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A COMPUTER PROGRAM FOR MEASURING FIBERS WITH THE ZEISS CSM 950 SCANNING ELECTRON MICROSCOPE

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A computer program designed for the stereological measurement of fibers was written for use with a ZEISS CSM 950 Scanning Electron Microscope (SEM). This program utilized the ZEISS' build-in Kontron Image Analyzer to access measurements of a variety of parameters for fiber analysis of images from a video camera, light microscope or SEM.

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PREFACE

In this report we describe the individual components and sequence of instructions of a computer program designed for the stereological measurement of fibers. Utilizing on-screen menu commands, we were able to design and execute a step-by-step analysis of a fiber measurement program. There was some difficulty in writing the program due to a lack of clear instructions in the operator's manual; however, conversations with company representatives helped us overcome these problems.

A COMPUTER PROGRAM FOR MEASURING FIBERS WITH THE ZEISS CSM 950 SCANNING ELECTRON MICROSCOPE

INTRODUCTION

The objective of this report is to describe a computer program designed for measuring stereological features of fibers by means of a Kontron image analysis system, which is built into a Zeiss CSM 950 Scanning Electron Microscope (SEM).

The program was written to permit interaction between the menu program displayed on the TV monitor and the keyboard or mouse. This increased capability permits the operator to change parameters within the measurement program depending on the type of fiber sample.

Since research presently being conducted in these laboratories on ballistic fibers, including biotechnologically derived fibers, requires information about fiber size, shape, etc., developing a menu-[illegible]led computer program facilitates that purpose.

METHODS

The sample model use was a drawing of 11 fibers (Fig. 1) which were visualized with a Dage MTI Model CCD 725 video camera with a Fuji C6 X 17.5 TV zoom lens. The images from the TV camera were transferred to a Zeiss CSM 950 SEM with a built-in Kontron image analysis system. The CSM 950 utilizes a CP/M operating system having a Z80 processor with a

FIGURE 1. Drawing of 11 Fibers to be Measured. The parameters measured (in pixels) were Area, Perimeter, Feret X (maximum projected length in the horizontal direction), and DCircle (area of fibers within a circle measuring 100 pixels in diameter).

20 Mbyte hard disk, 64 Kbyte random access memory (RAM) and a 16 Kbyte video RAM.

The image processing system performs evaluations in a series of steps. First, the image from a TV camera, SEM, light microscope or any other internal or external source is digitized and stored. Next, the grey image is processed for improved contrast and signal/noise ratio. Then, certain features are extracted, i.e., unwanted or overlapping features are eliminated, and features such as size, shape, etc., are analyzed. Finally, the derived data are processed statistically and a printout or photograph is obtained.

There are several major functions, which are grouped depending upon the specific sequence of a standard measuring procedure. The functions are:

1. Input - defines the type of input device and controls image digitization and storage.
2. Calibrate - controls the scaling factors, i.e., inches, microns, etc.
3. Enhance - rescales the grey levels, thereby enhancing image quality. Image noise reduction filters are used.
4. Image Edit - contains functions for the editing of images.
5. Segment - extracts the image background (distinguishes between objects and background).
6. Multiphase - enables further processing of binary (two grey levels) and multiphase (more than two grey levels) images.

7. Parameters - permits the selection of object-specific (single objects in a field of view), or field-specific (area of a visual field minus the area occupied by objects).

8. Evaluate - evaluates objects and outputs the results.

9. Advanced - performs geometric and arithmetic transformation of images, i.e., improves the quality of an image using Fast Fourier Transform methods.

10. Utilities - has general auxiliary functions.

11. Peripheral - permits control of peripheral devices, remote control, etc.

All the above functions have subfunctions that are displayed on the video monitor in the form of a menu. Dialogue with the program, allowing the operator to assign or delete numerical values as well as to control all of the system functions, is accomplished by utilizing the keyboard or a mouse.

The following is the selection sequence for the operator in order to obtain the desired program.

A. Calibrate

1. Scale - As all measurement data are indicated in the selected unit of calibration, the most important prerequisite for every geometric measurement is calibrating the measuring system. The calibration procedure is carried out according to the instructions in the operator's manual (Anonymous, 1986).

a. SCNO, the number of the scale factor to be activated, is set at 1.

B. Evaluate

1. Reset - Before starting a new measuring sequence, e.g., after loading a new program, some conditions such as data buffering, identification number and scanning stage can be reset. The system then has the same status as after initialization.

a. Single (on) - Clears the buffer into which all the data from several fields are accumulated.

b. Class (on) - Clears the data summed up over several evaluated fields to provide average values (e.g., mean area percentage).

C. Select Parameter

1. Area - Object specific area.

a. NCLS (20) - Number of classes when classification is selected. Classification refers to a range of measurement parameter values, i.e. area, perimeter, etc. into which classes (groups) of fibers would fit.

b. MODX (1-3) - Presentation and scaling mode of the X-axis in the histogram of the results.

c. MODY (1-10) - Presentation and scaling mode of the Y-axis in the histogram of the results.

d. Single (on) - Selection of a single list.

e. Class (on) - Selection of a classification.

f. Low 0.000 - Lower bound of classification.

g. High 5000 - Upper bound of classification.

D. Select Parameter

1. Feret - Feret diameters (maximum projected lengths) in X and Y direction.

a. NCLS (20) - Number of classes when classification is selected.

b. MODX (1-3) - Presentation and scaling mode of the X-axis in the histogram of the results.

c. MODY (1-10) - Presentation and scaling mode of the Y-axis in the histogram of the results.

d. Single (on) - Selection of a single list.

e. Class (on) - Selection of a classification.

f. Low (0.000) - Lower bound of classification.

g. High (200.00) - Higher bound of classification.

h. NCLY (20) - Number of classes when classification is selected.

i. MDYX (1-3) - Presentation and scaling mode of the X-axis in the histogram of the results.

j. MDYY (1-10) - Presentation and scaling mode of the Y-axis in the histogram of the results.

k. LO.Y (0.000) - Lower bound of the classification.

l. HI.Y (1000.0) - Upper bound of the classification.

E. Select Parameter

1. Perim - Object specific perimeter and length.

a. NCLS (20) - Number of classes when classification is selected.

b. MODX (1-3) - Presentation and scaling mode of the X-axis in the histogram of the results.

c. MODY (1-10) - Presentation and scaling mode of the Y-axis in the histogram of the results.

d. Single (on) - Selection of a single list.

e. Class (on) - Selection of a classification.

f. Low (0.000) - Lower bound of classification.

g. High (600.0) - Upper bound of classification.

h. NCLT (10) - Number of classes when classification is selected.

i. MDLX (1-3) - Presentation and scaling mode of the X-axis in the histogram of the results.

j. MDLY (1-10) - Presentation and scaling mode of the Y-axis in the histogram of the results.

k. LOLT (600.0) - Lower bound of classification.

l. HILT (0.000) - Upper bound of classification.

F. Select Parameter

1. DCIRCL - Diameter of area-equivalent circle (area of fibers within a circle which measures 100 pixels in diameter).

a. NCLS (20) - Number of classes when classification is selected.

b. MODX (1-3) - Presentation and scaling mode of the X-axis in the histogram of the results.

c. MODY (1-10) - Presentation and scaling mode of the Y-axis in the histogram of the results.

d. Single (on) - Selection of a single list.

e. Class (on) - Selection of a classification.

f. Low (0.000) - Lower bound of classification.

g. High (100.00) - Upper bound of classification.

G. Select Utilities

1. LAB: Label definition - Labels are used as markers in the sequence of a measuring program to which a jump (analogous to a GOTO function) can be executed.

a. LBL#(1) - Identifier of the label, which serves as designation for a jump.

H. Input

1. TVON - Switches the color monitor directly to a TV signal.

   a. Online (on) - Switches the online status on or off.

I. Select Utility

1. SYNC - The Image Processing System can be synchronized externally (e.g., by using a TV camera) or internally. In the internal mode, no TV input is possible.

   a. Internal (off), Internal (on), or External (off).

J. Select Utility

1. Pause - Interrupts run of program.

K. Input

1. TVINP - This function stores:

   a. TV Input - A TV image in the memory indicated by INP

   b. INP (1) - Image memory into which the image is to be stored.

L. Choose Segment

1. DISC2L - This function separates objects from the background, by setting two thresholds. Either the grey levels inside or those outside the entered limits are set to grey value 0 (black) and constitute the background. Depending on the variable BINARY, the remaining object points either keep their original grey levels or are set to white (255).

   a. INP (1) - Image memory containing the image to be processed.

   b. OUT (2) - Image memory into which the discriminated image is stored.

   c. LEV1 (0) - Lower discrimination limit.

   d. LEV2 (118)- Upper discrimination limit.

e. BINARY (on) - The object pixels become 255 (white), i.e. a binary image is produced.

M. Calibrate

1. MFRAME - This function is used to create a rectangular or circular measuring frame, which is sometimes necessary to correct errors (eliminate overlapping images) caused by the finite size of the field of view during the measurement process.

a. INP (2) - Memory containing the image to be processed.

b. WDSX (508) - X-side of the rectangular frame.

c. WDSY (467) - Y-side of the rectangular frame.

d. XO (4) - X position of the (rectangular or circular) frame.

e. YO (6) - Y position of the (rectangular or circular) frame.

f. RAD (100) - Radius of the circular frame.

N. Evaluate

1. IDENT - This function serves two purposes: Identification of discriminated images and elimination of objects following the conditions set by the measuring frame (function MFRAME, group Calibrate).

a. INP (2) - Image memory containing the image to be identified.

b. OUT (3) - Memory containing the identified and frame-corrected image.

c. MARG (1) - Controls the meaning of the measuring frame defined by MFRAME (function group Calibrate). This determines whether or not the fiber touching the rectangular or circular frame on the video monitor is measured. For example, one might not want to measure a fiber extending beyond the frame's margin.

d. 8-CONN (on) - Method of identification, on = 8 CONN.

O. Evaluate

1. MEASURE - This function executes the measurement of selected parameters such as area, perimeter, etc.

a. OBJ - Must be switched on if object-specific parameters are among the previously selected parameters (AREA-TIME).

b. INP (3) - Image memory containing the discriminated OBJ image.

c. GRIM (1) - Image memory containing the original grey image.

d. AUX1 (9) - Auxiliary image memory.

e. AUX2 (9) - Auxiliary image memory.

f. CHAN (1) - Channel selection.

g. SPEC (1) - Distance in pixels between consecutive lines of the "line grid". The lines are simply lines of the image memory, or pixel addresses with varying X, but constant Y coordinates.

h. FLG# (1) - Logical flag indicating a buffer overflow.

i. REJECT (on) - Interactive rejection of undesired objects, preceding an automatic measurement.

P. Evaluate

1. OUTCLS - (Output of Classification) This function displays the results of the classification in the form of a list and/or as a histogram.

a. HISTO (on) - Provides measured data in histogram form.

b. HALT (on) - Presents data on the monitor; the sum histogram can be manipulated.

c. NFAC (0.000) - Defines the area (in user units) into which the absolute counts should be normalized. A value of 0.0 gives normalized absolute frequencies.

Q. Evaluate

1. OUTSGL - (Output of single list) This function displays the individual lists of all measured data.

a. HALT (on) - Displays lists on the data monitor after each measurement.

R. Select Utility

1. Pause - This function interrupts the running of a measuring program.

RESULTS

The computer program designed and written for the stereological analysis of several fibers (Table 1) was utilized to measure their Area, Perimeter, Feret X and D Circle. The inputs to the parameters of this program were as follows:

Area (high upper bound of classification) - 5000

Feret X (higher bound of classification) - 200

Feret X (upper bound of classification) - 1000

Perimeter (high upper bound of classification) - 600

DCircle (high upper bound of classification) - 100

The printout of the computer program can be seen in Table 1, the plots for each parameter can be seen in Figures 2 to 5 and the printout of the statistical data can be seen in Table 2.

CONCLUSIONS

A computer program was designed and written to measure certain stereological parameters of fibers and to generate subsequent statistical analysis of the results. The program was developed to be driven by a series of menu commands, which allowed for direct interaction between the image appearing on the video monitor and the keyboard.

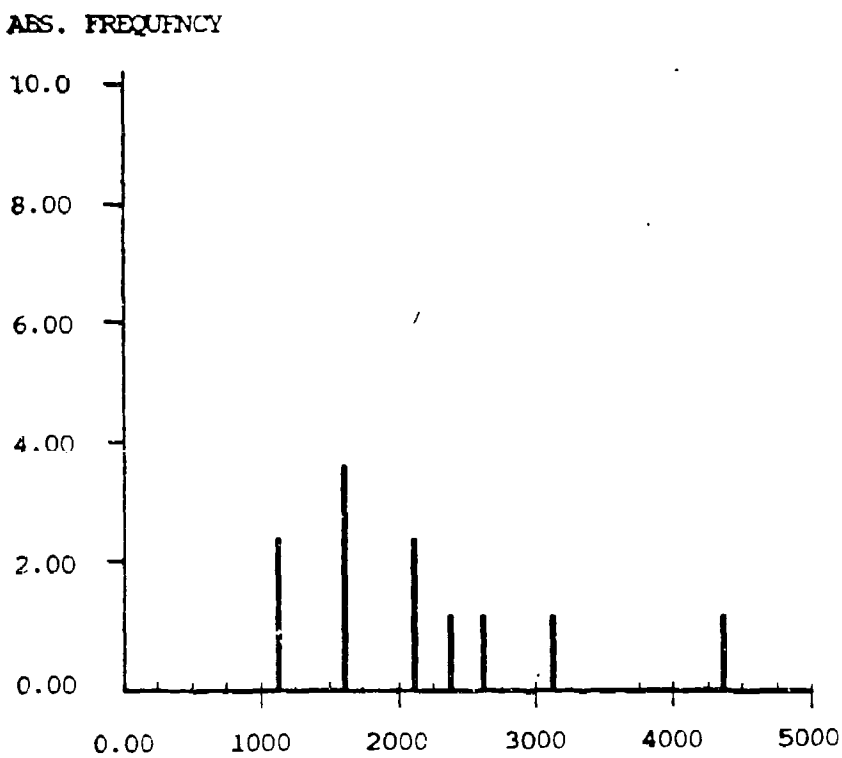
TABLE 1. Image Analysis Program for Measuring Fibers Using the Zeiss CSM 950 SEM with a Built-in Kontron Image Analyzer.

MAJOR FUNCTIONS	SUB FUNCTIONS	INTEGER VALUES	VARIABLES	SCALE VALUES
A. CALIBRATE	SCALE			
		SCNO 1		
B. EVALUATE	RESET			
			SINGLE	
			CLASS	
			PARAM	
C. PARAMETERS	AREA			
		NCLS 20	SINGLE	LOW 0.000
		MODX 1	CLASS	HIGH 5000.0
		MODY 1		
D. PARAMETERS	FERET			
		NCLS 20	SINGLE	LOW 0.000
		MODX 1	CLASS	HIGH 200.0
		MODY 1		LO.Y 0.000
		NCLY 20		HI.Y 1000.0
		MDYX 1		
		MDYY 1		
E. PARAMETERS	PERIM			
		NCLS 20	SINGLE	LOW 0.000
		MODX 1	CLASS	HIGH 600.0
		MODY 1		LOLT 600.0
		NCLT 10		LILT 0.000
		MDLX 1		
		MDLY 1		
F. PARAMETERS	DCIRCL			
		NCLS 20	SINGLE	LOW 0.000
		MODX 1	CLASS	HIGH 100.0
		MODY 1		
G. UTILITIES	LABl			
		LBL# 1		
H. INPUT	TVON			
		ONLINE		
I. UTILITIES	SYNC			

(Continued)

TABLE 1. Image Analysis Program for Measuring Fibers Using the Zeiss CSM 950 SEM with a Built-in Kontron Image Analyzer. (Continued)

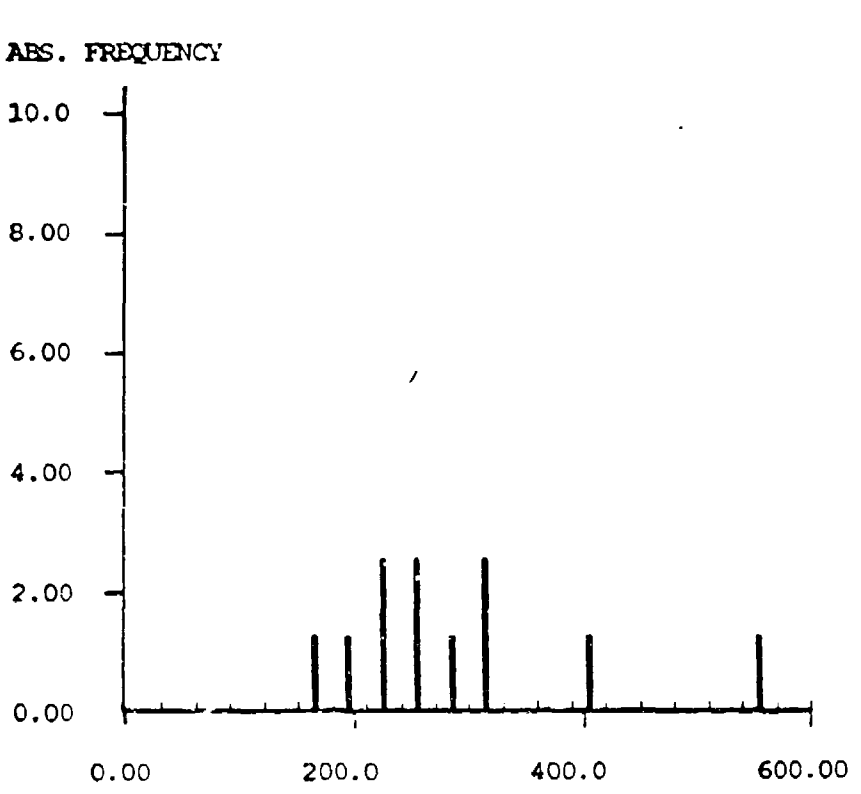
MAJOR FUNCTIONS	SUB FUNCTIONS	INTEGER VALUES	VARIABLES	SCALE VALUES
J. UTILITIES	PAUSE			
K. INPUT	TVINP			
		INP 1		
L. SEGMENT	DISC2L		BINARY	
			LEV1 0	
		LEV1 0		
		LEV2 118		
M. CALIBRATE	MFRAME			
		INP 2		
		WDSX 508		
		WDSY 467		
		XO 4		
		YO 6		
		RAD 100		
N. EVALUATE	IDENT			
		INP 2	8-CONN	
		OUT 3		
		MARG 1		
O. EVALUATE	MEASUR			
		INP 3		
		GRIM 1		
		AUX1 9	OBJ	
		AUX2 9		
		CHAN 1		
		SPAC 1		
		FLG# 1	REJECT	
P. EVALUATE	OUTCLS			
			HIST	NFAC 0.000
			HALT	
Q. EVALUATE	OUTSGL			
			HALT	
R. UTILITIES	PAUSE			
TOTAL PROGRAM LENGTH		340 BYTES		



COUNTS	11
UND. FLOW	0
OVERFLOW	0
CLASSES	20
%-IL CLASSES	
25%	7
MEDIAN	9
75%	11
95%	18
MODUL	
250.0	
L. BOUND	
0.000	
U. BOUND	
5000.	
MINIMUM	
1142.	
MAXIMUM	
4254.	
MEAN	
2177.	
SD	
901.3	

AREA

FIGURE 2. Histogram of Area Measurements from Figure 1.
The X axis values are in pixels.
The Y axis represents the frequency distribution.



COUNTS	11
UND. FLOW	0
OVERFLOW	0
CLASSES	20
%-IL CLASSES	
25%	8
MEDIAN	9
75%	11
95%	19
MODUL	
30.00	
L. BOUND	
0.000	
U. BOUND	
600.0	
MINIMUM	
169.7	
MAXIMUM	
547.3	
MEAN	
289.4	
SD	
108.2	

PERIM

FIGURE 3. Histogram of Perimeter Measurements from Figure 1.
The X axis values are in pixels.
The Y axis represents the frequency distribution.

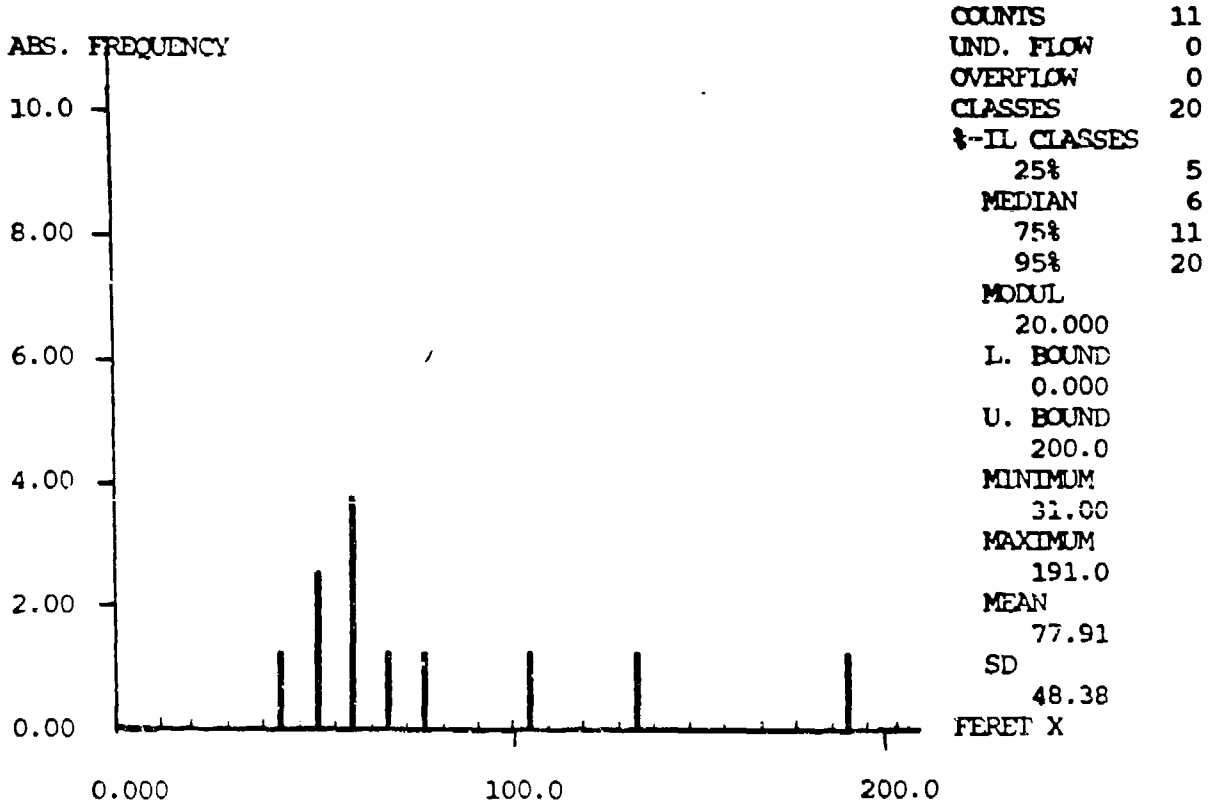


FIGURE 4. Histogram of Feret X Measurements from Figure 1.
The X axis values are in pixels.
The Y axis represents the frequency distribution.

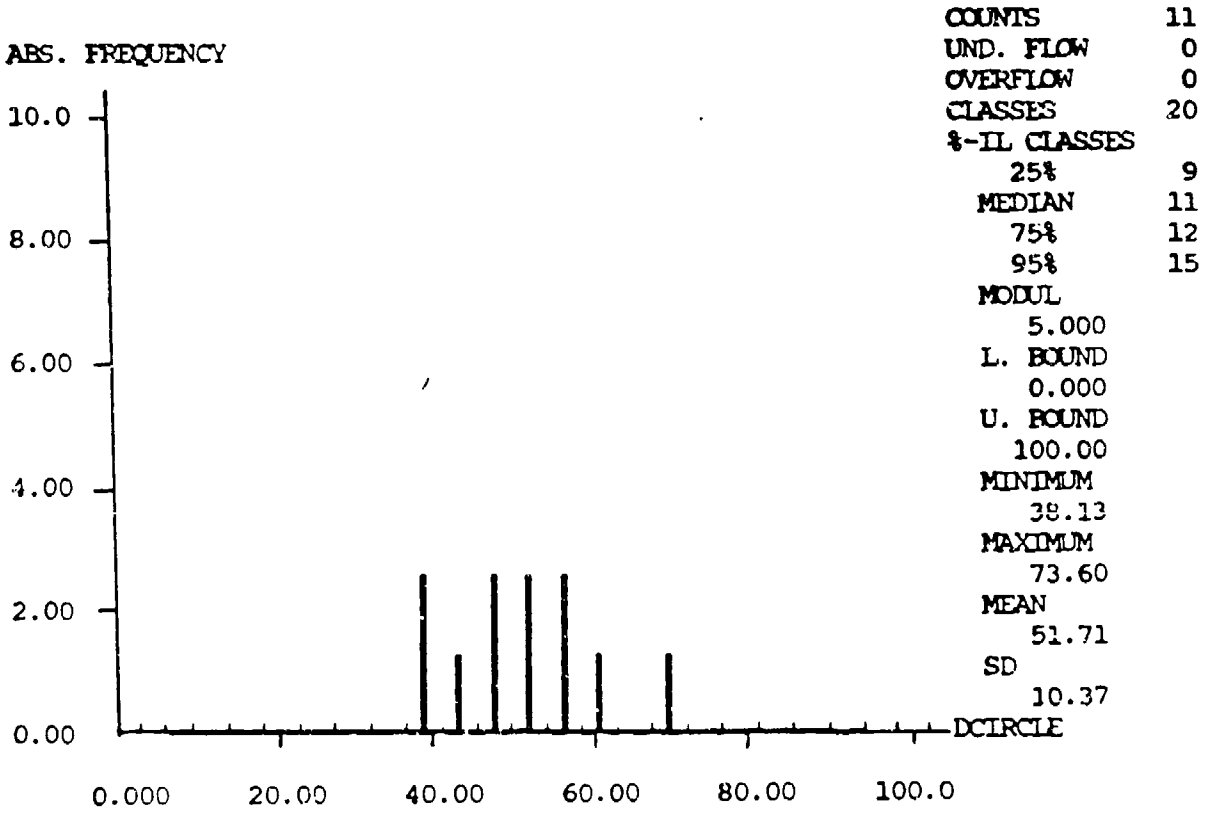


FIGURE 5. Histogram of an Area Equivalent to a Circle (called a DCircle) within Figure 1. The X axis values are in pixels. The Y axis represents the frequency distribution.

TABLE 2. Statistical Data (in Pixels) for 11 Fibers Measured

COUNT	AREA	PERIM.	FERETX	DCIRCLE
1	3111.0	413.4	137.0	62.94
2	2134.0	270.0	53.0	52.13
3	2512.0	318.4	58.0	56.55
4	1731.0	250.8	103.0	46.95
5	2378.0	302.6	52.0	55.03
6	1691.0	226.0	41.0	46.40
7	2196.0	269.5	44.0	52.88
8	1142.0	169.7	68.0	38.13
9	1217.0	196.7	79.0	39.36
10	1582.0	219.2	31.0	44.88
11	4254.0	547.3	191.0	73.60

REFERENCE

Anonymous, 1986. "Kontron SEM-IPS Operator's manual," Volume II, Release 4.4, 410 pages.

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