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Quantitative Analysis of Gross Adhesive Penetration in Wood Using Fluorescence Microscopy

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An optimum amount of adhesive penetration is desirable for economy of production and development of bond strength in wood composites. A general method that allows quantitative measurement of gross adhesive penetration in wood is described. Staining techniques have been developed that can provide sharp contrast between a cured adhesive and the wood substrate using fluorescence microscopy. An image analysis system utilizes this contrast to quantify gross adhesive penetration in wood. An example of this technique is provided, whereby the effect of molecular weight distribution of phenol formaldehyde prepolymers on gross adhesive penetration into yellow poplar (*Liriodendron tulipifera*) flakes is observed and quantified. Adhesive penetration into wood flakes was shown to be correlated with the molecular weight distribution of the prepolymer, decreasing with higher weight average molecular weight. Gross adhesive penetration into hardwoods is likely to be dominated by flow into vessel elements, as demonstrated by the wood species studied here.

KEY WORDS thermosetting adhesives; wood-based composites; gross adhesive penetration; liquid resole phenol formaldehyde; fluorescence microscopy; adhesive molecular weight distribution.

INTRODUCTION

The amount of adhesive penetration into the wood substrate during the manufacture of plywood has been known to affect bond quality. Speculation concerning the flow characteristics of these thermosetting adhesives has been based primarily on qualitative observations. Excessive adhesive penetration into the veneer will leave “starved” bondlines. Shallow penetration allows little internal surface contact for chemical bonding or mechanical interlocking. An optimum amount of adhesive penetration is needed to repair processing damage to the wood surface and allow

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better stress transfer between laminates. Other wood composites also benefit from optimum adhesive penetration, which promotes strong bonds and more efficient use of the adhesive. The objective of this research was to develop a method that allows quantitative measurement of gross adhesive penetration in wood.

Adhesive movement through wood is usually categorized as gross penetration and cell wall penetration. Gross adhesive penetration refers to movement of the adhesive through the large voids in the porous structure, whereas cell wall penetration involves movement of adhesive through the microvoids within the wood cell walls. Some gross adhesive penetration normally occurs upon wetting of the wood surface. Wood anatomy, surface energetics, adhesive viscosity, and wood moisture content are primary variables involved in the wetting and penetration of wood surfaces. In the production of wood composites with thermosetting adhesives, hot-pressing variables such as compaction pressure, press temperature, press closing rate, and particle geometry are introduced. These variables interact to create a dynamic environment involving temperature and vapor pressure gradients in the mat during hot-pressing. It is under these conditions that the adhesive must flow and cure properly.

It is desirable to develop methods to quantify adhesive flow and isolate the effect of individual variables. Microscopic methods have been developed to analyze adhesive penetration qualitatively.¹⁻⁵ Manual counting techniques have also been developed based on microscopic methods.^{6,7} Techniques, in which a scanning electron microscope is interfaced with an energy-dispersive analyzer for x-rays (SEM/EDAX), have been applied to measure chemically-labeled adhesive concentration in wood.⁸⁻¹⁰ The present research project focused on developing computer-assisted techniques to quantify gross adhesive penetration into wood through use of a fluorescence microscope interfaced with a digitizing image analysis system. An example of the technique that was developed is provided. In the example the effect of molecular weight distribution of phenol formaldehyde prepolymers on gross adhesive penetration into wood flakes is observed and quantified.

METHODS AND MATERIALS

Section Preparation

Flakes of yellow poplar (*Liriodendron tulipifera*) were sequentially sliced from a water-saturated block. Care was taken to slice the flakes from sapwood, with the width orientation as close to the pure tangential plane as possible. Flakes were dried in a convection oven at 104°C to an oven-dry condition. Target flake dimensions were 25.4 mm by 25.4 mm by .635 mm. Five minutes after removal from the oven, three drops of liquid phenol formaldehyde adhesive were placed across the width of each flake with a micropipette set at 0.5 microliters volume. The flake with each successive adhesive drop was weighed on an analytical balance. The flake/adhesive specimens were allowed an additional five-minute open assembly time after placement of the third droplet and then heated in a convection oven at 104°C overnight.

The three liquid-resole, phenol formaldehyde adhesives used were commercially prepared (Neste Resins, Springfield, Oregon, U.S.A.). The primary difference

TABLE I
Analysis of liquid resolite phenol formaldehyde adhesive parameters with molecular weight parameters measured by gel filtration chromatography

	Adhesive 1	Adhesive 2	Adhesive 3
pH	9.20	9.20	9.20
Nonvolatiles (%)	45.81	46.18	46.28
Viscosity (Cp at 25 C)	82	138	200
Gel Time (min)	23.50	20.90	19.96
NaOH (%)	1.45	1.45	1.45
Molecular weight averages:			
Weight Average (Mw)	2964	3680	4366
Number Average (Mn)	1322	1298	1153
Z Average (Mz)	6159	7713	10440
Polydispersity (Mw/Mn)	2.241	2.835	3.786
Molecular weight range:			
	Area adhesive 1	Area adhesive 2	Area adhesive 3
73,500-35,000	0.00	0.00	0.11
35,000-18,000	0.40	1.14	2.95
18,000-10,000	3.89	6.50	8.94
10,000- 5,000	12.70	15.50	16.47
5,000- 3,000	15.06	16.33	13.89
3,000- 2,000	15.27	15.63	14.63
2,000- 1,000	25.95	24.17	21.13
1,000- 500	20.29	16.22	15.58
500- 100	6.44	3.46	7.73

between the three adhesives was the degree of polymerization. Table I lists information on the adhesives, including an analysis of the molecular weight distribution by gel filtration chromatography (GFC). The gel packing material was Sephacryl S-200 (HR) and an aqueous 0.1N NaOH eluent was used. Sodium polystyrene sulphonate and phenol were used as calibration standards. A UV detector with a 280 nm filter was used and the output was relayed into a chromatographic data acquisition system. The analysis was performed according to the method described by Sellers and Prewitt.¹¹

Transverse cross-sections of the cured adhesive specimens were cut using an American Optical Company Model 860 sliding microtome. Strips of low density balsa wood were placed on both sides of the thin flakes for support during sectioning. Cross-sections which contained three adhesive drops were cut 80 μm thick from water saturated flakes. Thinner sections would work equally well if they could be removed complete. The sections were placed on glass slides, rewet with distilled water, straightened, and a cover slip applied. The slides were placed in a convection oven and allowed to dry at 104°C for 30 minutes. After drying, the cover slips were removed and each section was stained with a pipette drop of 0.5% Toluidine Blue O solution. After soaking for 15 minutes, the sections were rinsed twice with distilled water, a drop of glycerin was added, and the cover slip replaced.

Under UV light, the lignin component of wood will readily fluoresce whereas the semi-crystalline structure of the wood cell wall is translucent. Internal fluorescence

and reflectance creates a background illumination when observing adhesive on a wood surface under UV light. Under these conditions, optical resolution of the adhesive is poor. Greater resolution is achieved when the background illumination is inhibited using a fluorochrome depressor dye (Toluidine Blue O solution) that is selectively absorbed by the wood. The result is a brightly illuminated adhesive phase against a dark wood background.

Fluorescence Microscope and Image Analysis System

A Carl Zeiss Axioskop microscope, with a 50 W mercury burner and halogen bulbs, was used for observing the sections. The slides were then viewed under incident light with a $10\times$ Plan Neofluar objective, providing a total magnification of $80\times$ in the microscope and $320\times$ on the image analysis monitor. An UV G365/LP420 excitor-barrier filter set was used. Measurements were performed with a (Universal Imaging) Image 1/AT Image Processing and Analysis System. The system consisted of a CCD solid state camera, a 30+ lines per mm 330 mm color monitor, a WIN 386 personal computer with monitor, a frame grabber board, and the Image 1/AT software.

Image Analysis Technique

Each image captured by the Image 1/AT system had a resolution of 512 pixels wide by 480 pixels high. At $320\times$ magnification level, the video monitor screen corresponded to an area $800\ \mu\text{m}$ wide by $623\ \mu\text{m}$ high with a resolution of $1.67\ \mu\text{m}$ per pixel. The flake cross-section edge which contained the adhesive drops was lined up with the left side of the monitor screen, the flake thickness being in the wide direction of the screen. With a flake target thickness of $635\ \mu\text{m}$, most sections fit completely on the screen in the thickness direction. The target flake width of $25,400\ \mu\text{m}$ gave a possibility of 41 separate full screen images in the width direction.

Three adhesive drops had been placed across the width of each flake. Images alternately came from the adhesive drops on the outside edge and the center of each section. The width of each adhesive drop was measured in the flake-width direction and the midpoint of this distance was picked as the location for analysis. Upon lining up the sections on the screen, $100\ \mu\text{m}$ wide segments of the image were analyzed across the flake thickness (Figure 1).

The effect of machining damage was apparent on the wood surface. Processing indentations of up to approximately $75\ \mu\text{m}$ were seen on the transverse plane of the flake surface. Adhesive area measurements taken in the first $100\ \mu\text{m}$ segment were therefore primarily in this zone of machining damage. The initial grid line on the surface edge of the first $100\ \mu\text{m}$ segment was positioned at a location deep enough in from the flake surface such that a minimum of 50% of the surface in this segment was not damaged. Figure 1 is a photomicrograph of adhesive 1 on a transverse flake section showing a typical grid placement (see also Color Photograph 1).

For each image, measurements for area of penetrated adhesive were made in as many as eight segments, depending on the actual flake thickness at that point. Adhesive objects that fell on boundaries between segments were tallied in the

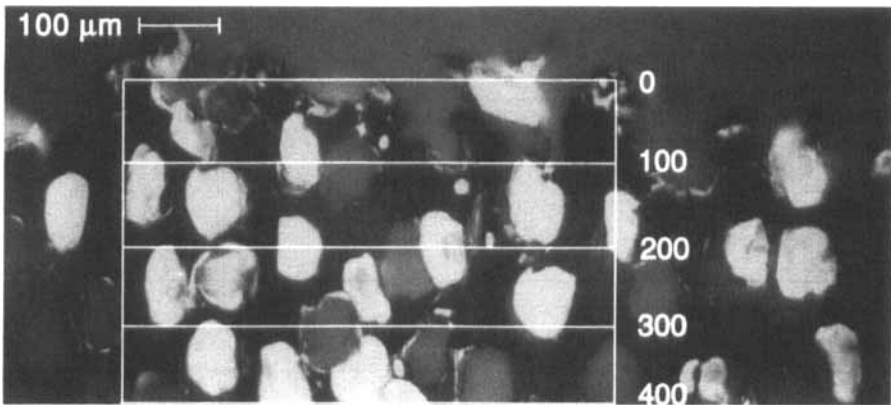
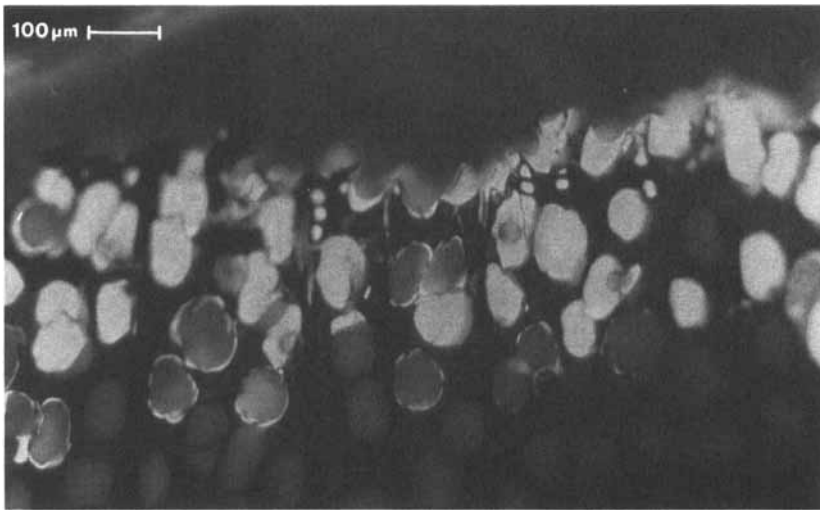


FIGURE 1 Cured resole phenol formaldehyde adhesive 1 on transverse plane of *Liriodendron tulipifera* flake with example of grid used to segment zones of adhesive penetration area.



PHOTOGRAPH 1 Cured resole phenol formaldehyde adhesive 1 on transverse plane of *Liriodendron tulipifera* flake (Blue BP450/490 - LP520 excitor-barrier filter set). See Color Plate I.

segment which contained over 50% of the object. Separate measurements of the area of unpenetrated adhesive on the flake surface were made. Figure 2 is a photomicrograph representative of adhesive 3 (see also Color Photograph 3). A large area of adhesive which did not penetrate into the wood substrate is present on the flake surface. Much of the volume of adhesive 2 also did not penetrate into the wood substrate (see Color Photograph 2). The sums of adhesive penetration area for all sample analyses shows that three times as much adhesive 1 was measured in the wood substrate as adhesive 2 or adhesive 3 (Table II). The sums of adhesive penetration area for adhesive 2 and adhesive 3 were very similar.

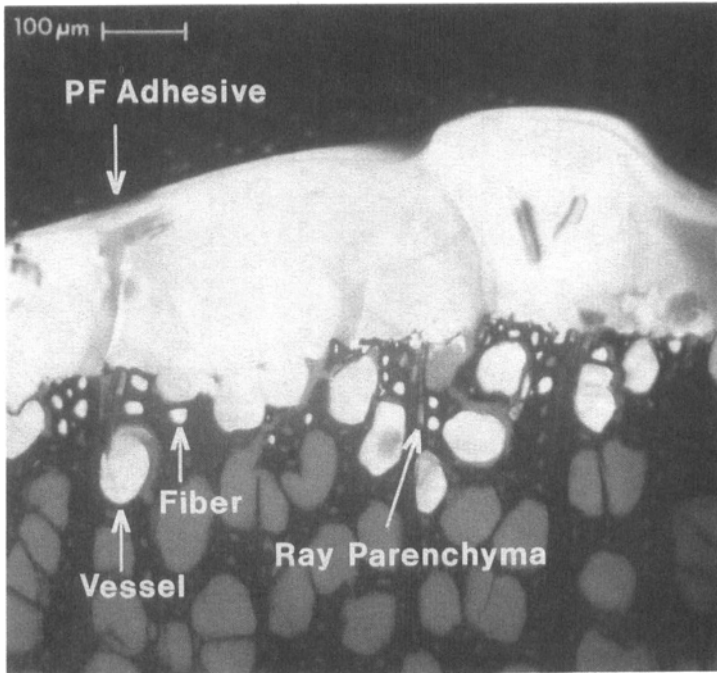
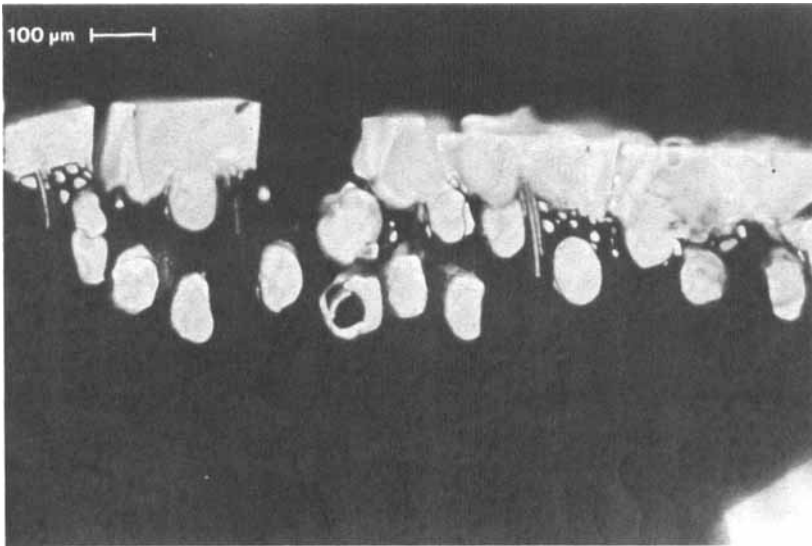
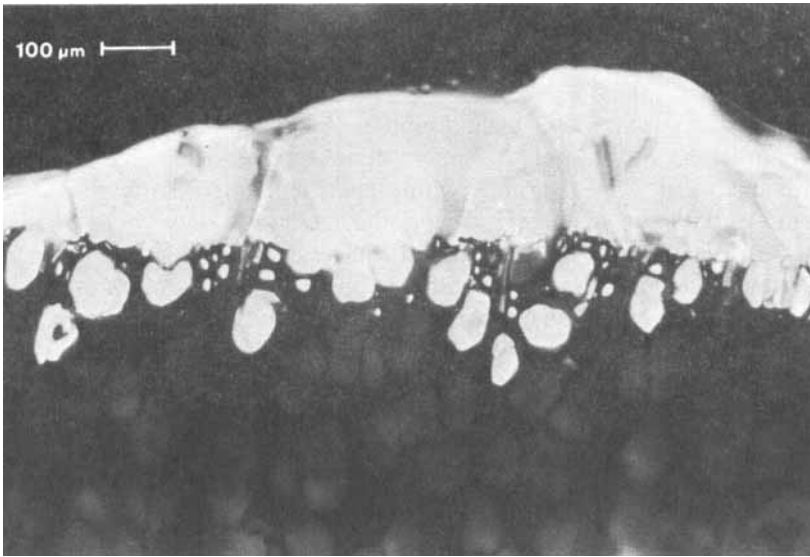


FIGURE 2 Cured resole phenol formaldehyde adhesive 3 on transverse plane of *Liriodendron tulipifera* flake with examples of major types of adhesive-filled wood cells in porous structure.



PHOTOGRAPH 2 Cured resole phenol formaldehyde adhesive 2 on transverse plane of *Liriodendron tulipifera* flake (UV G365/LP420 excitor-barrier filter set). See Color Plate II.

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PHOTOGRAPH 3 Cured resol phenol formaldehyde adhesive 3 on transverse plane of *Liriodendron tulipifera* flake (UV G365/LP420 excitor-barrier filter set). See Color Plate III.

TABLE II
Adhesive droplet measurements

Adhesive #	Mean droplet weight (mg)	Mean droplet length (μm)	Mean penetrated adhesive (%)	Sum ¹ adhesive penetration area (μm^2)
1	4.13 (.228) ²	2170 (249)	85.9 (5.68)	680,651
2	3.99 (.408)	2035 (208)	18.7 (15.1)	199,787
3	4.38 (.146)	2008 (283)	9.93 (4.72)	203,993
Mean	4.17 (.198)	2069 (83.9)	38.2 (41.6)	361,477 (276,421)

¹Sum of all penetrated adhesive area; Total N = 30, 10 for each adhesive.

²Standard deviations in parenthesis.

Each image was acquired as the average of 256 frames to compensate for random noise. The image was then sharpened, a software function that utilizes a Laplacian operator to enhance object edge features and deliver a "crisper" picture. A byte of memory contains an average value of brightness for each pixel element, with a gray level range of between 0 and 255. A value of 0 corresponds to completely black, while a value of 255 corresponds to completely white. A gray level range was determined for each individual image which visually corresponded to adhesive penetration areas. Thresholding sets the appropriate range of gray level values over which area measurements can be made. Measurements of adhesive were then made and recorded.

RESULTS AND DISCUSSION

Adhesive Movement through Wood

Image analysis systems have been found to be useful for research techniques involving microscopy as well as macro lens applications.¹² Manipulation and measurement of gray level groups within specified ranges provides quantitative information about the image. However, the technique only works if regions of interest, or objects, are well defined and can be contrasted to irrelevant regions. The staining technique described above suppressed the autofluorescence of the wood, while allowing the nonabsorbent adhesive to fluoresce. Therefore, measurements conducted in this experiment are of adhesive area on the transverse plane, herein referred to as adhesive penetration area.

Adhesive droplet length measurements show that adhesive 1 spread slightly further across the flake width than did adhesive 2, and adhesive 2 spread further than adhesive 3 (Table II). As also seen in Table II, 85.9% of adhesive 1, 18.7% of adhesive 2, and 9.9% of adhesive 3 penetrated into the wood substrate. The higher weight average molecular weight adhesives penetrated the least distance into the wood and the lower molecular weight adhesive penetrated the most. Figure 3 shows the distribution of the adhesives across the flake thickness as a percentage of the total amount of penetrated adhesive. Of the adhesive that penetrated into the wood, 82.4% of adhesive 3 and 75.8% of adhesive 2 did not penetrate past the first 100 μm . Only 24.8% of the less developed, lower viscosity adhesive 1 is contained within the first 100 μm of the flake thickness. More of adhesive 1 is contained in the segments between 100 μm and 300 μm than in the first 100 μm .

The adhesive penetration area as a percent of the total cross-sectional area is shown in Table III, as well as the other information from measurements of the individual 100 μm wide segments. In the first 100 μm segment, the percent adhesive penetration area as an average for the three adhesives was 39%. The wood should have approximately 73% void volume according to calculations using the green specific gravity of the species and the commonly-accepted value of 1.5 as the specific gravity of the solid cell wall substance.¹³ As judged by area measurements, approximately 53% of this void volume was filled by adhesive in the first 100 μm segment.

An object refers to the area of interest which you are able to contrast, threshold, and therefore quantify. In this study, the objects consist of the adhesive penetrated through the porous structure and measured on the transverse plane of the wood flake. The adhesive object area is the area mean of all the adhesive objects measured within each 100 μm segment. Table III shows that for all three adhesives, the mean adhesive object area is larger in the segment 100–200 μm from the flake surface than in the first 100 μm . Adhesive 1 has the largest mean adhesive object area in the segment range from 200 μm to 300 μm , while adhesives 2 and 3 have the largest mean adhesive object area in the segment range 100 μm to 200 μm deep. Figure 4 graphically shows the mean adhesive object area across the flake thickness. Reported measurements for fiber and vessel lumen areas for *Liriodendron tulipifera* are approximately 100 μm^2 and 4000 μm^2 , respectively.¹⁴ For all three adhesives, the average adhesive object area increased as distance from the flake surface increased,

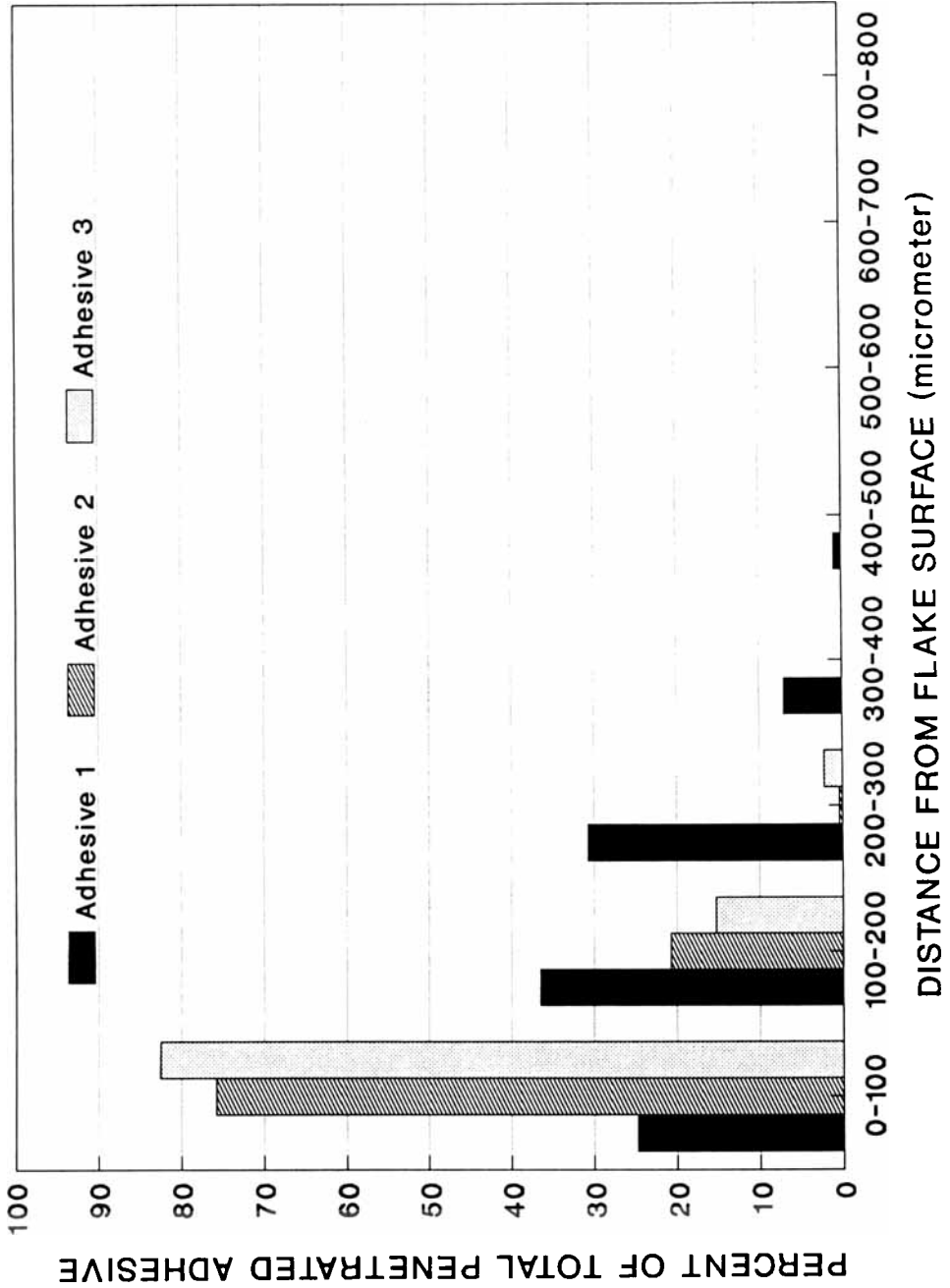


FIGURE 3 Mean percent of total penetrated adhesive as a function of distance from the flake surface.

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TABLE III
Image analysis measurements performed on 100 μm thick segments of flake cross-sections at specified distances from flake surface¹

Adhesive number	Segment range ² (μm)	Mean adhesive penetration area (μm^2)	Mean percent adhesive penetration area (%)	Mean adhesive object area (μm^2)
1	0-100	18,000	28.9	500
	100-200	24,400	39.2	1,490
	200-300	20,600	33.1	2,250
	300-400	6,010	9.6	750
	400-500	860	1.4	680
	500-600	0	0	0
	600-700	0	0	0
	700-800	0	0	0
2	0-100	27,500	44.2	1,260
	100-200	11,500	18.4	2,420
	200-300	234	.38	780
	300-400	0	0	0
	400-500	0	0	0
	500-600	0	0	0
	600-700	0	0	0
	700-800	0	0	0
3	0-100	27,100	43.4	810
	100-200	6,980	11.2	2,540
	200-300	1,190	1.9	1,660
	300-400	0	0	0
	400-500	0	0	0
	500-600	0	0	0
	600-700	0	0	0
	700-800	0	0	0

¹Total N = 30, 10 for each adhesive.

²Segment range is measured as distance from the flake surface.

reached a maximum size, and then decreased. One possible explanation is that a higher percentage of fiber lumens were filled close to the flake surface. Due to the large vessel lumen size and relatively permeable perforation plates between adjoining vessel elements, bulk flow of the adhesive deeper into the flakes was more easily accomplished. The mean adhesive object area declined at further depth into the wood, probably as a result of fewer fully-filled vessel lumens. Another possible influence on mean adhesive object area is the effect of machining damage to the surface region of the flake. In this study, that effect would be contained in the first 100 μm segment.

Figures 5 and 6 graphically show the distribution of adhesive object area sizes by adhesive. In Figure 5, the ordinate is the percentage of total number of adhesive objects recorded in each object size range. In Figure 6, the ordinate shows the percentage of total adhesive penetration area represented by each object size range. For both figures, the abscissa is divided into three size ranges. The first group has adhesive object areas from 0 μm^2 to 50 μm^2 , the second group has object areas from 50 μm^2 to 300 μm^2 , and the third group has an object area range from 300 μm^2 to 15,000 μm^2 . According to measurements performed by the authors, likely ob-

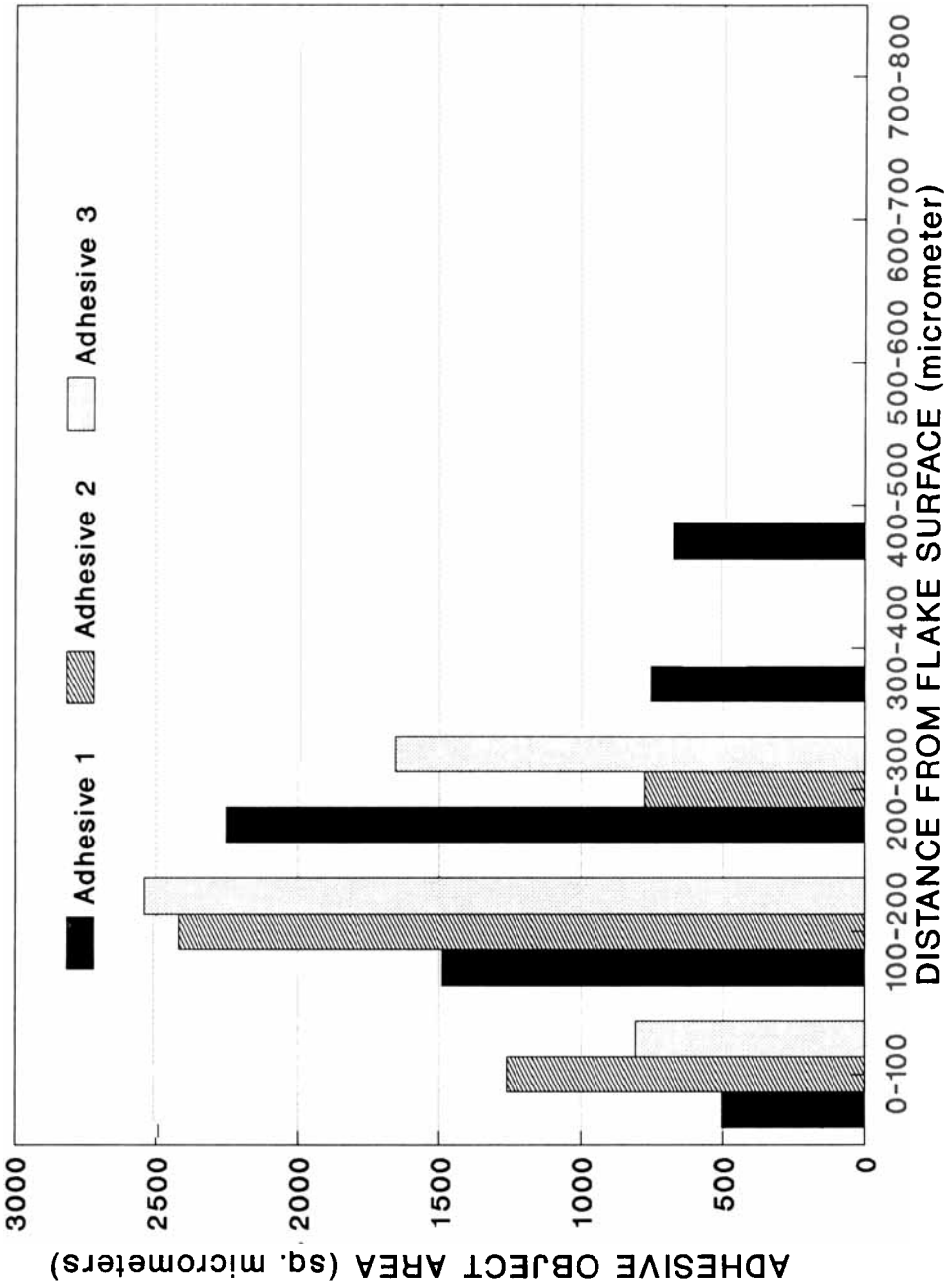


FIGURE 4 Mean adhesive object area as a function of distance from the flake surface.

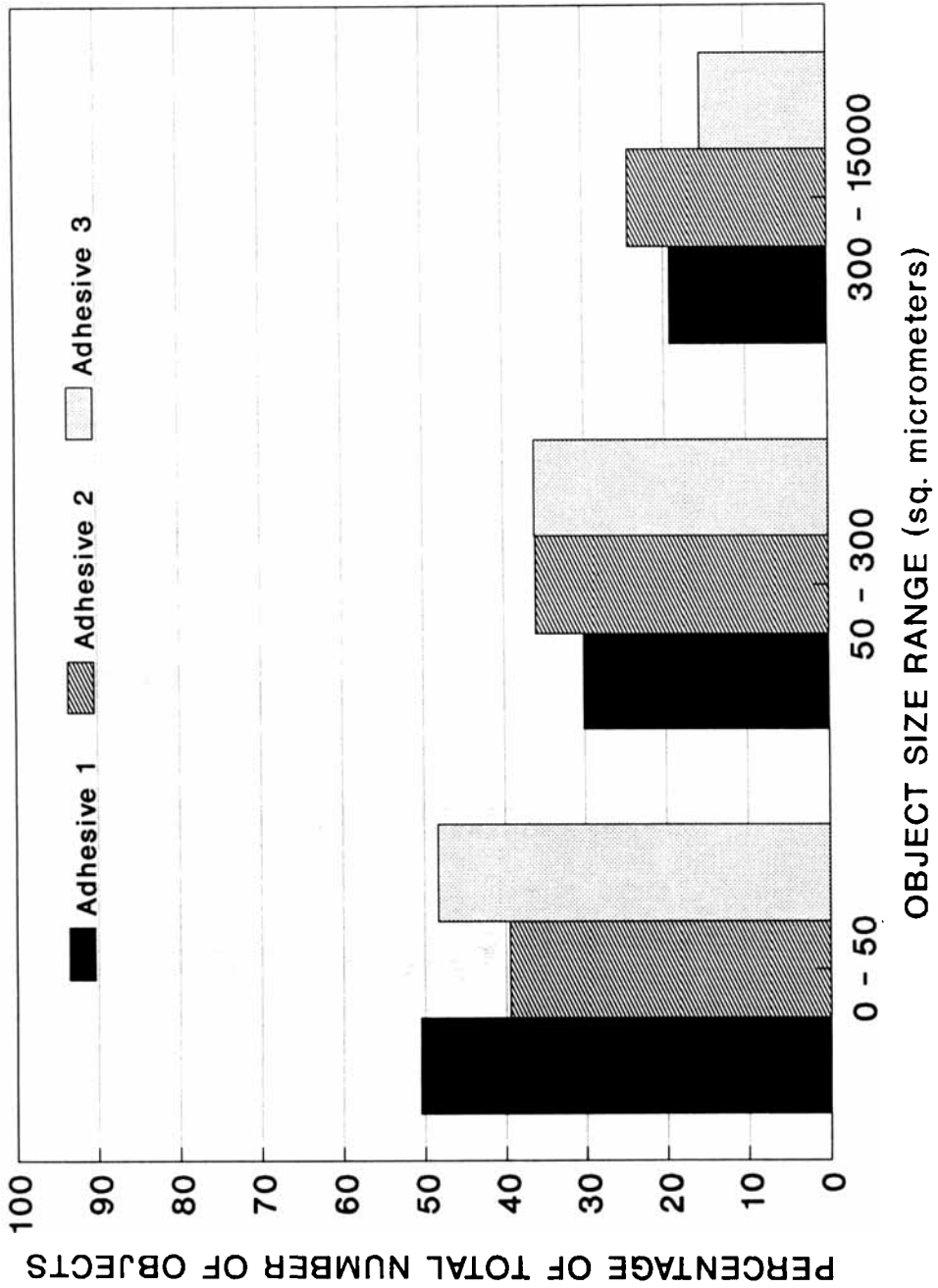


FIGURE 5 Percentage of total number of adhesive objects as grouped by adhesive object size ranges.

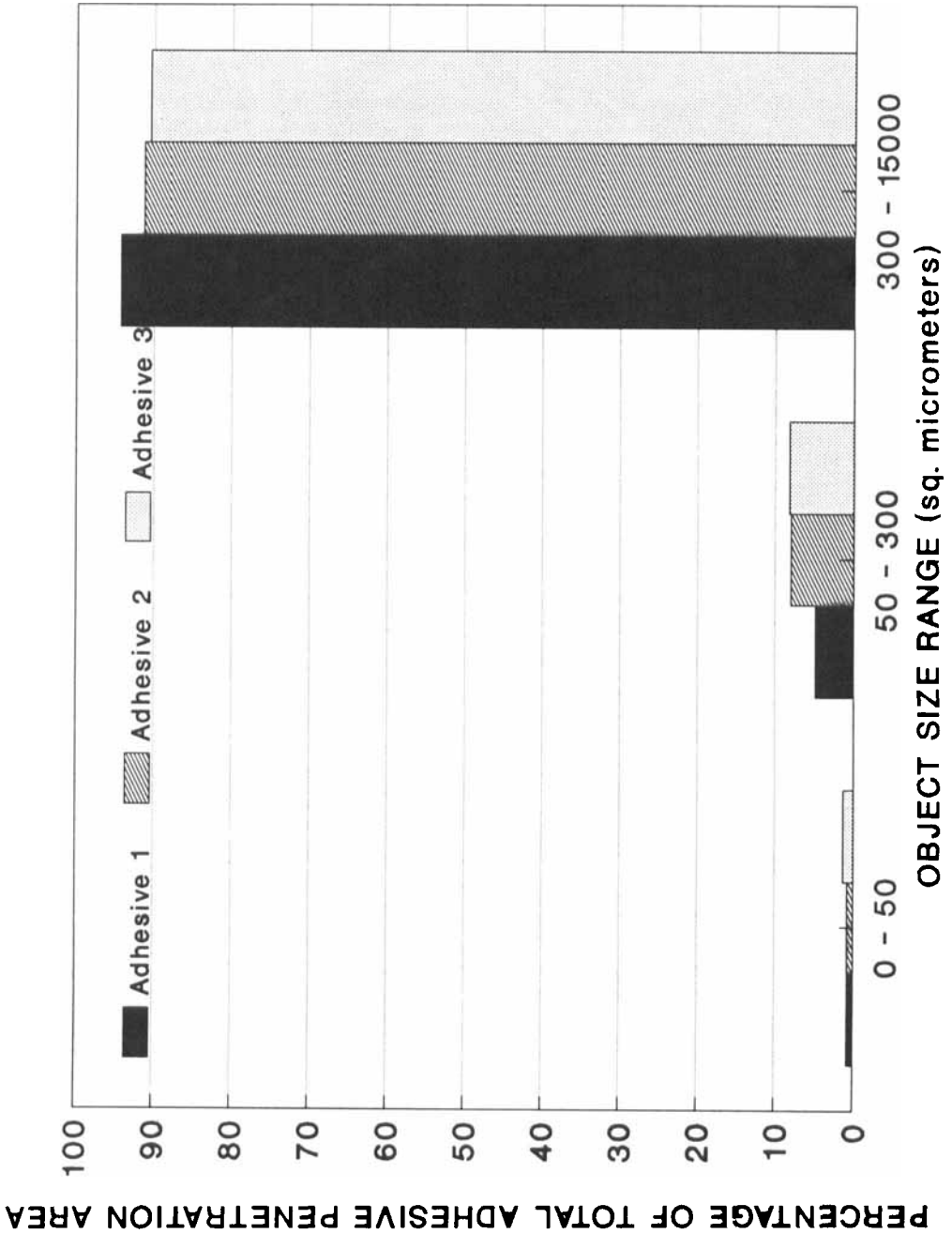


FIGURE 6 Percentage of total adhesive penetration area as grouped by adhesive object size ranges.

jects in the first group include partially-filled fiber or longitudinal parenchyma lumens, and any noise present in the image. The group from $50 \mu\text{m}^2$ to $300 \mu\text{m}^2$ would represent the majority of the completely-filled fiber cell lumens and any of the smaller number of longitudinal parenchyma lumens present. The majority of objects which have areas over $300 \mu\text{m}^2$ are partially or fully-filled vessel lumens and combinations of vessel lumens. A smaller number of adhesive-filled sections of the ray parenchyma lumens could be present in any one of the three size groups, although most of these objects would be larger than $50 \mu\text{m}^2$. Koch gives the relative proportions of *Liriodendron tulipifera* cell types by volume as follows: vessel elements (43.3%), fibers (42.6%), rays (11.4%), and longitudinal parenchyma (2.7%).¹⁴ Figure 2 identifies examples of adhesive-filled fiber, vessel, and ray parenchyma lumens. For all three adhesives, the vast majority of the number of adhesive objects is in the first two size ranges ($0\text{--}300 \mu\text{m}^2$). However, over 90% of the adhesive penetration area for all three adhesives is represented by the size range above $300 \mu\text{m}^2$. This indicates that vessels play a dominate role in adhesive flow in this wood species. Although the proportion of vessel elements and fibers by volume is similar, the ratio of void space to cell wall substance in vessels is much greater than in fibers. Vessel lumens are much larger than fiber lumens and permeable perforation plates between adjoining vessels facilitates greater flow of adhesive. Pit openings into fiber lumens are much more restrictive.

Limitations of Technique

The wood staining and image analysis technique described herein does have limitations. The type of adhesive used must fluoresce, and the species of wood must be able to retain enough stain to suppress its autofluorescence. Measurements, such as those to determine wood density, must necessarily be of wood in a saturated environment. Measurements are prone to a certain amount of subjective decision making, especially when visually picking threshold values for gray levels through which the computer analysis is made. This technique has sufficient resolution for measuring gross adhesive penetration, but another method must be used to detect cell wall penetration by the adhesive. Careful selection of mercury burner and excitor-barrier filter sets is necessary to optimize adhesive-to-wood contrast.

CONCLUSIONS

Staining techniques have been developed that can provide sharp contrast between an adhesive and the wood substrate. A fluorescence microscope and image analysis system can utilize this contrast to measure, quantitatively, gross adhesive penetration in wood. This technique was utilized to measure adhesive flow and penetration differences in wood as affected by adhesive molecular weight distribution. Adhesive penetration into wood flakes was shown to be correlated with the molecular weight distribution of the prepolymer, decreasing with higher weight average molecular weight. Gross adhesive penetration into hardwoods is likely to be dominated by flow into vessel elements, as demonstrated by the wood species studied here. Much

of the penetration of lower molecular weight liquid resole phenol formaldehyde adhesives, such as those used in the flakeboard industry, into wood likely takes place before the flakes are exposed to the hot-pressing environment. This measurement technique in combination with a method to measure cell wall penetration would be very useful for quantifying flow behavior of adhesives or coatings in wood.

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