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FROM IMAGE TO ANALYSIS: Object detection and measurement

IMAGE ANALYZERS, both manual and automated, allow users to identify objects and to categorize them using various geometric parameters. Densitometric identification and categorization is also possible with some image analysis equipment. Although image analyzers are not a recent development, many of their applications are new. Technological developments in the last decade have extended the capabilities of image analyzers by increasing speed, accuracy, and flexibility. The instruments have also become significantly more affordable.

With the expanded capability and increased affordability of image analysis systems, applications for their use have multiplied. In the life sciences, image analysis is used in microbiology, for cell and bacteria counting and for determining zones of inhibition of antibiotics; in marine biology, for counting plankton and measuring the growth of ocean bacteria; in virology, for counting viral plaques; in toxicology, for counting cells in trachea sections, and in measuring areas of glands and alveolar cell sizes; and in pathology, for measuring muscle fibers. Image analysis has also been used to count red blood cells in immunology studies. In the field of agriculture, image analyzers have been used to study the size and distribution of insecticide spray droplets.

The physical sciences represent an expanding area for the use of automated image analysis equipment. Image analysis can be used in metallurgy to determine grain size, inclusion length distributions, porosity, and particle sizing. It has also been used to count and analyze the distribution of bubbles in glass products and the size and distribution of emulsion particles in photographic products.

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The grid matrix method has been automated for a number of applications using digitizer tablets. This method is a very effective means of obtaining geometric information from an image with a limited amount of human interaction and at a relatively low cost. The object of interest is outlined on an electronic grid pad using a pen stylus or cross-hair cursor. A computer provides geometric data based on grid point intersection information transmitted and stored during the outlining operation. This kind of

Figure 1 *Image analyzers.*

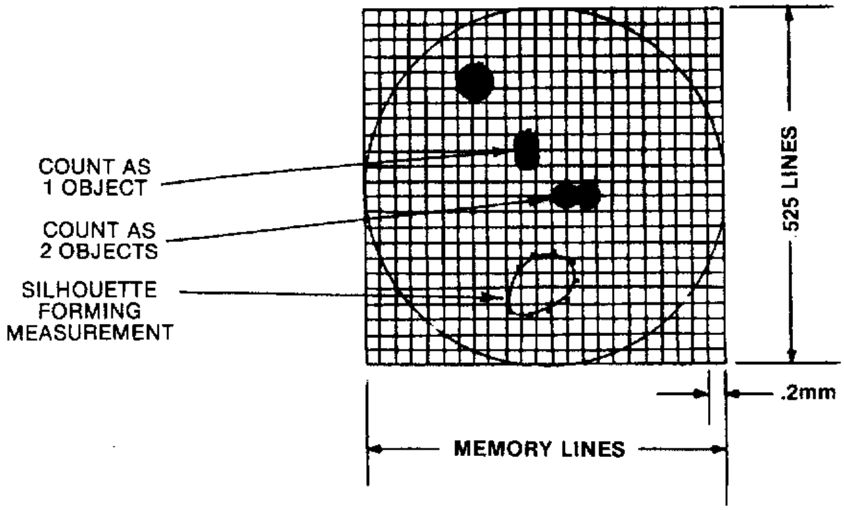

Figure 3 *Example of the use of leading and trailing edge detection.*

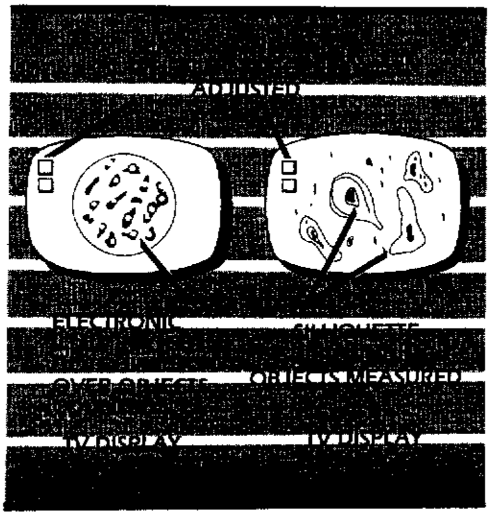

Figure 2 *Indicators showing incorrect sensitivity setting in manual mode.*

interactive system is very useful when objects of interest are not easily separated from the background, but it lacks the speed of more automated systems and cannot be used to distinguish grey levels.

In the last decade, refinements in video processing techniques have contributed to the development and introduction of more fully automated image analysis equipment. More samples can now be processed in shorter time periods, resulting in a reduction in both labor costs and operator fatigue. With new software developments, a wider range of statistical information is now available and statistical discrimination has been enhanced by these additional parameters.

The basic automatic image analyzer consists of a video camera, an image detector, a video monitor, and a light source. The video camera is usually mounted on a microscope or over a light source when working with macroscopic images. Using this type of system, the operator selects a suitable threshold to discriminate the objects of interest from other objects and the background.

The video camera used in the system functions much like any camera. Improper lighting will result in poor picture quality. Focus and aperture settings are also critical.

The Artek image analyzers (models 880 and 980) shown in *Figure 1* assist the operator in achieving optimum settings by automatically adjusting the sensitivity threshold. If manual sensitivity adjustment is desired, illuminated indicating boxes will flash when the sensitivity is set too high or too low (see *Figure 2*). The operator can also watch the monitor to see the objects that are being scanned, because each analyzed object is flagged with an illuminated dot.

Extremely successful results have been obtained using this type of analyzer for counting and measuring the area of bacteria, cells, grains, holes in paper products, leaves, and many other particles found in various industrial applications. The objects being analyzed can be of any shape.

Various illumination modes are possible, permitting analysis of a variety of object configurations. Transmitted or bottom light is useful when light is able to penetrate agar. Darkfield illumination is

Figure 4 *Modular image analysis system.*

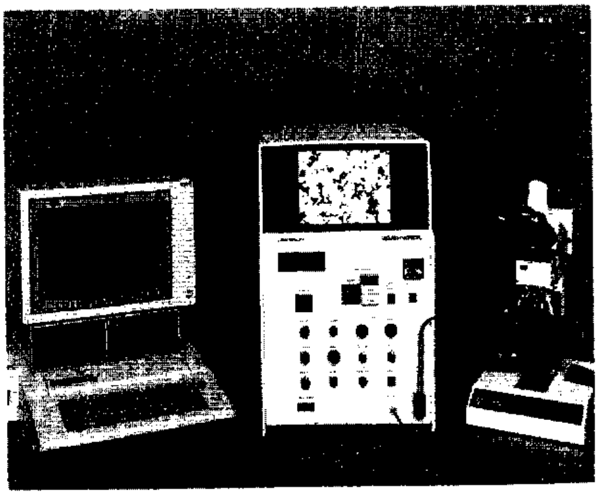

Figure 5 *A space-saving image analysis system.*

used for applications where contrast exists between objects and the background. A switch permits the operator to change from analyzing dark objects on a light background to light objects on a dark background. Top-lighting is available to permit quantification of opaque objects on an opaque layer. This type of analyzer allows a choice of scanning fields by shape, size, and position. A control dial provides a selection of circular, square, or rectangular apertures. The size and position of the aperture are adjusted by separate controls. The adjustable electronic aperture permits the isolation of an individual object or a group of objects for separate analysis. Size discrimination is easily set to eliminate objects that are smaller than the size threshold set.

There are various types of detection systems for electronically determining the boundaries of the objects from the video signal, and in the basic image analyzers described, edge detection (leading and trailing) is employed (see *Figure 3*). In less than one tenth of a second, the field of view is scanned with 525 horizontal scan lines. Vertical lines placed 0.2 mm apart have been stored in memory and these are used to identify the location of the object (or objects) to be counted. An object can be counted only if it touches one of the vertical grid lines.

When the system is initiated, the first horizontal scan line begins to scan from left to right and an internal clock begins counting vertical lines as soon as the first vertical line is intersected until the horizontal scan line strikes an object having a contrast different from the background. The information is placed in memory and the next line repeats the process. If there is no break in contrast between the previous line and the new horizontal line, the same information will go into memory. The object will be counted as soon as a successive scan line no longer finds a break in contrast.

This type of detection cannot discriminate between two objects which join each other in the vertical direction because the horizontal scan lines will not see a break in contrast. However, if the objects are joined in the horizontal direction, both objects will be counted separately because the horizontal scan line will have touched the top periphery of the first object, left the object, and then intersected with the top periphery of the adjacent object.

The vertical grid lines are placed 0.2 mm apart, and this defines the resolution of the system at a magnification of 1 ×. Increasing the magnification will increase the resolution; the objects to be analyzed only need to be 0.2 mm or greater at the detection point of the camera. With magnifications greater than 1000 ×, objects as small as 0.2 μm can be analyzed.

Edge detection in the Artek image analyzers works in a slightly different manner when measuring the area of objects in a field. The vertical grid lines are still used to identify the proximity of each object within the field. However, as the horizontal scan line intercepts the leading edge of the object, it superimposes a tiny illuminated dot at the point of intercept. Using the internal clock, the system will store the time it takes for the horizontal scan line to reach the trailing edge of the object and it will superimpose another dot at the point of intercept. This process is repeated with all succeeding scan lines. Regardless of the object's shape, it will have been completely silhouetted with dots around the periphery. Regardless of the number of configurations of the objects, this entire process is accomplished within one tenth of a second.

The basic systems described are not suitable for applications involving numerous overlapping objects and objects of low contrast. Statistical information with these systems is limited to the number of objects, total object area, and the percentage of total aperture area occupied by objects. Many image analysis systems are now available with statistical software packages. Optional expansion modules permit human interaction with the images.

The Artek 810 image analysis system (see *Figure 4*) is a modular system that is easily interfaced with computers and peripherals. The basic component of the expandable image analysis system is the 982 counter which in addition to edge detection, features grey level (density) detection. This is particularly useful in the analysis of metallurgical samples and objects of low contrast. Automatic size histograms are also available. The circuit includes localized background correction which aids in compensating for local image abnormalities and nonuniform lighting.

In order to obtain additional geometric parameters and to enable the operator to manipulate images, the images must be digitized and sent to a video editor which memorizes the image as picture points or pixels. Using the 940 image editor and video memory module in conjunction with the 982 counter, the operator can isolate an object in the field, separate overlapping objects through erosion and dilation, add or remove areas to or from objects, delete objects, cut objects, create a special aperture, and fill in void areas of interest. Digitizing the image increases the number of scan functions possible so that a number of useful statistical calculations can be provided including: perimeter, area, vertical feret (tangent-to-tangent distance in a specified direction), longest horizontal chord, longest dimension, X bar and Y bar (coordinates of the centroid), longest dimension/longest chord, circularity, width, length, biovolume, and length/width.

The use of this dimensional information also permits discrimination by shape and size. With measurements on each object in the field, dimensional information can be obtained on: autoradiographs, nuclear tracks, roots, ores, component inspection, pigments, colonies, photographs, eggs, histology sections, abrasives, powders, cells, fibers, corrosion areas, hydraulic contaminants, paper contaminants, cement, x-rays, seeds, and pores.

User requirements for space and ease of operation in microscopic applications have brought about some basic design changes in the appearance of the instruments. Recently, UNITRON IMAGETEK Systems introduced a space-saving image analysis system that has all control functions arranged in a vertical configuration (see *Figure 5*). The monitor is located at the same level as the microscope eyepieces. Use of bench space has been reduced to a minimum and operator eye movement from the eyepiece to the monitor has been restricted to the same plane.

When selecting an image analysis system, special attention should be paid to the capability of the image processor; the software currently available for the system and the ease with which it can be modified; the various cameras and image tubes available; the detection modes used; and the warranty and the availability of service. Careful selection now can result in a system that is compatible with future requirements.