets as a fiber distribution index (FDI) and to analyze the relationship between FDI and the handsheet properties. The images of fiber distribution in z-direction were obtained by Confocal Laser Scanning Microscope (CLSM) and analyzed by image analysis technique. The proposed FDI had a good correlation with high R^2 values with various properties of paper, such as apparent density, scattering coefficient, burst index, tear index, tensile index, and folding endurance. The proposed FDI was shown as a good index to quantify paper properties.

1. Introduction

Paper can be seen as a network of self-bonding fibers. It contains fillers, chemicals, and other non-fibrous materials. Variations in the amount of these materials deposited through the thickness of a sheet are unavoidable in the practical sheet forming process. These variations in the z-direction can lead to curl, dimensional instability and printability problems. A quantitative measurement of their distributions at various layers of a sheet would be very useful as it could provide information on the underlying causes of these problems. In addition, a knowledge of these variations would be most useful in optimizing the interior structure of the sheet by assisting control of fines migration and filler retention, in the z-direction.[1] In this paper, we examine only fiber distribution in the z-direction of five kinds of handsheets that have different mixing ratios between kenaf *(Hibiscus cannabinus)* bast and core fibers.

CLSM is widely being used in the field of pulp and papermakingscience, which enables non-destructive optical sectioning of a sheet. Since the introduction of the confocal theory by Minsky,[2] CLSM has been used for obtaining high resolution images and three-dimensional reconstruction of pulp

• This work was supported (in part) by the Korea Science and Engineering Foundation (KOSEF) through the Research Center for the Development of Advanced Horticultural Technology at Chungbuk National University and was supported (in part) by the Institute of Industrial Technology Policy, Ministry of Commerce, Industry, and Energy, "Development of Alternative Fiber Resources for Korean Traditional Papermaking" (1996).
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fibers.[3-7] Nowadays, CLSM and its related techniques have been developed in order to show the fines distribution within a sheet and the two-sideness of laboratory handsheets.[8] Furthermore, it came to get the fiber distribution as a numerical format in a z-direction from a sheet using CLSM and image analysis.[1] In this study, CLSM technique was adapted to observe the different structure along the z-direction of handsheets made by kenaf core and bast fibers with different mixing ratios. Fiber distribution index (FDI) was suggested to examine whether there is any correlation among FDI and conventional properties of a sheet.

2. Materials and Methods

Detailed materials and methods were already described in the previous article.[9] Noteworthy descriptions about materials and methods are as follows;

2.1 Dyeing of pulps

Both kinds of pulps for sheets of CLSM observation were lightly dyed with a fluorescent dye (Acridine Orange) solution for about 30 minutes.

2.2 Handsheet making for CLSM observation

TAPPI Test Methods T205 sp-95 and T402 om-93 are followed to make handsheets. The formulation of handsheet is given in Table 1.

Table 1. Mixing ratios of kenaf bast and core fibers

Sheet name	Pulp Mixture (%)	
	Bast fibers	Core fibers
B100:C0	100	0
B75:C25	75	25
B50:C50	50	50
B25:C75	25	75
B0:C100	0	100

2.3 CLSM observation

A Bio-Rad™ MRC-1024 CLSM system was used to generate the cross-sectional images of sheets. The sheets were placed on the glass slide and covered with cover slips. Immersing oil was adapted to lessen the refractiveness, when the images were generated by CLSM. Observations were made with both 20× and 40× objective. Scan speed was normal, Kalman collection filter was adapted, and the scanning depth along the z-direction was 1.0 μm. One representative area of a specimen was observed to get an image.

2.4 Image analysis of test sheets

The images obtained from CLSM were transferred to a program for image analysis to quantify CLSM images. An ImagePro™, image processing program was operated to convert the raw gray images into black and white images and to measure the converted area as fiber distribution area at the same time.

2.4.1 Image processing for FDI

Each CLSM image of *.pic file format was copied into ImagePro program for further processing. Confocal Assistant™ program was used in order to select a desired discrete image from the accumulated images generated by CLSM and to project all accumulated images. The copied raw gray-scale

Table 2. Number of scanned layer of test sheets by CLSM

Sheet name	B100:C0	B75:C25	B50:C50	B25:C75	B0:C100
Scanned layer	60	51	33	31	26

images were converted by using threshold function of ImagePro software for accurate estimation from low quality CLSM images. Then, fiber distribution area of each scanned layer out of accumulative images in a sheet was measured in the unit of pixel.

2.4.2 Fiber distribution index

The scanning number for a sheet was used in calculation of FDI. Relationship between FDI and strength properties of a sheet is analyzed. FDI was defined as follows;

$$FDI = A_{FD} / N_{ML}$$

where, A_{FD} is a ratio of distributed fiber's area to total cross-sectional area.

N_{ML} is the scanning number for a sheet.

$A_{FD} = [A_S / A_T] \times 100$

A_S is a sum of fibers area from all layers.

A_T is a total area in calculation. This value is calculated by multiplying the maximum area out of all measured areas in a sheet by the number of measured layer.

3. Results and Discussion

3.1 CLSM observation of handsheets

To see the fiber distribution along z-direction in a sheet, cross-sectional images of each sheet were taken. Table 2 shows the number of scanned layer by CLSM, and Fig. 1 shows both accumulated images of x-y plane and z-directional images from test

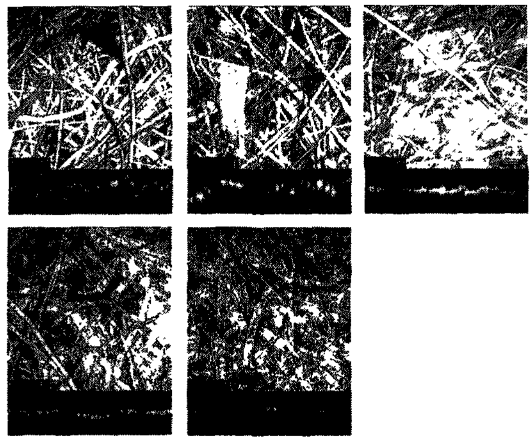

Fig. 1. CLSM image of test sheets. x-y plane image (upper) and z-directional image (lower). Scale bar is 100 μm. Sheet names refer to Table 1.
Upper left: B100:C0, Upper center: B75:C25, Upper right: B50:C50
Lower left: B25:C75, Lower center: B0:C100

sheets, which have different mixing ratios between bast and core pulps. The black and lower image in each small figure is the image along the z-direction of a sheet.

In B100:C0 (upper left image) sheet of Fig. 1, most of the fibers have a rather narrow width and long length. Also, considerable amount of fibers are straight. A rather white portion in the x-y plane is a higher part of the sheet in topographical, whilst dark and black portion is the part of the valley or void pore in the micro-structure of paper. White spots out of the black area in the z-directional image are the fiber distributed area.

Since B75:C25 (upper center image) sheet contained 25% of core fibers, it seems that the distributed fiber's area in z-directional image has a less discrete distribution than

Table 3. Fiber distribution index (FDI) derived from CLSM images and image processing

Item \ Sheet name	B100:C0	B75:C25	B50:C50	B25:C75	B0:C100
Number of measured layer, N_{ML}	60	51	33	31	26
Sum of measured area from all layers, A_S (pixel)	1221928	1402036	1269270	788140	602217
Maximum area from all measured layers (pixel)	54872	63983	119117	71193	69240
Total area in calculation, A_T (pixel)	3292320	3263133	3930861	2206983	1800240
Fiber distribution area, A_{FD} (%)	37	43	32	36	33
FDI	0.62	0.84	0.98	1.15	1.29

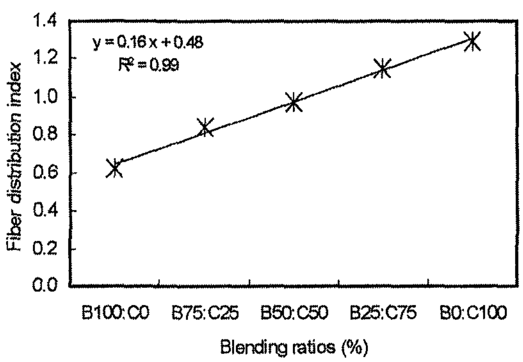

Fig. 2. Blending ratios of kenaf bast and core pulps and fiber distribution indices.

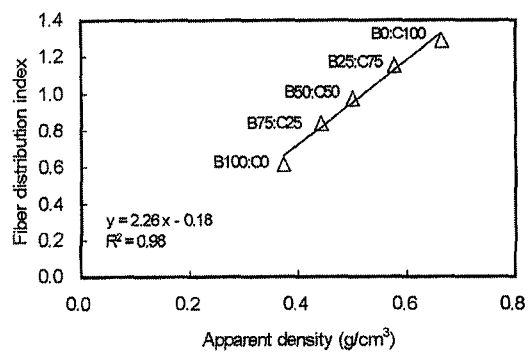

Fig. 3. Apparent densities and fiber distribution indices.

that of bast 100% sheet image.

As the sheet had a greater amount of core fiber addition, fiber distribution along z-direction became more or less flatter and seems to form a continuum (lower left image). Being composed by 100% core pulp, it has well shown the continuous and narrow fiber distributed area in cross-sectional image (lower center image). Note and compare the approximate thickness from each image defined by fiber area, which is white portion, in z-direction.

3.2 Fiber distribution index

At first hand, fiber distribution area was calculated in percent, dividing the sum of measured fibers distributed area from each x-y plane along z-direction by a total area from all layer in a sheet. As it seemed not to have any important relationship each other, another criterion was needed to inspect the relationship on strength properties. FDI of each sheet is calculated merely to compare sheet-to-sheet and to appreciate the relationship with strength properties.

Fig. 2 shows the relation between FDI and blending ratio of kenaf bast and core pulps,

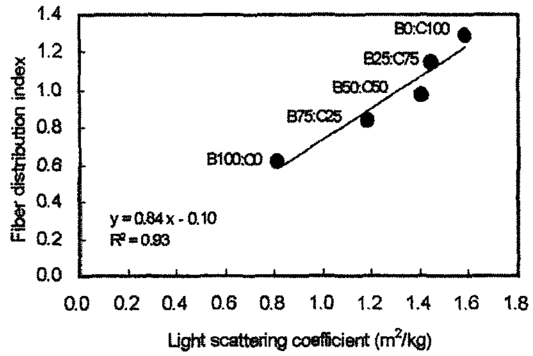

Fig. 4. Light scattering coefficients and fiber distribution indices.

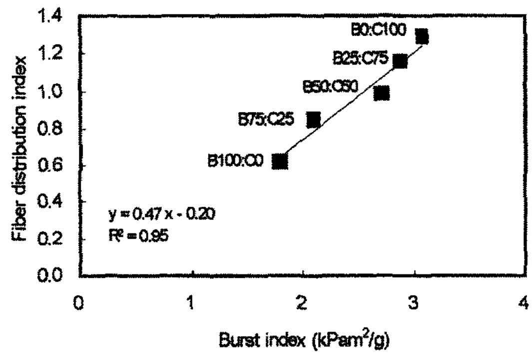

Fig. 5. Burst indices and fiber distribution indices.

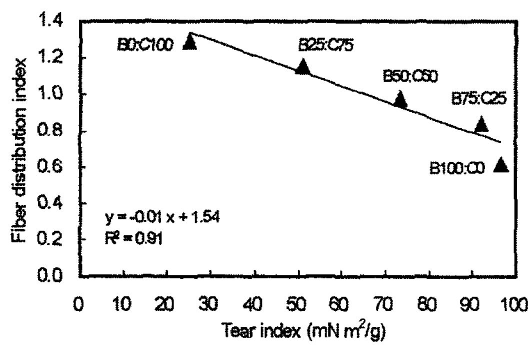

Fig. 6. Tear indices and fiber distribution indices.

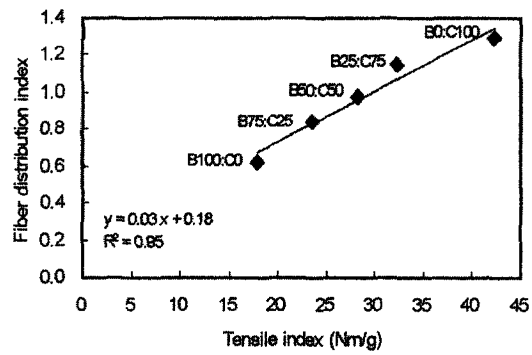

Fig. 7. Tensile indices and fiber distribution indices.

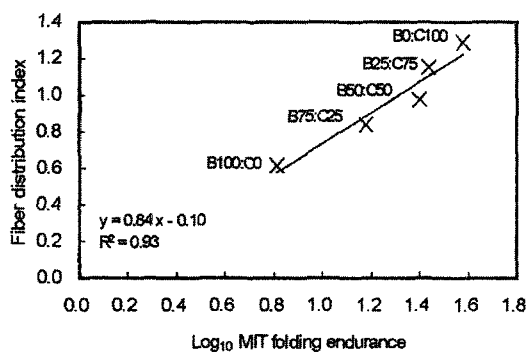

Fig. 8. Folding endurances and fiber distribution indices.

Table 4. R^2 values from fiber distribution index and each properties

Property	R^2
Apparent density	0.98
Scattering coefficient	0.93
Bust Index	0.95
Tear Index	0.91
Tensile Index	0.95
Folding Index	0.93

and Fig. 3 is drawn to show the relationship with apparent density of FDI. Generally, the value of R^2 is a descriptive measure of the utility of the regression equation for making prediction. The R^2 values in Table 4 and Figures 4~8 suggest that FDI correlates to strength properties well. These results imply that FDI is able to be used to estimate the paper properties using CLSM image, although the procedure of presumption and calculation for FDI are needed to be elaborate. With further investigation, it is speculated that FDI can be related to various properties of paper such as smoothness, porosity, penetration of water and air, and printing properties.

4. Conclusions

This study was performed to provide a usage of CLSM into paper science, especially to see the fiber distribution in z-direction of sheets that made by different mixing ratios of kenaf bast and core pulps. FDI was suggested in order to quantify fiber distributions in thickness direction of a paper.

4.1 Confocal laser scanning microscopy (CLSM) is proved as a good tool for investigation of paper structure along the z-direction in papers. Image analysis system was used to convert and quantify the images gained by CLSM.

4.2 Fiber distribution index (FDI) was introduced by quantifying the CLSM image to draw a relationship between paper structure and properties of a sheet. FDI had a good correlation with several properties of paper, and the relationship was supported by high R^2 values. Although the effects of self-shadowing in thick paper on obtaining good CLSM images have not been fully overcome, FDI was a good and useful index to predict and explain the properties of sheets.

Acknowledgement

The authors would like to thank to Jeong-Seon Cheon, Center for Research Instruments and Experimental Facilities, Chungbuk Nat'l Univ., for assistance of CLSM observation.

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