

VIDEOTEST-COLONYP COUNT

Version 4.0

Computer software for count and measure
the bacteria colonies
Windows 9x, 2000, XP

developed by VideoTest, Ltd.

SPECIFICATION

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1. INTRODUCTION

VideoTesT - Colony Count is designed to perform the acquisition, enhancement, counting, and measure the bacteria colonies. The analysis data are processed statistically. The program enables to analyze one, two, three or four different bacteria colonies in one image.

VideoTesT - Colony Count provides an ability to use four pre-installed methodics: «One Colony», «Two Colonies», «Three Colonies», «Four Colonies», or work in a free mode («Default»).

2. METHODICS

METHODIC: DEFINITION

Methodic - is the automatically performed sequence of operations upon an image, providing the needed data with the minimum interaction of the user. The methodic accelerates routine analysis making it less exhausting.

VideoTesT - Colony Count contains four pre-installed methodics:

1. «One Colony» methodic is used to threshold, count and measure the bacteria colonies of the same type. The thresholded colonies are classified by their size.
2. «Two Colonies» is used to threshold, count and measure the bacteria colonies of two types differing in brightness or color. The total count and percentage of two thresholded colonies are estimated.
3. «Three Colonies» is used to threshold, count and measure the bacteria colonies of three types differing in brightness or color. The total count and percentage of two thresholded colonies are estimated.
4. «Four Colonies» is used to threshold, count and measure the bacteria colonies of four types differing in brightness or color. The total count and percentage of two thresholded colonies are estimated.

Also, the program provides an ability to work in «Default» mode. Using this mode you can acquire an image and then select any program functions you want to be performed under the image (enhancement, annotation, thresholding, measurements, etc.)

«ONE COLONY»

This methodic allows the user to acquire, process, and measure the images of bacteria colonies of the same type. Right after the color or b/w image (depending on the image acquisition system employed) is captured or loaded the program starts its processing.

The bacteria colonies are thresholded. If you are not satisfied with their thresholding there is an ability to adjust it, select the different automatic thresholding method available in the program or perform the manual corrections. The thresholded colonies are measured and measurement data are displayed in the auto measurement data table (see Figure 1). Measurement parameters are selected by the operator.

The measured colonies are classified automatically by their size. The classification results are displayed in the class table. The class table contains all information on object classes and their percentage. You can process and measure the unlimited number of images, and all statistical data will be accumulated in the class table. The number of classes, the parameter for classification, statistic parameters to be calculated and displayed can be easily specified by the operator.

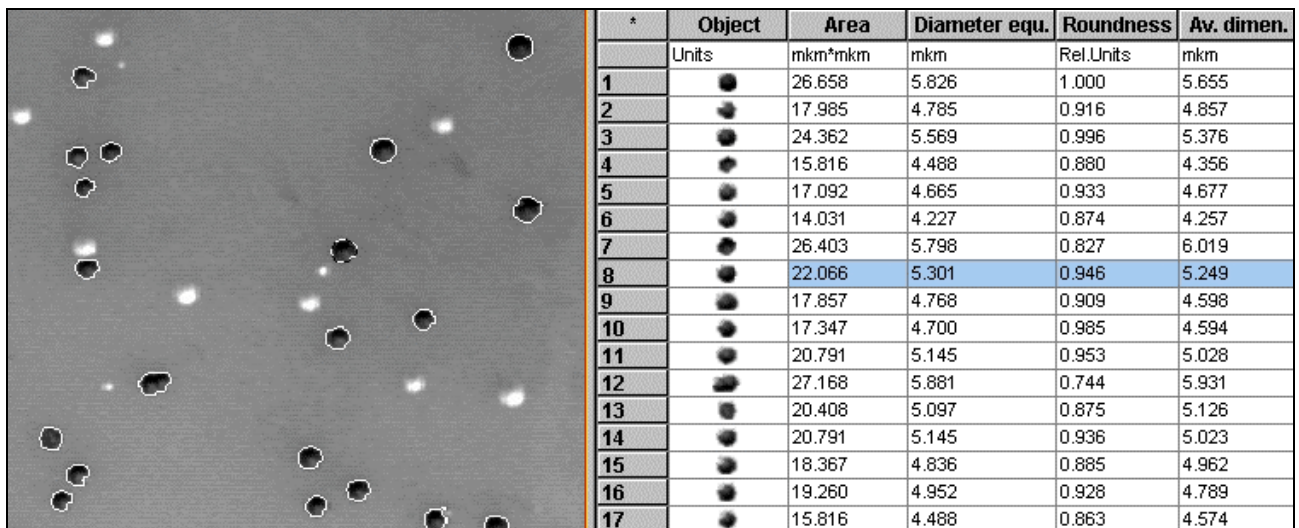


Figure 1

The window is split into two parts. Left: the image of bacteria colonies, right: the automatic measurements table. The active object is outlined with the running contour and highlighted in the table.

«TWO COLONIES»

This method allows the user to acquire, process, and measure the images of bacteria colonies of two types which differ in their brightness or color. Right after the color or b/w image (depending on the image acquisition system employed) is captured or loaded the program starts its processing.

First the bacteria colonies of the first type are thresholded. Then the bacteria colonies of the second type are thresholded. If you are not satisfied with their thresholding there is an ability to adjust it, select the different automatic thresholding method available in the program or perform the manual corrections.

The thresholded colonies are measured. The measurement data can be displayed in the auto measurement data table. Measurement parameters are selected by the operator.

The data on total count, specific quantity and percentage of the different colonies are displayed in the class table (see Figure 2). The operator can process and measure the unlimited number of images, and all statistical data will be accumulated in the class table.

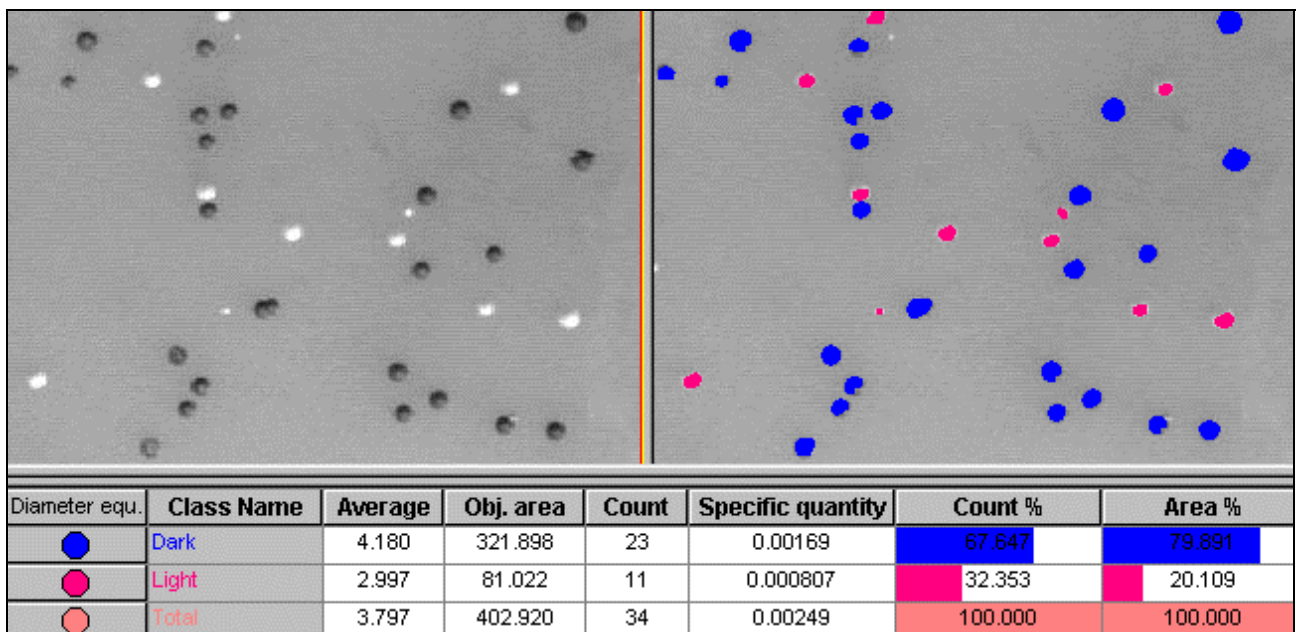


Figure 2

The bacteria colonies of two types («dark» and «light») are thresholded and counted. In the bottom: class table with data on bacteria count and percentage.

«THREE COLONIES»

This methodic allows the user to acquire, process, and measure the images of bacteria colonies of three types which differ in their brightness or color. Right after the color or b/w image (depending on the image acquisition system employed) is captured or loaded the program starts its processing.

First the bacteria colonies of the first type are thresholded. Then the bacteria colonies of the second type are thresholded. After that the bacteria colonies of the third type are thresholded. If you are not satisfied with their thresholding there is an ability to adjust it, select the different automatic thresholding method available in the program or perform the manual corrections.

The thresholded colonies are measured. The measurement data can be displayed in the auto measurement data table. Measurement parameters are selected by the operator.

The data on total count, specific quantity and percentage of the different colonies are displayed in the class table. The operator can process and measure the unlimited number of images, and all statistical data will be accumulated in the class table.

«FOUR COLONIES»

This methodic allows the user to acquire, process, and measure the images of bacteria colonies of four types which differ in their brightness or color. Right after the color or b/w image (depending on the image acquisition system employed) is captured or loaded the program starts its processing.

First the bacteria colonies of the first type are thresholded. Then the bacteria colonies of the second type are thresholded. After that the bacteria colonies of the third type and the bacteria colonies of the fourth type are thresholded. If you are not satisfied with their thresholding there is an ability to adjust it, select the different automatic thresholding method available in the program or perform the manual corrections.

The thresholded colonies are measured. The measurement data can be displayed in the auto measurement data table. Measurement parameters are selected by the operator.

The data on total count, specific quantity and percentage of the different colonies are displayed in the class table (see Figure 2). The operator can process and measure the unlimited number of images, and all statistical data will be accumulated in the class table.

«DEFAULT»

The «Default» mode does not provide any pre-defined sequence of operations. After an image is acquired, you can use any of the program functions which are selected from the toolbars or from the menu.

NEW METHODICS

The number of methodics is not limited by six pre-installed. The program provides the following abilities to create additional methodics:

1. Copy any methodic with a new name, modify it without changing the general analysis procedure, and save.
2. Install a new methodic created by the developers.

3. IMAGE: GENERAL INFORMATION**IMAGE ACQUISITION**

VideoTesT-Colony Count provides the following abilities of image acquisition. The images can be acquired with a TV-camera, scanner, other acquisition devices TWAIN/MCI compatible or having special capture driver, pasted from the clipboard, opened from disk, loaded from the **VideoTesT - Album** Image Database. **VideoTesT - Colony Count** processes 8 and 24 bit images of .bmp, .tiff, .gif, .pcx, .jpg, .avi formats.

IMAGE DISPLAYING

The program provides different modes of the image displaying: original image, image with object contours (see Figure 3-a), image with the object masks on the original background (see Figure 3-b), objects in false-colors corresponding to class colors, on the original background (see Figure 3-c). To have objects displayed in false-colors it is first required to classify them.

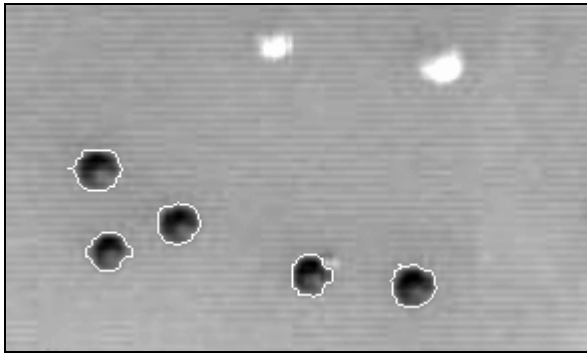


Figure 3-a. The contoured objects (blood cells) on the original background.

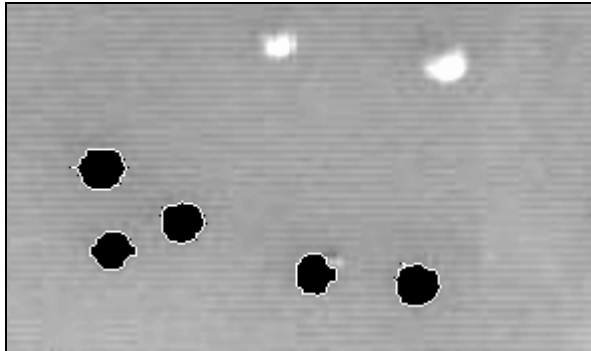


Figure 3-b. Object masks on the original background

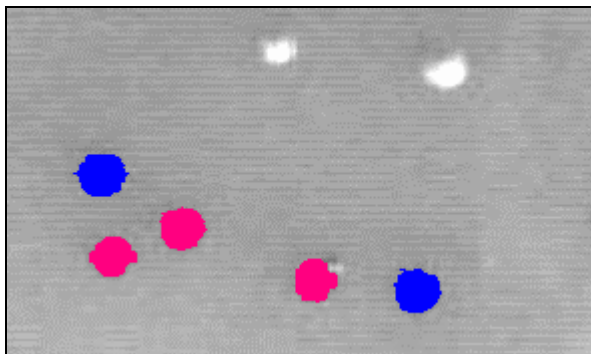


Figure 3-c. Objects in false-colors on the original background.

Figure 3

An image or its area within a frame can be zoomed in or out.

SAVING IMAGES AND DATA

An active image can be saved to .bmp, .tiff, .gif, .pcx, .jpg file. If it is necessary to save images together with data (e.g. measurements data) use .vti file format.

Also, the image and data can be sent to the **VideoTesT - Album** Image Database and saved there.

4. IMAGE ENHANCEMENT

VideoTesT - Colony Count allows a user to enhance the original image: enlarge or reduce its size, enhance it by filters, annotate it (text and graphics).

To enhance the image within an area of interest, you need to set frames. The frame may be of rectangular, ellipse or free shape. You can add areas of any shape to the frame, or subtract them from the frame.

The area of interest can be cut or copied to the clipboard, moved or copied within an image or between different images.

RESIZING AND ROTATION

The program allows the user to change image proportions (resize operation) and rotate the whole image or its area within a frame.

FILTERS

The program includes a set of enhancement filters. The filtration is performed upon the whole image, an active object, or within an area of interest.

- **Brightness and contrast**

The filter allows the user to change brightness and contrast of the whole image, its area within a frame (see Figure 4), or a separate object.

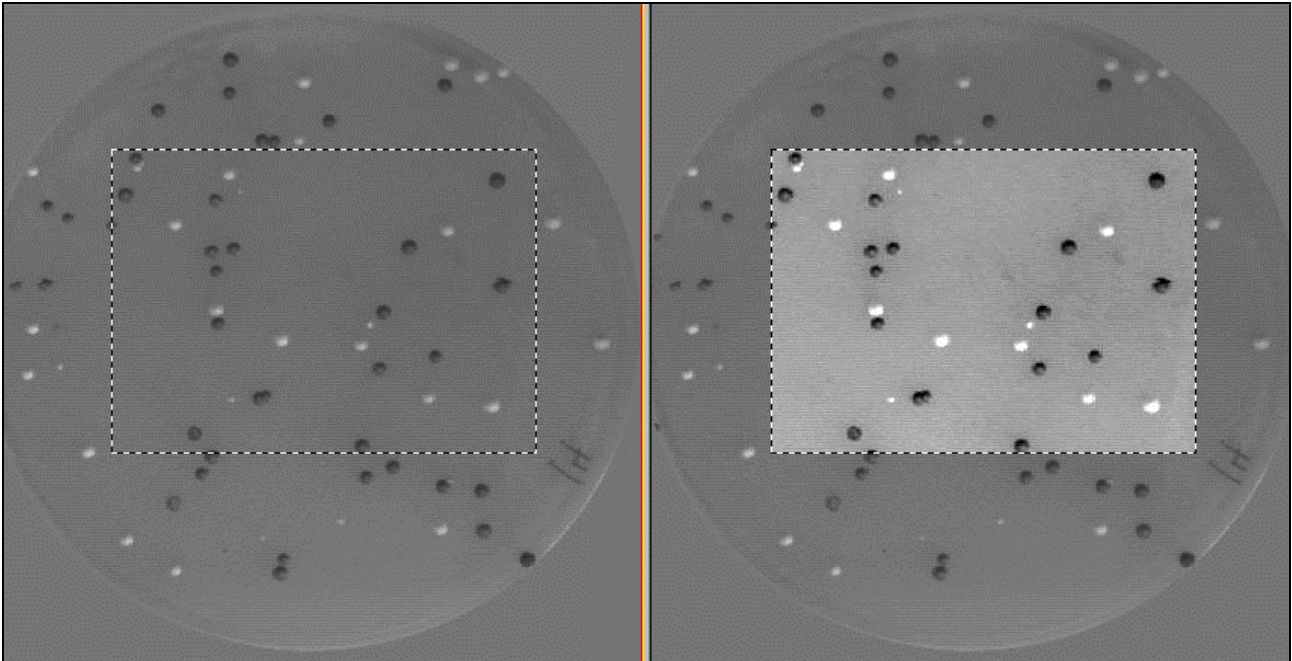


Figure 4

Left: original image, right: contrasted image area within a frame.

- **Background subtraction**

This filter is used for the elimination of uneven image background.

- **Special filters**

These filters are used to increase the image sharpness or convert it to negative.

IMAGE EDITING

The image editing functions are used to draw straight, freehand and spline lines on the image, type text, fill in a framed image area with a chosen color, resize and rotate image, copy or move the whole image or its area, etc.

Also, there is an ability to overlay the image with a calibration bar.

5. OBJECTS (COLONIES) THRESHOLDING AND MEASUREMENTS

VideoTesT - Colony Count allows a user to perform the automatic measurements on the images.

Automatic measurements are performed in all methodics. The measure results are displayed in the automatic measurements data table.

The objects (cells, nuclei) can be automatically measured only when they are thresholded on the image.

OBJECTS THRESHOLDING

In all pre-installed methodics the objects or image areas are automatically thresholded on the image. If you are not satisfied with the pre-defined automatic thresholding you can adjust it or select the other thresholding method.

The program provides two methods of objects thresholding: **B/W Automatic** and **B/W or Color Manual** (by brightness histogram or by sampling).

- **Automatic thresholding**

There are four methods of the automatic objects thresholding:

Simple	Objects thresholding by original image.
With smoothing	Objects thresholding by an image with equalized background. This option should be used when the objects are on the uneven background.
With histogram correction	Objects thresholding by an image with contrast enhancement performed.
With smoothing and histogram correction	Objects thresholding by an image with the background equalization and contrast enhancement performed.

To compare the thresholded image with the original one, the image window can be split vertically into two parts. Then the result of objects' thresholding will be displayed in one part of the window and the original image will be shown in another part (see Figure 5).

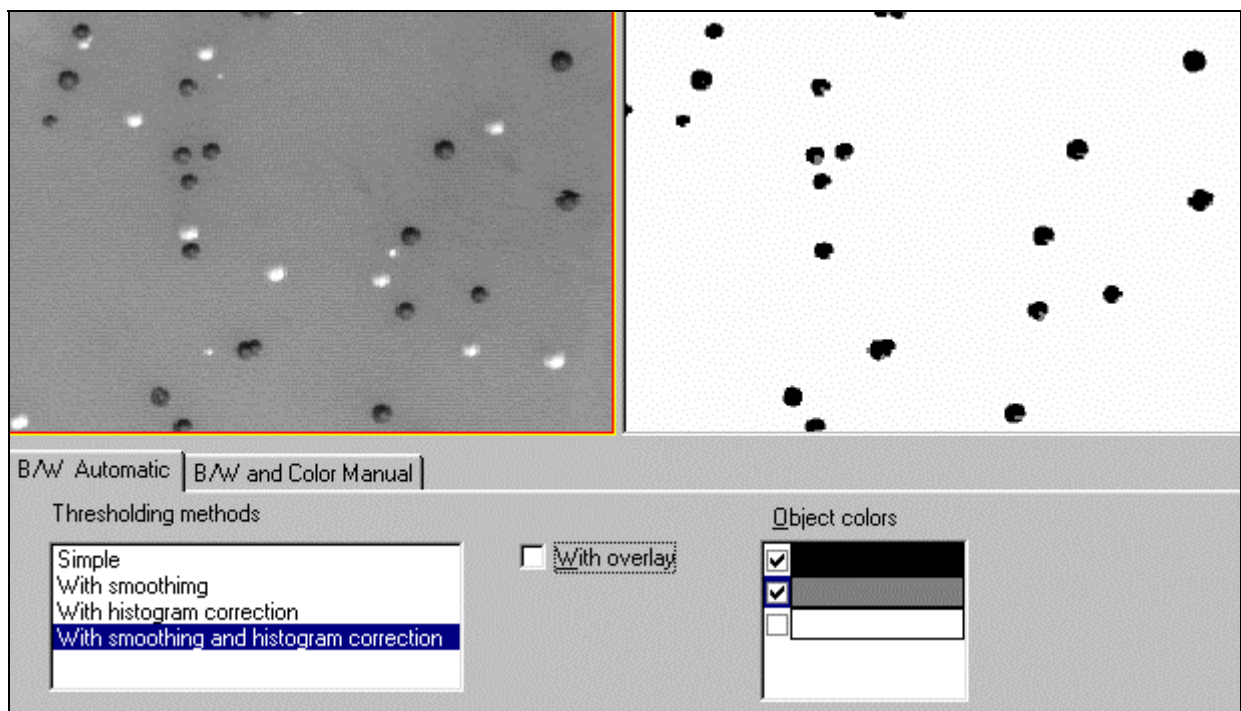


Figure 5

The window is split into two parts. Left: the original image, right: the image with thresholded objects (black and gray).

- **Manual thresholding**

In order to threshold objects (image areas) you can either move the thresholds on the brightness histogram or train the system by sampling the object regions.

To compare the thresholded image with the original one, the image window can be split vertically into two parts. Then the result of objects' thresholding will be displayed in one part of the window and the original image will be shown in another part.

The program also provides an ability to draw objects (which can not be thresholded by above methods) with a mouse pointer.

MEASUREMENTS

VideoTesT - Colony Count allows a user to perform the automatic measurements (as it was mentioned above, in order to be measured automatically the objects should be first thresholded).

Automatic measurements of morphological parameters are performed in all methodics. The measure results are displayed in the automatic measurements data table.

The following parameters can be measured automatically:

- Area: Area
- Perimeter: Perimeter
- Sizes: Length, Width, Average Dimension, Size by X, Size by Y, Average Chord, Diameter Feret Max, Diameter Feret Min, Diameter Feret Average
- Sizes by models: Diameter Equivalent, Max Ellipse Axis, Min Ellipse Axis
- Position: X Coordinate, Y Coordinate
- Density: Average Brightness, Brightness Deviation, Minimum Brightness, Maximum Brightness, Integral Brightness
- Color: Red, Green, Blue, Hue
- Complex objects: Number of Internal Object, Area of Internal Objects, Area Ratio.

Not all available parameters are measured by default in pre-installed methodics. If necessary the operator can select additional parameters to be measured.

6. CLASSIFICATION

The measured objects can be grouped into classes.

To classify the objects **VideoTesT-Colony Count** allows the user to use any of measured morphological and density parameters.

Numerical class limits can be set either automatically or by the operator who can employ one of the following methods:

- filling in the class table by training the system,
- setting the numerical limits in the class table.

In «One Colony» methodic the threshold and measured colonies are classified automatically by their size.

In «Two Colonies», «Three Colonies», and «Four Colonies» methodics the colonies are grouped into classes after thresholding (depending on their brightness or color). Their percentage is calculated.

The statistical data are displayed in the class table. The class table contains information on object classes and statistical data for each class.

The class table can be displayed in three modes: **Classes** (see Figure 6), **Statistics** and **Diagram**.




Manual	Class Name	Diameter equ.	
	Class 1	2.000	3.000
	Class 2	3.000	4.000
	Class 3	4.000	5.000

Figure 6
Class table in the Classes mode.

The **Classes** mode allows the user:

- to change (or set) numerical class limits for each parameter,
- to add/delete classes,
- to change class names and colors.

The **Statistics** (see Figure 7) and **Diagram** (see Figure 8) modes display statistical results for the class data.





Diameter equ.	Class Name	Average	Count	Count %
	Class 1	2.611	6	15.789
	Class 2	3.648	22	57.895
	Class 3	4.407	10	26.316
	Total	3.684	38	100.000

Figure 7

Class table in the Statistics mode.

The Statistics mode displays results of statistical treatment for each class and for all classes in total.

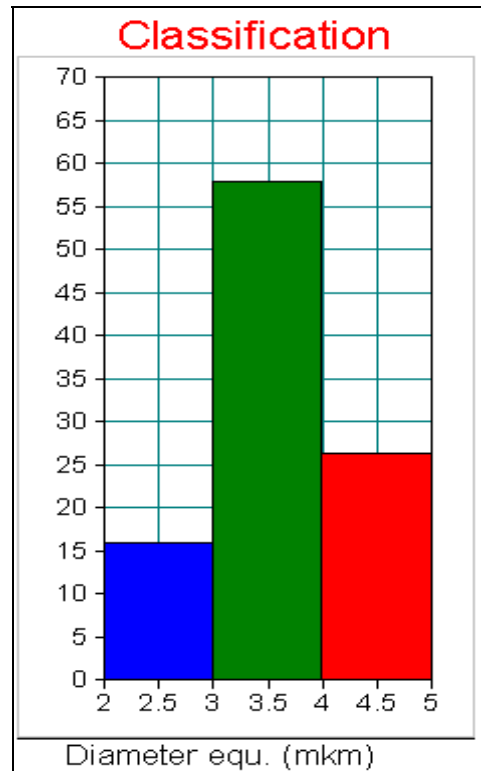


Figure 8

Class table in the Diagram mode.

The following statistical parameters can be calculated with **VideoTesT-Colony Count**:

- Average Mean value of the chosen parameter.
- Median Parameter value which is in the middle of all values (a number of smaller values equals a number of greater values).
- Av. Error Absolute error of the parameter average value.
- Std. Dev. Standard deviation of the average value.
- Min The parameter minimum value.
- Max The parameter maximum value.
- Var. Coeff. Variation of the parameter average value. Counted by %.
- Count The number of objects in a class.
- Obj. Area Total area of all objects.
- Specific quantity Number of objects per area unit.
- Excess Describes the relative irregularity of a distribution compared with the normal distribution.
- Asymmetry Distribution asymmetry around its average.

The relative percentage parameters are also calculated with the program. They include Area Percentage, Area Percentage Error, Count Percentage.

Also, the stereological parameters can be calculated if it is required to characterize three-dimensional microstructural features of the material based on measurements made on two-dimensional planes. The

stereological parameters include Volume Fraction, Surface Area, Specific Surface, Average Chord, and Average Distance.

7. DATA EXPORT

VIA CLIPBOARD

The images and data can be exported to other Windows applications (MS Word, MS Excel, etc.) via clipboard.

TO MS EXCEL

All measure data can be sent to MS Excel for further treatment. **VideoTesT-Colony Count** is installed with the MS Excel book (file VTBOOK.XLS in VideoTesT catalogue) to process measure data in MS Excel 7.0.

TO VIDEOTEST-ALBUM IMAGE DATABASE

For archiving purposes you can send the original or enhanced images, and data to VideoTesT-Album image database. This is a separate software which is installed together with **VideoTesT-Colony Count** program. VideoTesT-Album provides an ability to store images with compression, sort them, create queries, generate and print reports. For more details refer to VideoTesT-Album User Guide.

8. ABOUT DEVELOPER

Since 1990 VideoTesT, Ltd. (former Ista-VideoTesT, Ltd.) has been focusing its activity on development of image analysis software, integration of PC-based VideoTesT Image Analysis Systems, automation and implementation of the image analysis methods in the Life Sciences, Medicine, Material Sciences, and Manufacturing fields.

VideoTesT software includes several universal and specialized programs.

Universal programs:

- *VideoTesT 4.0* - new software package for image analysis. On the basis of VideoTesT 4.0 three programs are developed:
 - VideoTesT-Capture 4.0 (image acquisition, editing and enhancement),
 - VideoTesT-Size 4.0 (image acquisition, editing, enhancement and *manual measurements on the images*),
 - VideoTesT-Master 4.0 (image acquisition, annotation, enhancement, manual and *automatic measurements on the images, and ability to create applications - methodics - for automatic image analysis*).
- *VideoTesT-Size 5.0* – new software for image acquisition, annotation, enhancement and manual measurements on the images.
- *VideoTesT-Album 3.0* - image database.
- *VideoTesT-Album 4.0* - image database.

Special programs:

- *VideoTesT-Master (Morphology) 4.0* - VideoTesT-Master with pre-installed bio-medical applications.
- *VideoTesT-Master (Structure) 4.0* - VideoTesT-Master with pre-installed applications for material analysis.
- *VideoTesT-Karyo 2.1* - chromosome analysis: karyotyping and ideograms.
- *VideoTesT-Karyo 3.1* – new software for chromosome analysis: high accuracy of karyotyping.
- *VideoTesT-Sperm 1.2* - sperm analysis (motility and morphology).

- *VideoTesT-FISH* - FISH analysis.
- *VideoTesT-Colony Count* - count and sizing the bacteria colonies.

For additional information on VideoTesT products, please contact:

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