Measurement of pore size distribution in a lamellar Bt horizon using epifluorescence microscopy and image analysis

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(Received January 15, 1991; accepted after revision March 26, 1991)

ABSTRACT


A staining technique suitable for epifluorescence microscopy of polished block surfaces was developed using proprietary dyes (Sanford Co.). The dyes greatly enhanced the contrast between particles and pores, thereby allowing, with only minimal processing, the production of images suitable for automated pore and particle size measurements.

An algorithm, the "multi-directional minimum chord" (MDMC) method, was developed for measurement of pore size as it relates to the hydraulic properties of soils. Pore chord lengths were measured in two or more directions through a set of points on a grid. The smallest dimension was retained as being most representative of the effective capillary diameter, or hydraulic diameter, of the pore. Chord length distributions measured by the MDMC method were compared to those obtained by the unidirectional chord intercept method.

These techniques were applied to a study of lamellar Bt horizon formation in sandy glacial outwash in the Anoka Sand Plain, Minnesota, U.S.A. The lamellae are nearly horizontal, occur from 0.6 to 2 m depth, and vary in thickness from 0.5 to 6 cm. It was hypothesized that differences in pore size distribution initially present in the sands caused changes in soil hydraulic properties that may have been responsible for the initial formation of lamellae. Undisturbed samples containing lamellae and the underlying interlamellar horizons were collected using Kubiena boxes, oven-dried at low temperature, cast in resin, sawed in half, polished, and stained. Digitized images of lamellae and the uppermost part of the interlamellar horizons were taken using epifluorescence microscopy and then edited to remove clays to simulate conditions present before the formation of the lamellae.

Pore and particle size distributions measured on the images by the MDMC method showed that significant differences existed between the lamellae and the uppermost part of the underlying interla-

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mellar horizons. In all cases a coarser over finer pore size discontinuity existed at the bottom of the lamellar horizon, in support of the proposed mechanism for the initiation of lamellae formation.

INTRODUCTION

Image analysis and image processing applications in soil science have gained increasing acceptance over the last few years. The development of sophisticated computer systems that provide fast, easy handling and processing of complicated data make these applications much more successful and attractive.

A number of soil properties have been studied by image analysis techniques. Significant contributions have been made in the study of pedological features (Murphy et al., 1977a, b; Bui and Mermut, 1987), soil structure and pore space analysis (Moran et al., 1989; Bui et al., 1989; Spaans et al., 1989), and root morphology (Ruark and Bockheim, 1988; Lebowitz, 1988). The soil materials analyzed included not only thin sections but also polished and unpolished resin-impregnated blocks, varying in size from a few cm$^2$ to several dm$^2$, as well as whole soil profiles (Grevers and de Jong, 1992).

The use of image analysis techniques to characterize soil pore space is finding growing acceptance. Traditionally, pore space has been described and evaluated visually in the field, estimated indirectly from measurements of various soil physical properties, or manually measured in thin sections. Visual evaluation is only qualitative and is limited by the resolution of the naked eye. Indirect estimation of pore space characteristics through measurements of soil physical properties fails in many cases to produce true values because of problems such as sample disturbance, especially in poorly aggregated sandy soils. Techniques using soil thin sections have been described by several workers (Murphy et al., 1977a, b; FitzPatrick, 1984; Bullock et al., 1985). Although these techniques are quantitative, the time and effort required to produce good-quality thin sections is considerable, especially if sufficient numbers of samples are required for statistical verification of results.

Polished block surfaces have also been used for pore space characterization (Ringrose-Voase and Nortcliff, 1987; Moran et al., 1989). Even though less mineralogical information can be obtained from polished blocks than from thin sections, their use in pore space characterization and plasma quantification can save considerable time and effort. Unlike thin sections, examination of polished blocks requires the use of epifluorescence microscopy, in which a short-wavelength light source (ultraviolet or blue) of high intensity is used as an excitation source and produces fluorescence of a longer wavelength. The nature of the fluorescence depends on the properties of the resin used for impregnation and on special treatments of the sample. Some resins are autofluorescent; others require the incorporation or surface application of special
dyes or stains to produce or increase their fluorescence. For details on this subject, the reader is referred to Altemüller and van Vliet-Lanoe (1990).

The hydraulic properties of a soil, particularly its water-holding and flow characteristics, are related to the pore structure of the soil. Due to the capillary effect, the smallest dimension of a pore is the most important dimension for water-energy relationships and water movement in porous media with nonuniform pores, such as soils (Bouma, 1977; Hillel, 1980; Toledo et al., 1989). This dimension is referred to as the effective capillary diameter because water-energy relations of pores can be predicted from pore dimensions through the capillary equation. For any point within a pore, the smallest diameter through that point, regardless of its orientation in the three-dimensional structure of the pore, is the effective capillary diameter, and half that distance is the hydraulic radius.

The method most widely used for measuring pore size by image analysis is the pore-chord intersection technique (Jongerius et al., 1972a, b; Ismail, 1975; Murphy et al., 1977a, b; Bullock and Thomasson, 1979; Yanuka and Elrick, 1985; Bui et al., 1989). In this technique, images are commonly scanned horizontally, either randomly or systematically, by a number of transect lines, and the chord lengths (the length from one side of the pore to the other along the transect line) intersected by pores are measured and tabulated into frequency distributions. This method is known for its simplicity and rapidity, but it does not accurately predict the hydraulic characteristics of soils because it does not find the minimum chord length at most points of measurement. Yanuka and Elrick (1985) reported that predictions of soil water permeability from chord length distribution determined by the horizontal chord length method were two orders of magnitude larger than the measured values unless calculations rejected measured chord lengths > 300 μm. Bui et al. (1989) also found that pore size distributions estimated from a soil moisture retention curve were not in good agreement with those determined on the same materials by image analysis using the horizontal chord technique.

To better approximate the pore dimensions that influence hydraulic properties, an algorithm was developed that measures the smallest chord lengths at a series of points within the pore space. By this algorithm, the chord length is measured in several different directions through a point within a given pore, and only the smallest chord length is retained as being most representative of the effective capillary diameter of the pore at that point. If measurements can be taken in three dimensions, this method will accurately measure the effective capillary radius of a pore. When measurements are restricted to two dimensions, as in images of thin sections or polished blocks, this algorithm may be expected to overestimate effective capillary diameter to some degree because it does not account for potentially shorter chord lengths in the third dimension. Even in two dimensions, however, it should more accurately measure the effective capillary diameter distribution of a soil than the unidirec-
tional, pore-chord intercept method does, and consequently be able to better predict its hydraulic properties.

Measurements made in two dimensions are related to the properties of the three-dimensional network from which the two-dimensional image was obtained (Weibel, 1980). Direct extrapolation of measurements taken in a two-dimensional image to three-dimensional space can only be achieved for some types of measurements, such as total porosity. The relationship between measurements taken in two and three dimensions for other types of measurements, such as particle size distribution, is less simple and less obvious. This is also the case with chord length distributions, where there is no known direct extrapolation between the chord length distribution measured in two dimensions and that existing in three dimensions.

A case study in soil genesis will be used here to illustrate the use of these methods. Some sandy soils (Psammentic Glossoboralfs) formed on glacial outwash in the Anoka Sand Plain, Minnesota, U.S.A., have nearly horizontal, distinct, iron- and clay-rich lamellae that occur from 0.6 to > 2 m depth and that vary in thickness from 0.5 to 6 cm. These lamellae display highly oriented, well-developed clay bridges between sand grains, a feature indicative of pedogenic origin. Studies of lamellae in similar soils have also shown a pedogenic origin.

We hypothesized that the outwash deposits were bedded, and that lamellae could have initially formed by clay deposition at particle size and pore size discontinuities in the sands. Outwash and other types of water-laid sediments are commonly deposited in graded beds, wherein the coarsest material occurs at the base of the bed and the material becomes gradually finer upward. This type of grading is called “normal” grading, as opposed to inverse grading, which is coarser upwards. Particle size discontinuities may occur at the contact between beds, where the finer, upper part of one bed contacts the coarser base of the overlying bed. Because particle size changes are commonly associated with changes in pore size distribution, and soil hydraulic properties change across pore size discontinuities, associated changes in flow could have caused the deposition of clays carried in suspension at the discontinuity. Once lamellae formation was initiated, the presence of the finer-textured material in the lamellae would further enhance the pore size discontinuity, thus providing a positive feedback mechanism for continued deposition and growth of lamellae. Likewise, any subtle textural stratification that might have been present before lamellae formation began, would be masked by the presence of clays subsequently deposited in the lamellae.

To test this hypothesis, image analysis techniques were used to measure and compare the porosity and pore size distribution of lamellae and the uppermost part of their underlying interlamellar horizons on images taken from resin-impregnated polished blocks by epifluorescence microscopy. In order to simulate the conditions present before the initial formation of the lamellae,
i.e., before illuviation began, the plasma was removed from the images by image processing and manual editing so that pore and particle size distributions could be measured on a clay-free basis.

The objectives of this study were: (1) to collect high-quality, digitized images of pores and sand grains from polished block faces of lamellar and interlamellar horizons; (2) to introduce a new method for the measurement of pore size by image analysis and compare the results obtained with this method to those obtained from the horizontal chord method; and (3) to determine if differences in pore size distribution existed between lamellae and the uppermost part of their underlying interlamellar horizons prior to the formation of the lamellae.

MATERIALS AND METHODS

Sample collection and preparation

Samples were collected from two pedons at two different sites in the Cedar Creek Natural History Area located on the Anoka Sand Plain near Bethel, Minnesota. One site was selected under a maple–basswood community and the other was selected under a red pine–white pine community. The pedons were described and classified using standard methods (Soil Survey Staff, 1975). Although both soils have been mapped as Alfis Udipsamments, the pedons described were reclassified as Psammemic Eutroboralfs. Numerous lamellae (Fig. 1) occur in these soils at depths between 0.6 and >2 m. The lamellae are nearly horizontal, vary in thickness from 0.5 to 6 cm, and have higher iron and clay contents than the intervening horizons. The slope was between 0 and 4% for both sites, and the depth to the water table was >3 m.

Undisturbed soil samples containing a lamella and the uppermost part of the underlying interlamellar horizon were collected from pedon faces using Kubiena boxes of 10×6×5 cm (Fig. 2). The samples were air-dried at room temperature, evacuated for 24 h at 30°C and for another 24 h at 40°C to ensure complete drying with minimal disturbance. The samples were impregnated with Scotchcast resin (3M Co., St. Paul, MN, U.S.A.) at 60°C under vacuum to ensure complete entry of the resin into all pores. This particular resin is autofluorescent. The impregnated samples were cured at 40°C for 3 to 4 days, and then sawed into blocks parallel to the face of the pit. One vertical face of each sample was polished on a lapping plate. Sample orientation was preserved throughout the study; thus, directional references in this manuscript are correct with respect to sample orientation prior to sample collection.
Fig. 1. The upper portion of a representative pedon from Site 1 showing the iron- and clay-rich lamellae (L). Although an irregular lamella is visible near the top of the profile (uppermost arrow), most lamellae were nearly level.

Epifluorescence microscopy

The soil blocks were examined by epifluorescence microscopy using an Olympus BH2 microscope equipped with a mercury vapor ultraviolet light source. Preliminary examination of unstained samples showed very poor contrast between the particles and the pore space. During this preliminary examination, however, it was observed that areas of the block surface that had been marked for sample identification with a permanent marker had much
Fig. 2. The face of an undisturbed soil sample collected in a Kubiena box (actual size 10 by 6 cm). A lamella and parts of the overlying and underlying interlamellar horizons are clearly visible. Note the level upper boundary and wavy lower boundary of the lamella.

better contrast than unmarked portions of the image. This serendipitous discovery led to the development of a surface staining technique that used ink extracts from either red or brown Sharpie® permanent markers (Sanford Co., Bellwood, IL, U.S.A.). These ink extracts contain a combination of the active dyes Solvent Red #109, Solvent Red #208, Solvent Brown #52, and Solvent Yellow #161. Because these dyes are proprietary to Sanford Co., we were unable to obtain their chemical names or formulas; however, small samples can be obtained directly from Sanford Co. Either stain was applied to the surface
of the polished blocks and left to dry for 3 to 4 min. The blocks were then washed with 95% ethanol and immediately dried with a stream of compressed air. Both stains enhanced the contrast of the images and produced similar results. The use of a blue filter further enhanced the contrast between particles and pores.

Image acquisition, digitization, and processing

Two representative soil blocks, each containing a lamella (L) and its underlying interlamellar (IL) horizon, were taken from two depths at each site. Three fields of view were randomly selected in each L and in the uppermost part (0 to 2 cm) of each associated IL horizon for a total of 24 images. The samples were observed under relatively low magnification (90×) because of their coarse texture, thereby allowing observation of large-scale interconnections of the pore complex which would have been difficult to observe at higher magnification. The images were acquired directly from the microscope using a fluorescence-sensitive video camera (SIT, Series 66), and then digitized (256 grey levels) and filtered by a Mass Comp computer equipped with a high-resolution monitor (International Imaging System). Each image consisted of a 512×512 pixel array. At 90× magnification, the pixel size was equivalent to 3.8×3.8 μm and the overall field of view was approximately 2×2 mm.

The digitized images were transferred to a Macintosh IIci workstation equipped with a public-domain image-processing program, IMAGE, Ver. 1.31p.* The images were initially processed to enhance contrast and reduce noise, thereby allowing a better distinction between skeletal grains, plasma, and pore space.

Image analysis

To test the hypothesis that a discontinuity in pore size distribution existed at the lamellar-interlamellar boundary before the initiation of lamellae formation, minimum chord length distributions of the lamellae and the uppermost part of the underlying interlamellar horizons were compared on a clay-free basis to simulate the initial conditions of their formation, i.e., before there was any deposition of clays in the current lamellar horizons. Pore infillings, clay coatings, and clay bridges were removed from the image by thresholding and manual editing of the grey-scale images using IMAGE. Following this step, the images were segmented to produce binary digital images composed essentially of skeletal grains (black) and pore space (white). The final binary

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images were visually compared to the original grey-scale images to ensure that artifacts or distortions were not introduced during processing and editing.

Pore and particle size measurements

A new method for measurement of pore size, herein referred to as the “multidirectional minimum chord” (MDMC) method, was developed. A grid was laid over the image, and at each grid line intersection occurring in a pore, the lengths of chords through the point within the pore were measured in two or more directions (Fig. 3). For two directions, the chords ran N–S and E–W. For four directions, NW–SE and NE–SW directions were added. Additional directions may be added to expand the method to 8, 16, or more directions. The smallest chord length was retained as being most representative of the effective capillary diameter or hydraulic diameter of the pore at that point. If any chord through a point intersected an edge of the image, that point was excluded from the results. The output data were transferred to a spreadsheet for statistical evaluation. The MDMC algorithm has been coded as a subroutine in Pascal; copies are available at no cost from the second author.

The number of points at which pore chord lengths were measured was varied in order to determine the sampling density required to yield consistent and representative results. Spacings of 10, 15, 25, and 35 pixels between grid lines yielded 2601 (51×51), 1156 (34×34), 400 (20×20), and 196 (14×14) grid intersections per image, respectively. The total number of pore

Fig. 3. A magnified portion of a binary image illustrating the four directions in which pore chord lengths were measured using the four-direction MDMC method.
measurements taken per image was approximately equal to the number of
grid intersections multiplied by the porosity minus those points excluded be-
cause their chords intersected the edge of the image.

The total porosity of the images was calculated both before and after re-
moval of plasma by image processing and manual editing, and the percentage
of plasma was determined by difference. Clay and silt contents were deter-
mimed by the pipet method (Gee and Bauder, 1986) and sand particle size
fractions by sieving. Iron and aluminum oxides were removed by the citrate-
bicarbonate–dithionite (CBD) method (Jackson, 1969) and estimated from
the concentrations of Fe and Al determined by atomic absorption on the ex-
tracts. The plasma was assumed to consist of clay plus silt plus sesquioxides,
and this sum was compared to the results of plasma analysis obtained by im-
age analysis.

Particle size was also determined using IMAGE. Some manual editing was
required to completely separate individual sand-sized grains in the images.
Individual particle areas measured on the clay-free images were used to cal-
culate the diameter and mass of each particle, assuming that the grains were
spherical. The diameter, \( d \), of each particle was \( d = 2 \text{(area/}\pi)^{1/2} \). The mass
of the grains was calculated assuming a uniform particle density, and grain
frequencies were tabulated into size classes similar to those of the sand sieve
data.

Statistical comparisons between chord length distributions were performed
using the Kolmogorov-Smirnov (K-S) test (Press et al., 1986). This test is
useful when comparing cumulative distributions that are functions of a single
independent variable. It poses as its null hypothesis that two data sets were
drawn from the same parent distribution. The rejection of the null hypothesis
indicates that two cumulative frequencies are significantly different. A level
of statistical certainty is established based on the number of observations and
on the maximum value of the absolute difference between the two cumulative
distribution functions.

RESULTS AND DISCUSSION

Staining technique

A fluorescence micrograph of an interlamellar horizon is presented in Fig.
4. The contrast between particles and pore space in the unstained (right) por-
tion of the image was so poor as to make image analysis very difficult. The
poor contrast and resolution were mainly due to emanation of fluorescence
from below the surface due to the translucent nature of quartz grains, which
comprised more than 70% of the sand fraction of these samples. The stained
(left) portion of the image (Fig. 4) shows greatly increased contrast between
the solid phase and pore space, eliminating much of the need for preprocess-
Fig. 4. A fluorescence micrograph showing the difference in contrast observed on stained (left) and unstained (right) portions of a polished block face cut from an interlamellar horizon. A blue filter was used to enhance image contrast. Photographic print "dodging" techniques were used to approximately equalize the light intensity on both halves of the photographic print.

ing before image analysis. The use of a blue filter increased the contrast over that observed in the unfiltered image and made the preprocessing step easier.

This staining technique allowed the user to easily discriminate between sand particles (dark brown), clays (bright red), and pores (light yellow). When observed through a blue filter, however, the contrast between clays and pores was greatly decreased. This effect was particularly useful in this study, because we wanted to be able to remove the clays from the image. In many cases, the reduction of contrast between the clays and pore space was sufficient to allow the removal of most of the clays from the image by thresholding, thereby eliminating much of the manual editing that would have otherwise been required.

Image analysis

With the exception of sample pairs L2 and IL2, the L horizons have higher total porosities, on a clay-free basis, than the uppermost part of the underlying IL horizons (Table 1). The total plasma contents, which were calculated
TABLE 1

Measurements of total porosity determined by image analysis, and plasma contents determined by difference and by the sum of pipet method plus CBD-extractable sesquioxides

<table>
<thead>
<tr>
<th>Sample</th>
<th>Depth (cm)</th>
<th>Total porosity by image analysis (%)</th>
<th>Plasma (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>before clay removal</td>
<td>after clay removal</td>
</tr>
<tr>
<td>Site 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L 1</td>
<td>88-90</td>
<td>39.7</td>
<td>47.7</td>
</tr>
<tr>
<td>IL 1</td>
<td>90-98</td>
<td>37.1</td>
<td>42.0</td>
</tr>
<tr>
<td>L 2</td>
<td>115-120</td>
<td>36.0</td>
<td>45.0</td>
</tr>
<tr>
<td>IL 2</td>
<td>120-134</td>
<td>39.2</td>
<td>45.3</td>
</tr>
<tr>
<td>Site 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L 3</td>
<td>110-112</td>
<td>42.7</td>
<td>50.7</td>
</tr>
<tr>
<td>IL 3</td>
<td>112-140</td>
<td>35.7</td>
<td>43.0</td>
</tr>
<tr>
<td>L 4</td>
<td>158-161</td>
<td>33.7</td>
<td>46.7</td>
</tr>
<tr>
<td>IL 4</td>
<td>161-170</td>
<td>34.9</td>
<td>41.7</td>
</tr>
<tr>
<td>Pooled standard deviation</td>
<td>1.9</td>
<td>0.9</td>
<td>1.9</td>
</tr>
</tbody>
</table>

*aPlasma = total porosity after plasma removal minus total porosity before plasma removal from images.
*bSilt and clay determined by the pipet method, sesquioxides by the citrate-bicarbonate-dithionite method.
*L = lamella; IL = interlamellar horizon.

Fig. 5. Pore chord length distributions determined by the four-direction MDMC using 2601, 1156, 400 and 196 grid intersections for an interlamellar horizon.

as the difference between the two porosities, are in good agreement with the sum of clay plus silt plus sesquioxide contents as determined by the pipet method (Gee and Bauder, 1986) and the CBD method (Jackson, 1969).
Pore size

Multiple measurements on the same image permitted direct comparisons among algorithm results. A four-direction MDMC algorithm and four grid densities were used to determine the minimum sampling density for reproducible and statistically valid results. Sampling grids of 2601, 1156, 400 and 196 points (Fig. 5) were tested; however, the actual number of points measured on an image was less, being equal to the total number of grid points times the porosity minus those points excluded because their chords intersected the edge of the image. No statistical differences \( P > 0.87 \) in pore size distribution, expressed as cumulative minimum chord length frequency, were observed among the sampling densities tested; however, the frequency distribution curves were less smooth at smaller sampling densities, particularly for the 196-point grid. The densest grid was used for all other analyses reported in this study in order to provide large numbers of observations for statistical accuracy.

Chord length distributions were measured by the unidirectional chord intersection technique for both horizontal and vertical transects, by the two-direction MDMC technique, and by the four-direction MDMC technique illustrated in Fig. 3. As expected, comparisons between these methods (Fig. 6) showed that the longest chord length distribution was measured by the unidirectional chord intersection method. Differences were present throughout the range of pore sizes observed, but they were particularly large for larger pore sizes. The maximum chord length measured by the unidirectional chord intersection technique was approximately twice that measured by the four-direction MDMC method. Significant differences \( P < 0.0001 \) were found between the unidirectional chord intersection and four-direction MDMC methods by the K-S test.

![Fig. 6. A comparison of pore chord length distributions of a lamellar horizon determined by the unidirectional (both horizontal and vertical) chord intersection method and the two- and four-direction MDMC method.](image-url)
The minimum chord length distribution measured by the two-direction MDMC method was also longer than that measured by the four-direction MDMC method (Fig. 6). The results were nearly identical for smaller pore sizes, and diverged only at larger pore sizes. The K-S test demonstrated that the minimum chord length distributions determined by the two methods were not statistically different ($P=0.404$), indicating that the two-direction MDMC method was sufficiently accurate for this task. In less isotropic soils, such as heavy-textured soils, in which the pores possess some degree of orientation, measurement of chord lengths in more than two directions may be necessary. The use of more than two chord directions may also be required in images having larger pore size to pixel size ratios or less uniform pore shapes.

Chord length distributions measured in both horizontal and vertical directions by the unidirectional chord intersection technique were nearly identical for pore chord lengths less than 300 $\mu$m (Fig. 6). For larger pores, the horizontal chord length distributions were significantly longer than the vertical chord lengths ($P=0.028$), indicating a degree of anisotropy.

Minimum chord length distributions were measured in three fields of view for each L and IL sample. Statistical analyses showed that there were no significant within-horizon differences ($P>0.1$) between different fields of view in any one horizon (Fig. 7), indicating that the fields of view were representative of the samples from which they were selected. However, differences in minimum chord length distributions between L and IL horizons were apparent (Fig. 7). Cumulative chord length distributions measured in the L and IL horizons are compared in Fig. 8. Each curve is a weighted average of the data collected from three fields of view in the sample and includes approximately 3000 measurements. The results show that the L horizons and the uppermost part of the underlying IL horizons have significantly different ($P<0.0001$) minimum chord length frequency distributions, with the uppermost part of
Fig. 8. Minimum pore chord length distributions of paired lamellar and interlamellar horizons from two depths for each site (site 1 and site 2) determined by the four-direction MDMC method.
the IL horizons having finer pores. For example, in the chord length range of 75 to 250 μm, the IL samples have 10 to 18% higher cumulative frequency values than their associated L samples (Fig. 8). Between the median and the third quartile of each pair of curves, the difference in terms of pore size between the L and the IL ranges between 30 and 60 μm for the four pairs of samples, with an overall average difference of about 40 μm.

The pore size distribution data indicate that a coarser-over-finer pore size discontinuity initially existed at the base of the lamellae, in agreement with the proposed hypothesis. These data do not necessarily imply, however, that the part of the interlamellar horizon lying directly above each lamella also has a finer pore size distribution than that initially present in the lamella. Rather, it is more likely that the pore size of the interlamellar horizons may become increasingly finer upward from the top of one lamella to the bottom of the overlying lamella, as would be expected for normally graded beds.

The observed differences in pore size distribution measured at the base of the lamellar horizons suggest that changes in water-holding capacities and flow characteristics occurred at the discontinuity (Hillel, 1980; Hanks and Ashcroft, 1986) and may have been involved in the formation of the lamellae. Presumably, dispersed clays and sesquioxides were deposited by sedimentation at the discontinuity, thus initiating the formation of the lamellae.

**Particle size**

The results of particle size distribution measurements made by image analysis are presented in Table 2 where they are compared to distributions determined by dry sieving. A relative comparison of particle size distributions determined by image analysis shows that, in general, the L horizons have less material in the 0.1–0.25 mm size fraction and slightly more material in the 0.25–0.5 mm size fraction than the IL horizons. These results support those obtained from pore chord length distribution, as the coarser textured L horizons have larger pore size distributions.

A comparison of grain size distributions determined by image analysis with those determined by sieving shows that the two methods do not agree very well (Table 2). Image analyses tend to underestimate grain diameters relative to sieve diameters. A similar discrepancy between the two methods was encountered by Bui et al. (1989). The observed discrepancy can mostly be attributed to underestimation of grain diameters because the two-dimensional section did not necessarily cut through the center of gravity of the grains (Friedman, 1958), and possibly because the assumption of sphericity was unwarranted.

The results of particle size measurements were more variable (pooled variance for various size fractions ranged from 13 to 32%) than results obtained for chord length distributions. The total number of particle observations
### TABLE 2

Sand size distributions of lamellar and interlamellar horizons

<table>
<thead>
<tr>
<th>Sample Depth (cm)</th>
<th>Sample Size</th>
<th>Sand size (mm) distribution (%) by sieving</th>
<th>by image analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2–1 1–0.5 0.5–0.25 0.25–0.1 0.1–0.05</td>
<td>2–1 1–0.5 0.5–0.25 0.25–0.1 0.1–0.05</td>
</tr>
<tr>
<td>Site 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L 1*</td>
<td>88–90</td>
<td>0.2 1.2 23.2 58.2 17.2 0 0 32.0 66.2 2.0</td>
<td></td>
</tr>
<tr>
<td>IL 1*</td>
<td>90–98</td>
<td>0.1 0.7 21.0 60.3 17.9 0 0 11.0 84.6 4.4</td>
<td></td>
</tr>
<tr>
<td>L 2</td>
<td>115–120</td>
<td>0.0 1.0 25.2 55.8 18.0 0 0 33.9 65.3 0.8</td>
<td></td>
</tr>
<tr>
<td>IL 2</td>
<td>120–134</td>
<td>0.1 0.6 19.4 61.5 18.3 0 0 8.6 88.2 3.1</td>
<td></td>
</tr>
<tr>
<td>Site 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>L 3</td>
<td>110–112</td>
<td>0.1 0.6 24.6 56.6 18.2 0 0 20.4 78.3 1.2</td>
<td></td>
</tr>
<tr>
<td>IL 3</td>
<td>112–140</td>
<td>0.1 0.5 25.0 58.8 15.5 0 0 15.5 82.1 2.5</td>
<td></td>
</tr>
<tr>
<td>L 4</td>
<td>158–161</td>
<td>0.0 0.9 23.8 58.0 17.3 0 0 33.7 65.7 0.6</td>
<td></td>
</tr>
<tr>
<td>IL 4</td>
<td>161–170</td>
<td>0.1 0.9 24.4 59.5 15.1 0 0 15.6 81.7 2.7</td>
<td></td>
</tr>
<tr>
<td>Pooled standard deviation</td>
<td></td>
<td>0 0 6.8 9.6 0.6</td>
<td></td>
</tr>
</tbody>
</table>

*L = lamella; IL = interlamellar horizon.

(~ 120 for three fields of view) was smaller than the number of chord lengths measured (~ 225 for three fields of view at a grid size of 196 points, proportionately higher for higher grid densities), however, suggesting that a greater number of fields of view, or larger sampling areas, were needed in order to generate sufficient data to reduce variability and allow statistically verifiable comparisons between different particle size distributions.

### CONCLUSIONS

The staining technique used in this study greatly enhanced the contrast between skeleton grains and pore space observed in polished blocks of sandy soil examined by epifluorescence microscopy. The improvement in image quality allowed the production of images suitable for pore size measurements with a minimum of additional processing. The relative contrast observed between different parts of the image (skeleton grains, plasma, and pore space) varied markedly depending on the wavelengths allowed for observation.

An algorithm, named the “multi-directional minimum chord” (MDMC) method, was developed to measure pore size as it relates to the effective capillary diameter or hydraulic radius more accurately than the horizontal chord intercept method. This algorithm should allow more accurate estimation of the effective capillary diameter, or hydraulic radius, of soil pores to provide better predictions of the hydraulic properties of soils. The algorithm can be easily extended to three dimensions, if appropriate images are available.
In a study of the formation of a lamellar Bt horizon in glacial outwash sands, the MDMC method was used to measure pore size distributions in images of lamellae and the uppermost part of their underlying interlamellar horizons obtained from polished blocks using epifluorescence microscopy. The results, determined on a clay-free basis, demonstrated that a significant difference in pore size distribution initially existed at the base of the lamellar horizons, with the upper part of the interlamellar horizons having finer pores. These data support the hypothesis that changes in water-holding capacities and flow characteristics at the pore size discontinuity may have initiated the formation of the lamellae. Once initiated, the deposition of clay would further enhance the pore size discontinuity, thus providing a positive feedback mechanism for continuing growth of the lamellae. These results are not conclusive, however, but are presented mainly to demonstrate the applicability of image analysis and the MDMC method to problems in soil genesis. A more definitive study of the mechanism of lamellae formation will require the analysis of pore size distributions along a vertical transect across one or more lamellae, from one interlamellar horizon to another.

ACKNOWLEDGEMENTS

The authors are grateful to Dr. Ahmet R. Mermut for his support and help during the preparation of samples for analysis, and to Dr. Michael L. Thompson for a careful and thought-provoking review of the manuscript. The authors thank Carter Carpenter for technical assistance with image analysis, Dr. H. W. Rines for providing us with IMAGE, and Dr. David A. Laird for stimulating and profitable discussions. Some financial support was provided by the USDA-ARS.

Scientific Journal Series Paper No. 18818, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul, MN 55108, U.S.A.

REFERENCES


and characterization of microporosity by image analysis and comparison with data from
book of Soil Thin Section Description. Value Research Publications, Alberington, Wolver-
hampton, 152 pp.
433 pp.
Friedman, G.M., 1958. Determination of sieve-size distribution from thin section data for sed-
Gee, G.W. and Bauder, J.W., 1986. Particle size analysis. In: A. Klute (Editor), Methods of
Soil Analysis, Part I. Agronomy Monograph 9, Soil Science Society of America, Madison,
Grevers, M.C.J. and de Jong, E., 1992. Soil structure changes in subsoiled Solonetoxic and Cher-
nozemic soils measured by image analysis. In: A.R. Mermut and L.D. Norton (Editors),
Digitization, Processing and Quantitative Interpretation of Image Analysis. Geoderma, 53:
289–307, this issue.
Ismail, S.N.A., 1975. Micromorphometric soil-porosity characterization by means of electro-
optical image analysis (Quantimet 720). Soil Survey Papers 9, Netherlands Soil Survey In-
inute, Wageningen, 104 pp.
Jackson, M.L., 1969. Soil Chemical Analysis, Advanced Course. 2nd ed., published by the au-
thor, Madison, WI, 895 pp.
Jongerius, A., Schoonderbeek, D. and Jager, A., 1972a. The application of the Quantimet 720
Jongerius, A., Schoonderbeek, D., Jager, A. and Kowalinski, St., 1972b. Electro-optical soil-
921–928.
Murphy, C.P., Bullock, P. and Turner, R.H., 1977a. The measurement and characterization of
voids in soil thin sections by image analysis, I. Principles and techniques. J. Soil Sci., 28:
498–508.
Murphy, C.P., Bullock, P. and Biswell, K.J., 1977b. The measurement and characterization of
Ringrose-Voase, A.J. and Nortcliff, S., 1987. The application of stereology to the estimation of
soil structural properties: A preview. In: N. Federoff et al. (Editors), Soil Micromorphology,
Ruark, G.A. and Bockheim, J.G., 1988. Digital image analysis applied to soil profiles for esti-
Soil Survey Staff, 1975. Soil Taxonomy: A Basic System for Soil Classification for Mapping and

