Densitometric Applications of a Flatbed Image Scanner

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Numerous flatbed scanners have been made for use in the desktop publishing industry.[1] Many of these have at least eight bits (256 levels) of grey scale capability. Some are suitable for use as scanning densitometers. The Hewlett-Packard ScanJet Plus™ was chosen for this work primarily because of its low cost, quality, ready availability and wide applicability among computers.

The Scanner

The Hewlett-Packard ScanJet Plus is approximately 345 x 480 x 94 mm (13.6 x 18.9 x 3.7 in). Beneath the scanner's lid is a glass platen upon which is placed the image to be scanned. During a scan, a fluorescent lamp is lit and moved beneath the glass platen. Simultaneously, a photodetector, lens and mirror system are moved beneath the platen to scan the image one line at a time. The image is transmitted from the scanner on a line-by-line basis to the host computer. The scanner and computer are under the control of a data acquisition program. Eight-bit data representative of reflectance of light from the image are saved to disk. The resolution of the scanner is nominally 300 pixels per inch (11.81 pixels/mm).

A cross-sectional view of the scanner is shown in Fig. 1. Light is emitted from the lamp at an angle of 45 degrees to the platen and the white, reflective surface located on the underside of the scanner's lid. The photodetector is positioned to read light reflected at an angle of 90 degrees with respect to the platen. When no light absorbing object is interposed between the lamp and the CCD, the scanner's output is calibrated to indicate a reflectance of 100%. When all the light directed toward the reflective surface is absorbed, the scanner will indicate a reflectance of 0%.

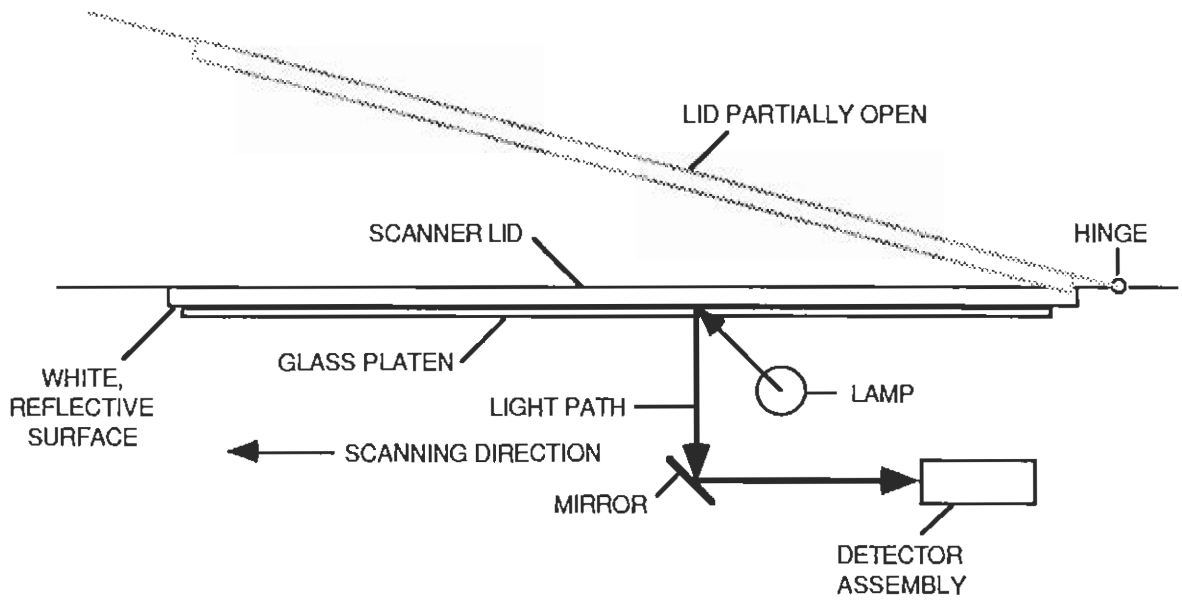

Fig. 1. Flat Bed Scanner, Cross-Sectional View

The Data Acquisition Program

The data acquisition program provided with the scanner's computer interface operates in two modes. A low resolution "preview scan" is usually done first. The user then has the option of rescanning all or part of the image at a higher resolution. This latter feature is of importance in this work. Images are stored on the disk in the standard, well-documented tagged image file format (TIFF)[2]. The numbers stored on the disk are representative of reflectance, or intensity. Image parameters such as contrast, brightness, resolution and scaling are available to the user. For the analyses considered here, we recommend setting both brightness and contrast near 50%. This will ensure that the final image is linear with reflectance.[3]

Application of the Scanner: Reflectance Images

Images on photographic paper and other opaque, reflective materials can be analyzed directly. During the preview scan phase, an image of the entire platen is shown on the computer's screen. The computer's mouse is used to select a rectangular region containing a line or lines to be quantified. The final image scan is then made and saved to disk.

Densitometric quantitation generally requires data in the form of optical density (O.D.), rather than reflectance. This requires a simple manipulation of the data saved from each scan. This manipulation is stored with each image in the form of a "photometric interpretation tag" (PIT), one of the "tags" in the TIFF file. The (PIT) is simply a look-up table in which reflectance values are stored with corresponding optical density values. The (PIT) provided with the scanner used in this work converts only numbers indicative of reflectance between 1 and 100%. Numbers below 1% are truncated. See Fig. 2 for a plot of the (PIT).

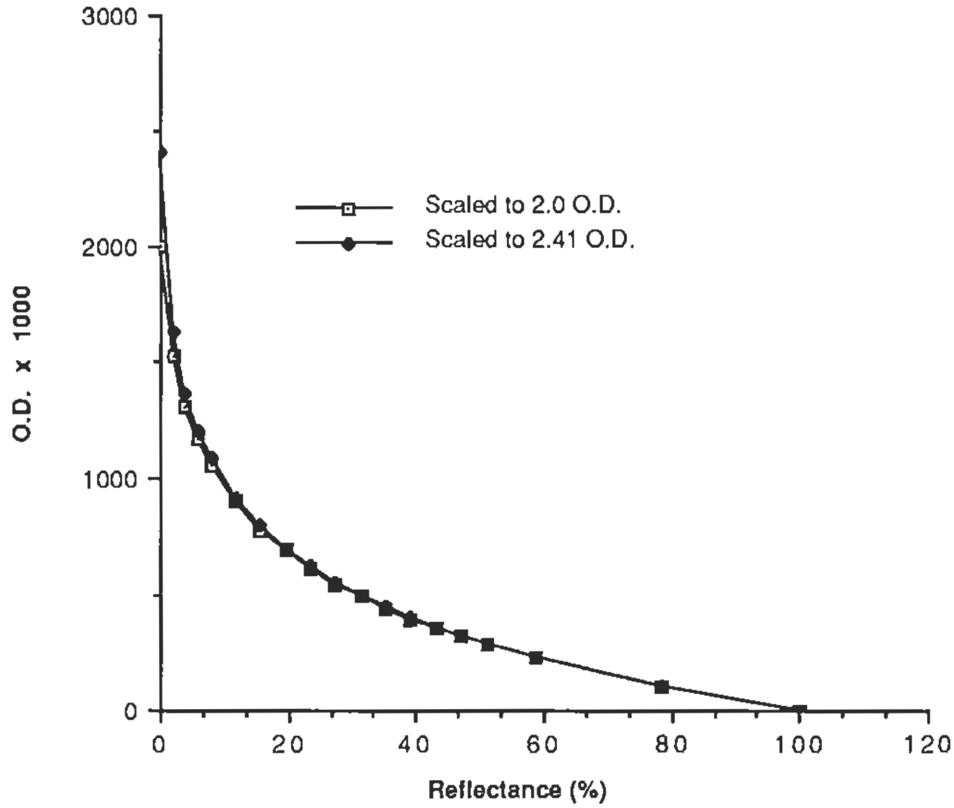

Fig. 2. Photometric Interpretation Tag (PIT)

A dynamic range of 255:1 represents an optical density range of 2.41:1. A more precise table can be generated, but we have found it to be of little additional value for two reasons: the two tables differ by a negligible amount at reflectances greater than 1%, and only a few bits of amplitude resolution are available for reflectances less than 1%.

A calibrated, grey scale reflectance step wedge was scanned and an image saved. (See Appendix.) The measured and actual O.D. values were compared. The result is shown in Fig. 3. "Full Scale" in the plot refers to the O.D. value obtained for the darkest part of the image. Scanned O.D. values are linear with the calibrated values up to an O.D. of approximately 1.61.

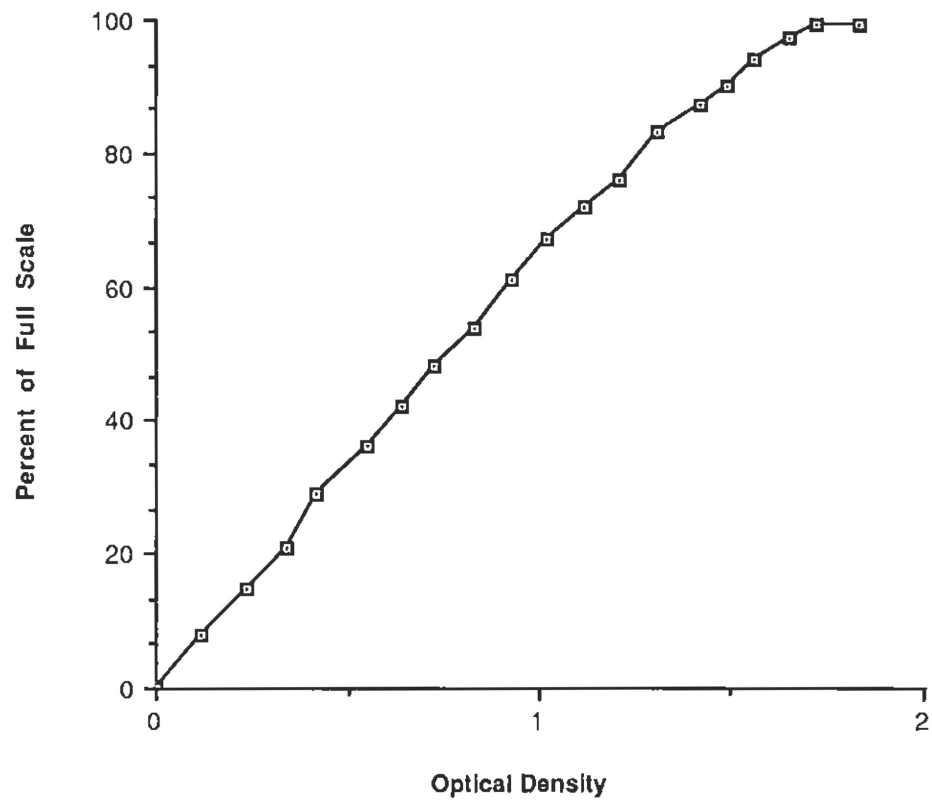

Fig. 3. The Reflectance Grey Scale

Application of the Scanner: Transmittance Images

Autoradiograms, other images saved on photographic film, and stained gels can also be analyzed provided certain limitations are observed. A calibrated, photographic film grey scale step wedge was scanned and an image saved. A plot of measured and calibrated values of optical density for the film strip is shown in Fig. 4. Note that the calibrated and measured values are linear for O.D. values below about 0.81.

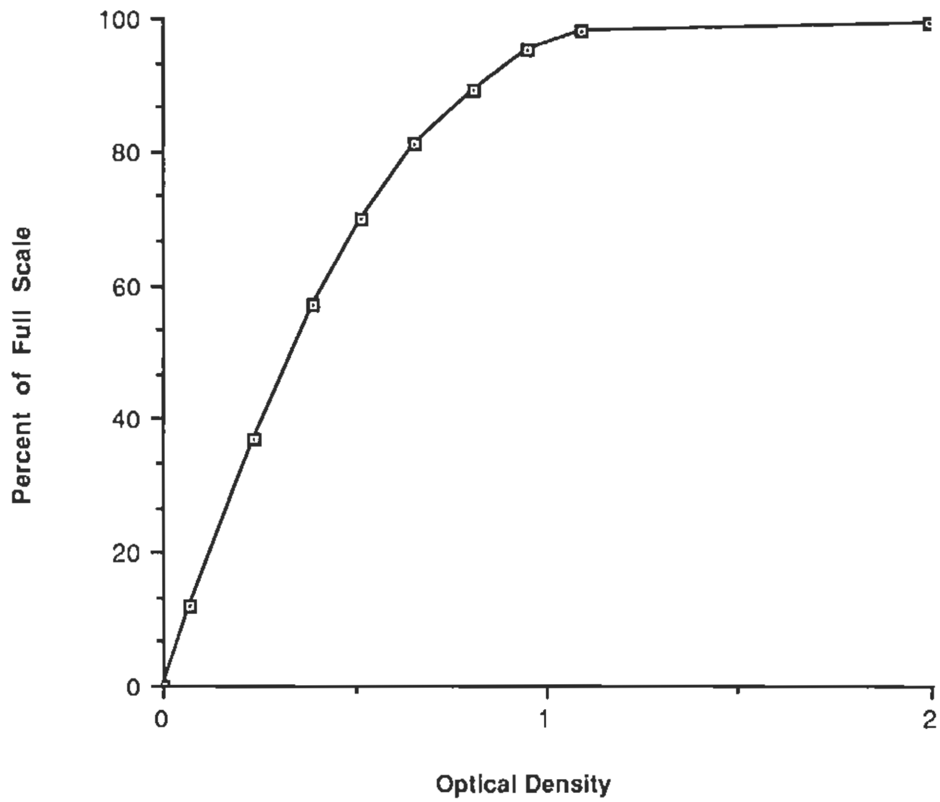

Fig. 4. The Transmittance Grey Scale

A reflectance of 100% corresponds to an optical density of zero. A reflectance of 1% corresponds to an optical density of 2.0. Because light from the lamp must travel through the film strip twice, the measured optical density is twice the actual O.D. of the film at any point. Thus since the scanner is limited to an optical density range of 1.6 in reflectance, it will be limited to an O.D. range of 0.8 in transmittance.

Another limitation on transmittance images is the possibility of parallax. If the thickness of the object being scanned is small compared to the extent of features in the image, no parallax errors will result. Images from photographic film, dried gels and other images saved on relatively thin media will not exhibit parallax.

In the case of a wet gel, more care must be taken. Parallax can be minimized by orienting the greater extent of a feature parallel with the scanning direction. Thus in the case of electrophoresis gels, the bands should be oriented so that they are parllel to the scanning direction. The degree of parallax which can be tolerated must be determined by the user. The preferred orientation is shown in Fig. 5.

1-D Analysis Software

The pictorial data in a TIFF file must be converted to data suitable for use by 1-D analysis software. An accessory program to the P-Scan1D software converts data contained in rectangular TIFF files to single line scans suitable for one-dimensional analysis.

A typical low-resolution, pre-scan from the ScanJet Plus is shown diagrammatically in Fig. 5. A region of interest is selected.

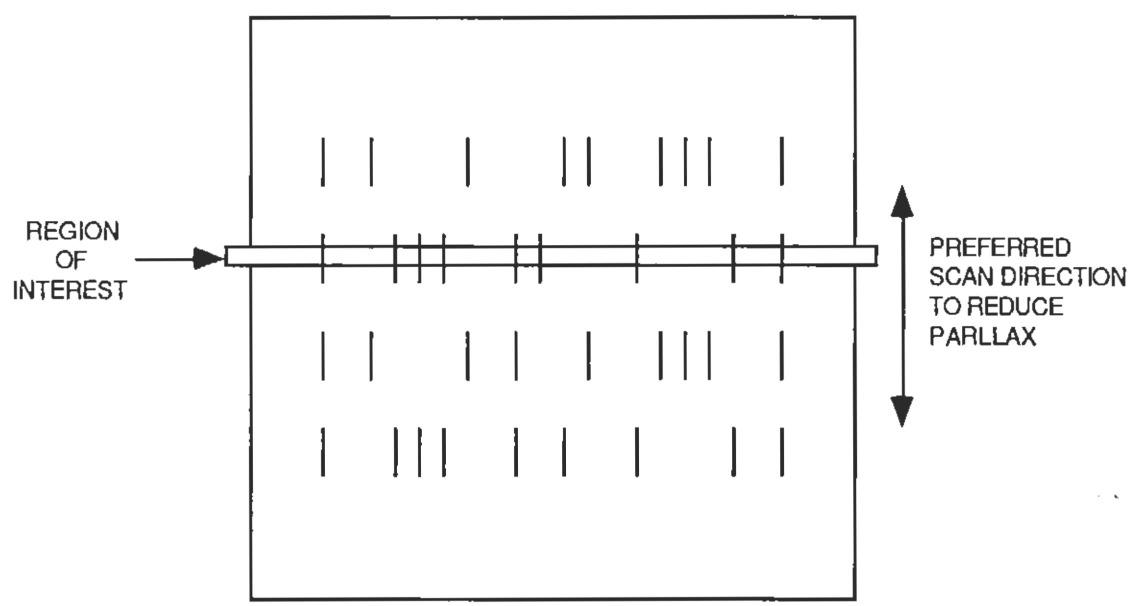

Fig. 5. A Full Scan With Region of Interest Outlined

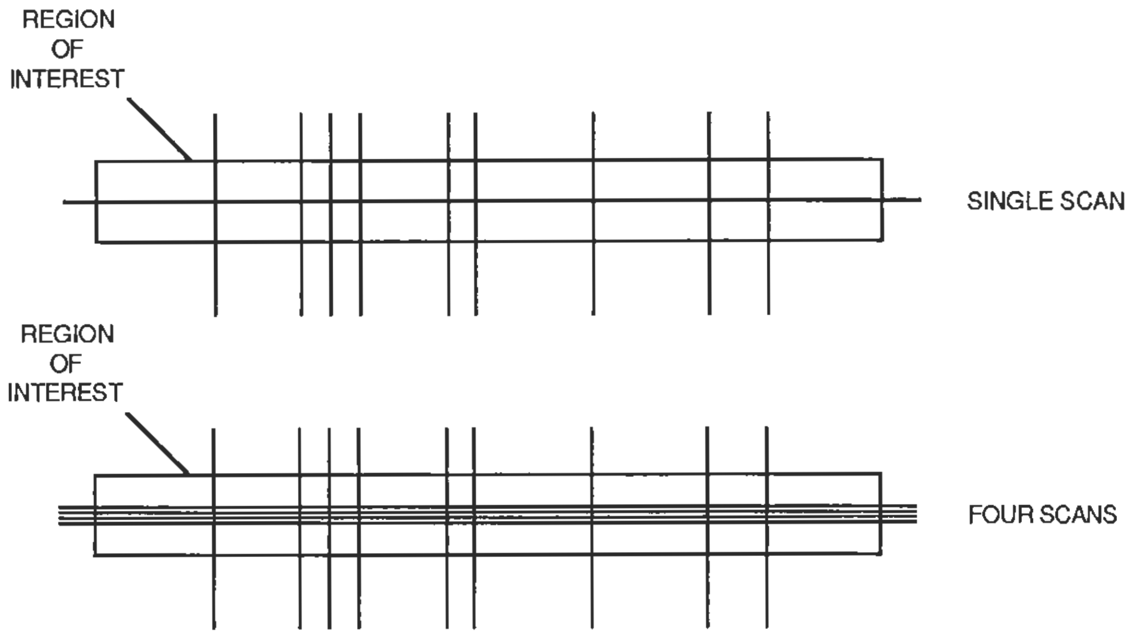

Fig. 6. Scans Taken Through the Region of Interest

All scans described herein were taken with the brightness and contrast controls set near 50%, and at a spatial resolution of 300 dpi. The rectangular region of interest must contain at least ten data points in the long direction, and the length must be at least twice the width. The long direction of the rectangle may be either horizontal or vertical. The TIFF to 1-D conversion program will automatically note the orientation and save 1-D data in a line or lines parallel to this long direction. The user can save either a single line scan down the center of the rectangle or average up to 20 adjacent, parallel scans about the center.

Two P-Scan1D programs are available: one for the Apple Macintosh® computer and the other for IBM®-style computers. P-Scan1D Macintosh version TIFF conversions are made using the following procedure: (1) scan and save a region of interest using the scanner's acquisition program, (2) use the TCONVERT program to make the TIFF to 1-D file conversion, (3) analyze the 1-D file using the P-Scan1D program. The user is prompted for the number of scans to average in the TCONVERT program.

P-Scan1D IBM version TIFF conversions are made automatically when a TIFF file is opened. The user simply saves a region of interest using the scanner's acquisition program, then opens the file in the P-Scan1D program. The user is prompted for the number of scans to average when the file is opened.

Both the scanner and the P-Scan1D programs can be present on the desktop at the same time. The user can quickly and easily switch back and forth among the applications.

Conclusion

A flatbed image scanner is suitable for use as a scanning densitometer provided certain limitations are observed. Objects with optical density ranges of about 1.6 in reflectance and 0.8 in transmittance can be analyzed. The combination of an 8-bit grey scale, flat bed scanner and P-Scan1D software results in a low-cost, versatile 1-D densitometric data analysis system. The user can calibrate the scans to optical density units and millimeters if desired. This makes possible the reporting of integrals in units O.D. x mm.

Appendix

Sample plots for various types of data are shown in the pages below. These data were obtained using the HP ScanJet Plus, Scanning Gallery Plus™ image acquisition software running under Microsoft Windows™ on an IBM AT™ computer with an EGA adapter. The plots were printed on an HP DeskJet printer.

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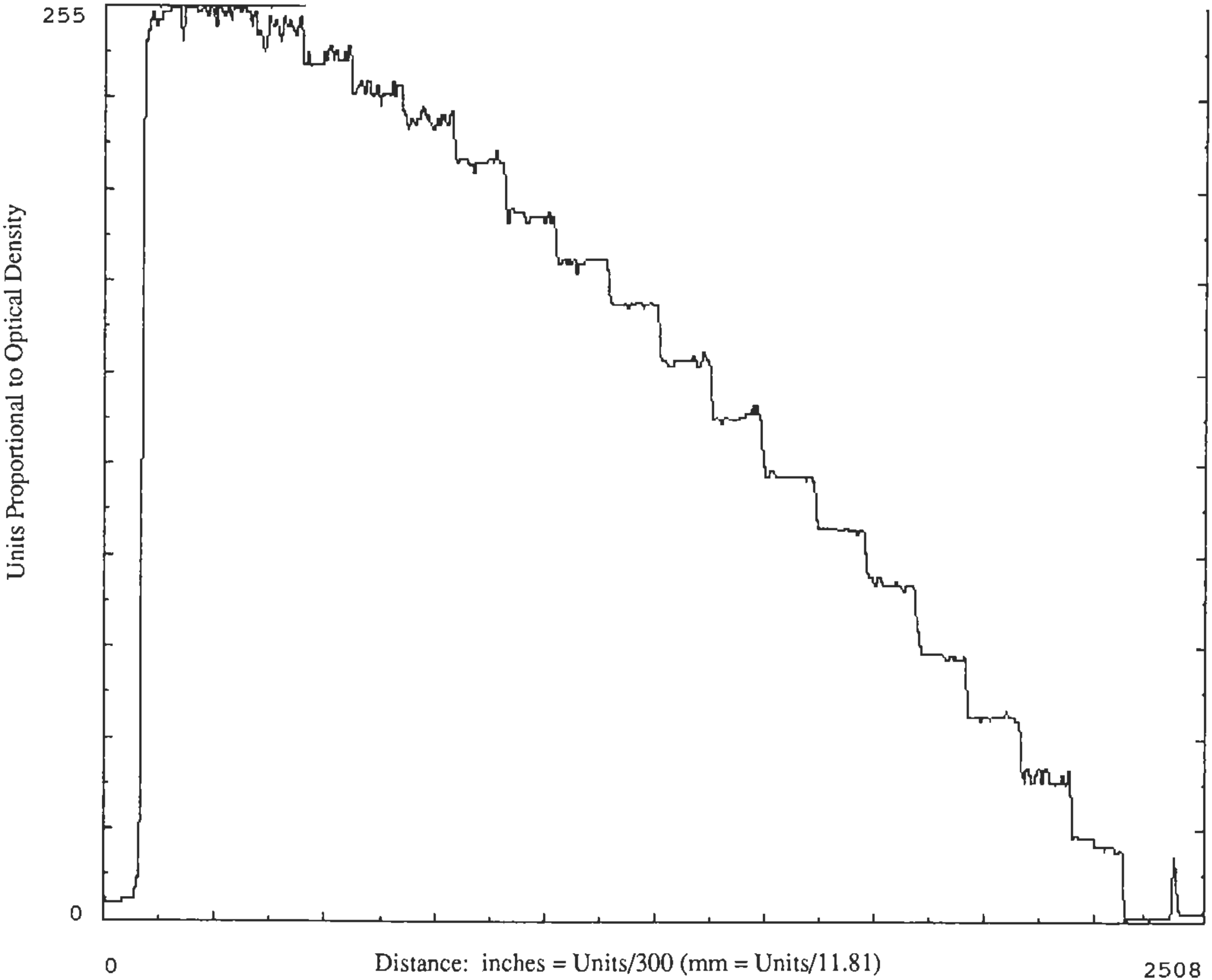
1. Roth, S., MACWORLD, pp. 124-133, May, 1989.
2. Aldus Corporation, "Tag Image File Format Specification, Revision 5.0", 1988.
3. Private communication.



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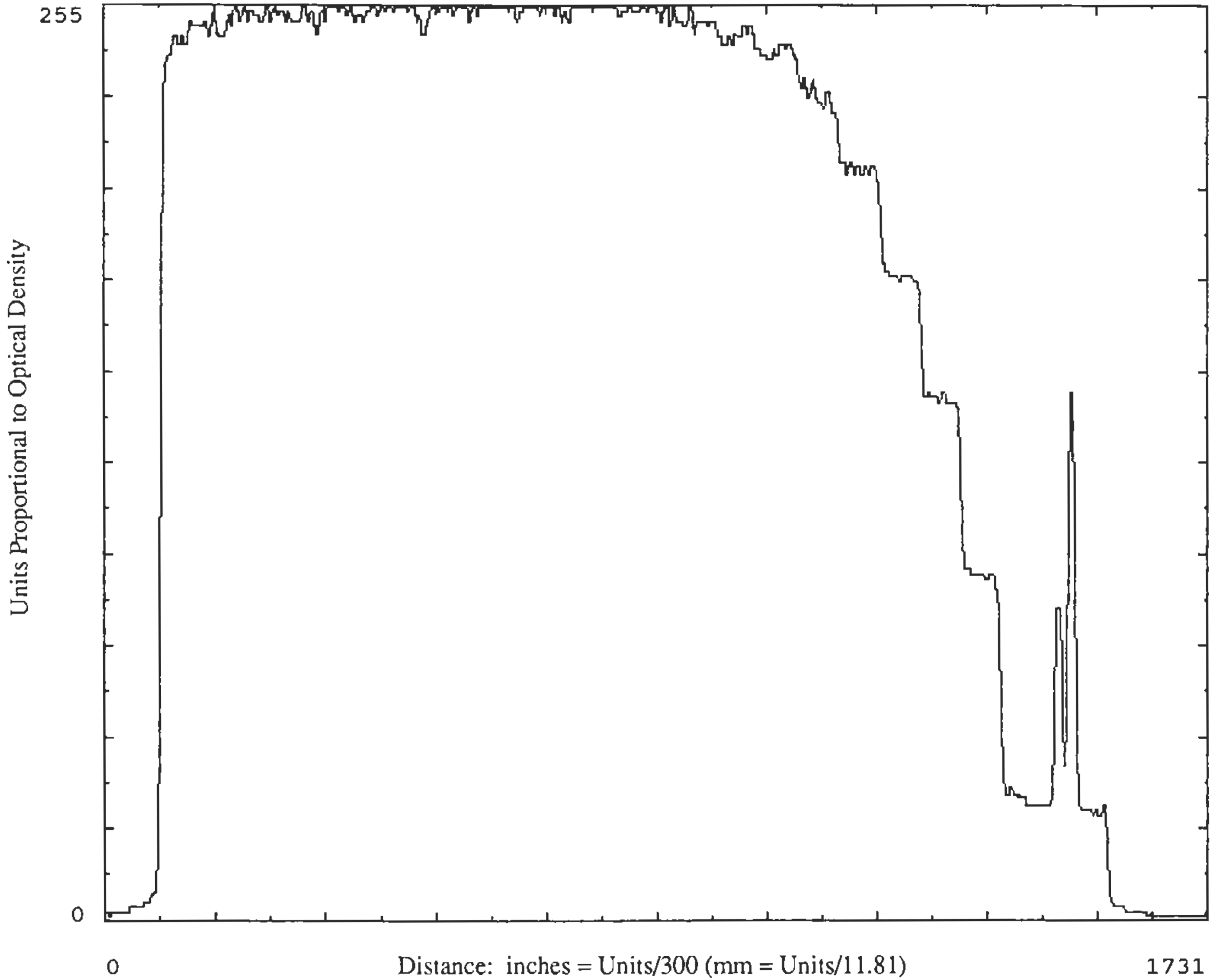

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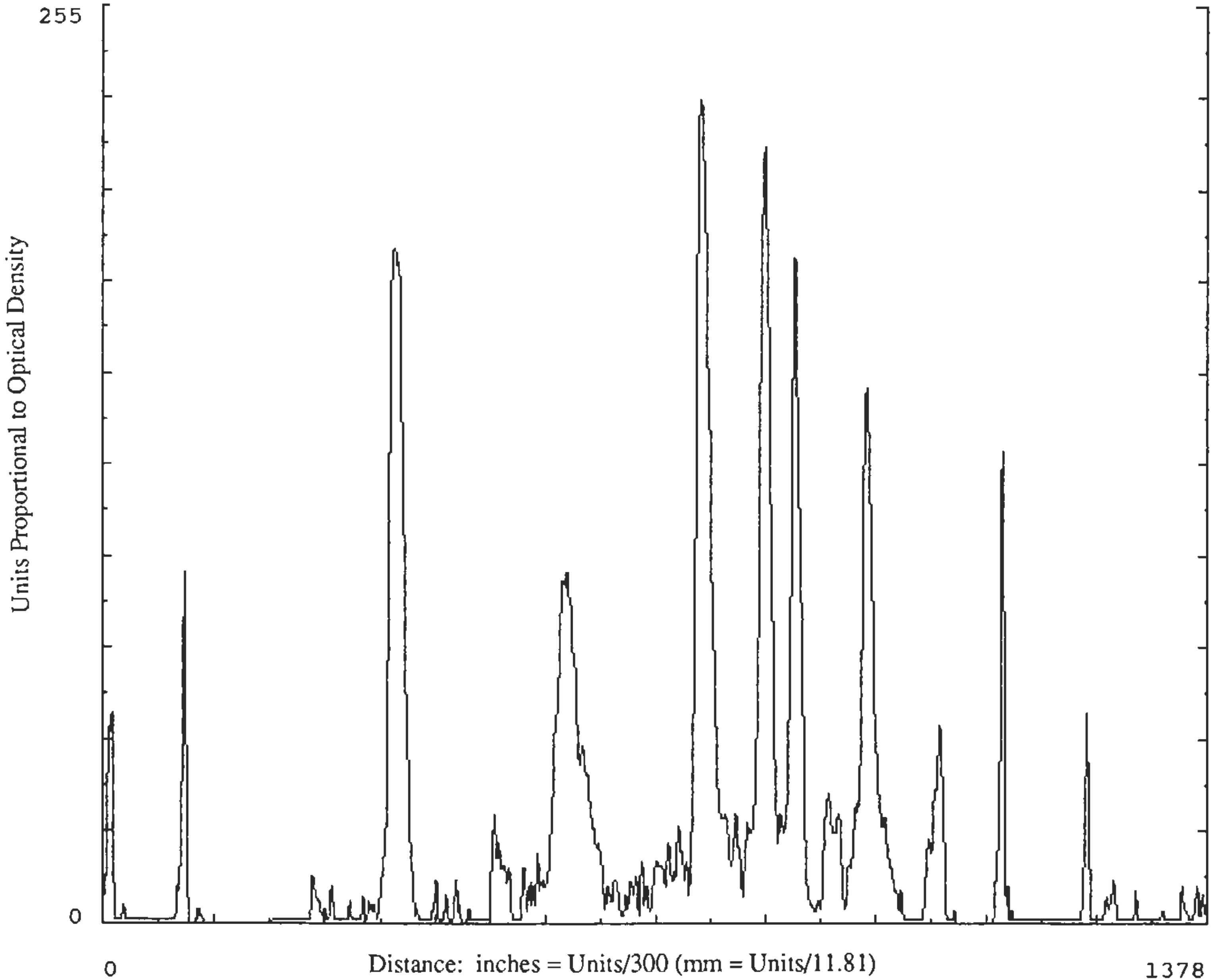

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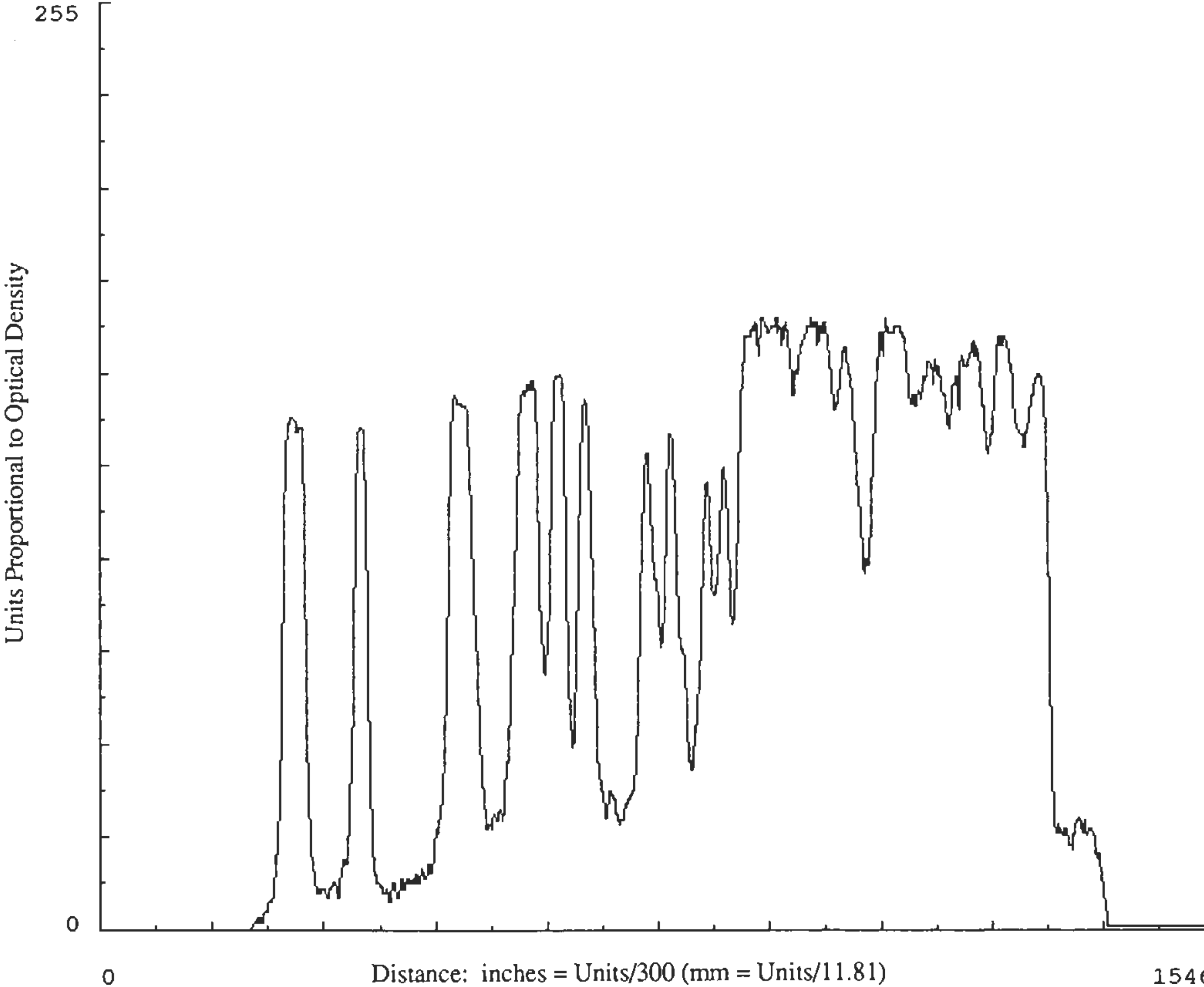

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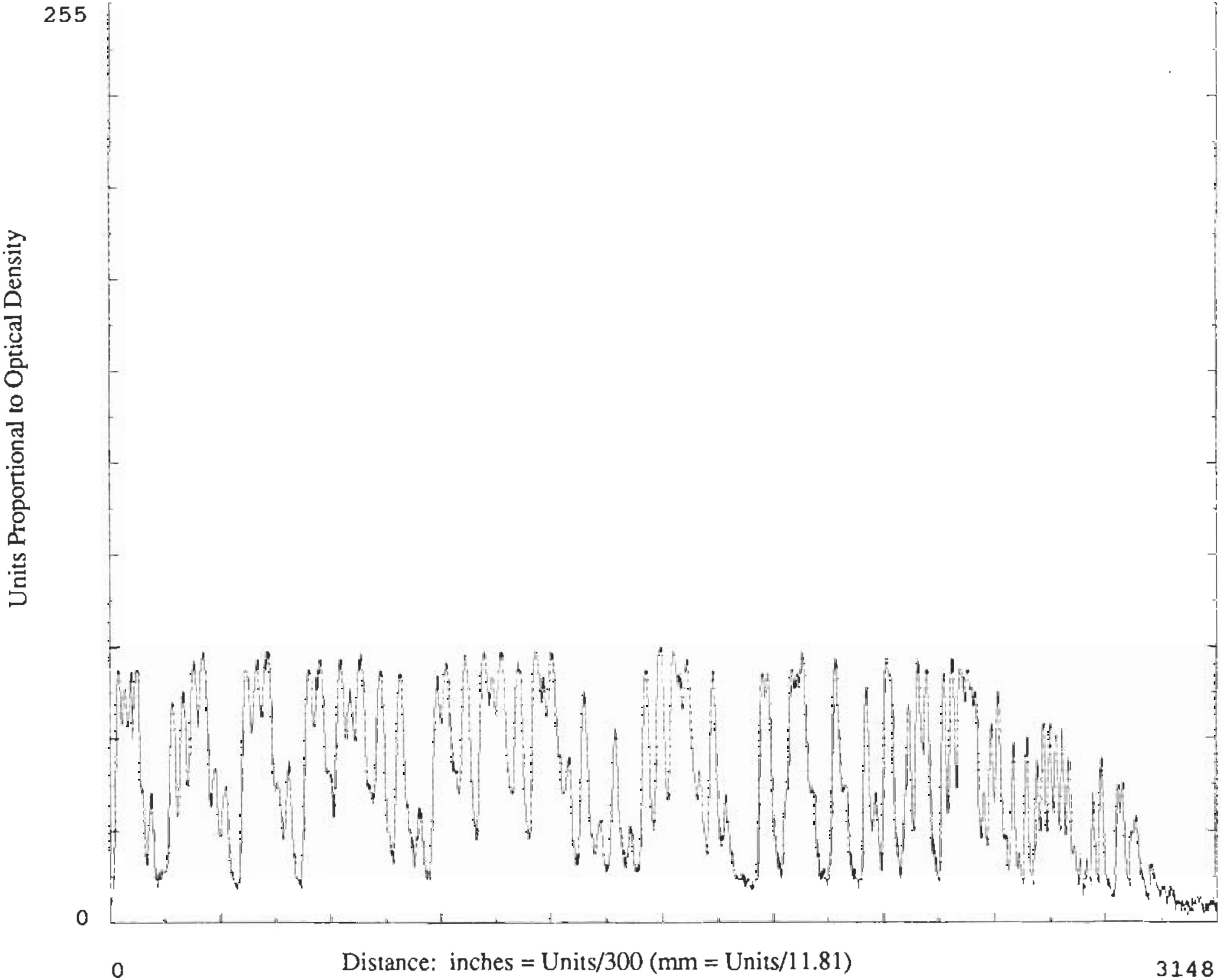

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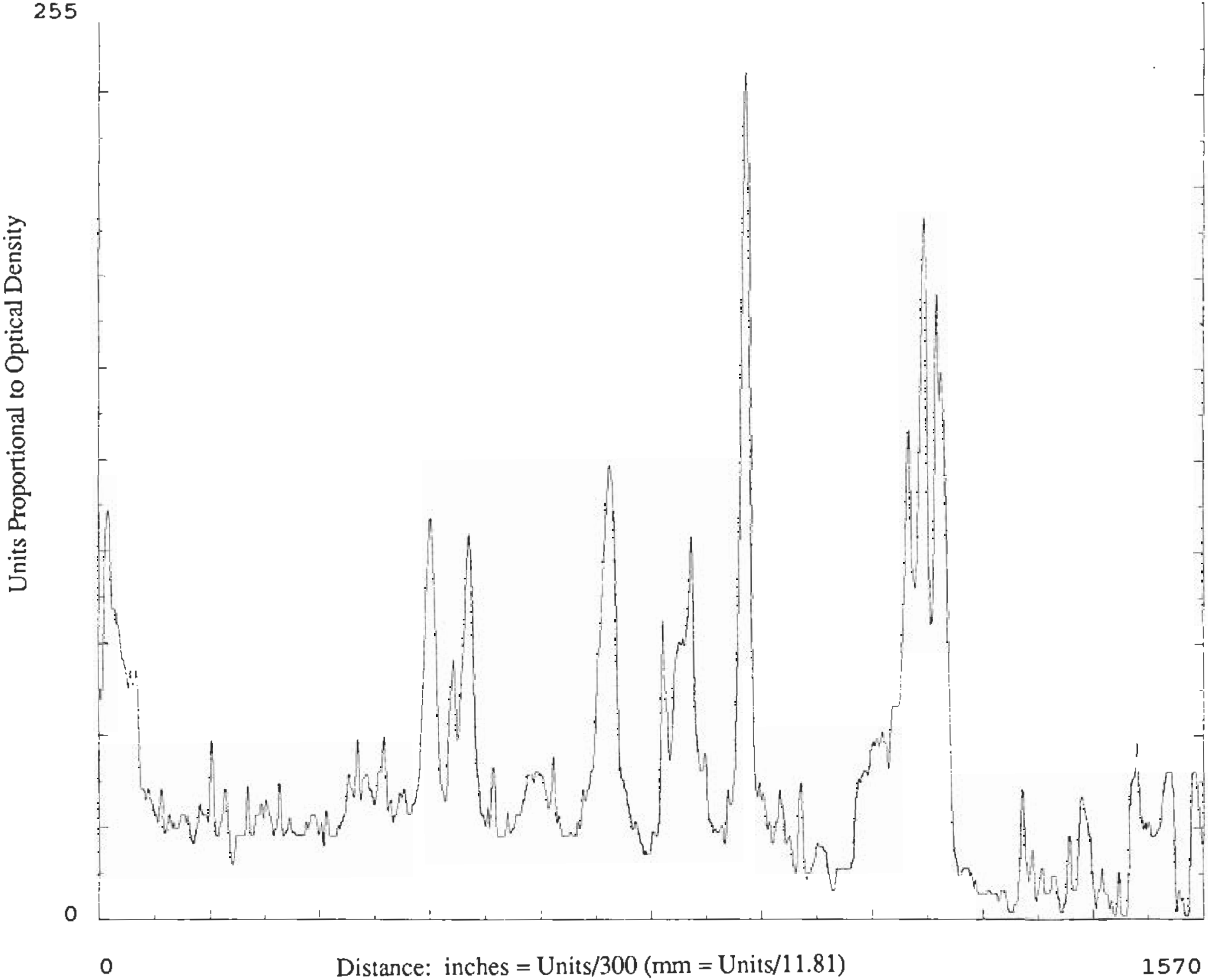

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