Image Analysis: Part 1: Where Is It Going?

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Classical image analysis programs do not imitate the way humans analyze images. By paying close attention to how humans extract information from images, more advanced, more powerful image analysis systems can be designed.

Most current image analysis techniques develop data by counting pixels included in a user-identified line or area. This data analysis technique is some-what limiting in that only rudimentary mathematical processing techniques can be used, primarily addition and subtraction. In contrast, human beings recognize size, shape, roughness, color, texture, and other image features. We process this data artlessly and we are able to describe the viewed objects with a wide variety of verbal descriptors and comparisons. Typically we describe an object's size by comparing it with an object of known size, golf ball-sized hail, for example. Similarly we describe an object's shape by comparing it with a known, familiar shape. We may use such terms as oval, round, rectangular, needle-like, irregular or even lumpy. We even add qualifiers such as very angular, rather spherical and so on. Thus humans have an inherent ability to recognize partial shapes and relative sizes. If similar techniques can be incorporated into advanced image analysis systems, analysis results can be generated that relate more closely to the human experience and at the same time, make possible more powerful mathematical analysis of the image.

Image Analysis

Although humans are very good at analyzing images, the process is very 'labor intensive'. If we can get a machine to do the analysis process for us, we are saved a lot of effort. Also there is a technical need for numerical descriptors for comparing and analyzing images that can be conveniently supplied by image analyzers. Such numerical descriptors add precision to the analysis process, a capability difficult to achieve using verbal terms.

Reviewing Classical Image Analysis

As of 1995, classical image analysis is a 40 year old technology concerned with the measurement of elementary image features. This is usually done by pixel counting. The actual analysis is simple, even primitive. For example, consider the object in Figure 1. Let us use an image analyzer to measure the following characteristics:

- The intercept length
- The perimeter of the object
- The area of the object

We know from simple algebraic geometry that the intercept is the length of the line between X_1Y_1 and X_2Y_2 is the absolute value of:

$$I = \sqrt{((X_1 - X_2)^2 + (Y_1 - Y_2)^2)}$$

Classical image analyzers do not determine the intercept using such equations. Instead they very simply and elegantly count the pixels. By knowing the size of each pixel - let's call it 'f', the line length can be determined by counting the number of pixels in the line and multiply it by the pixel width to determine the line length. Thus the line length is 'nf'. There is no need for algebra or more sophisticated processes, just a simple bit of arithmetic, as in multiplication, does the trick. Determining the perimeter of an object is a similar process. It is determined by counting the number of pixels making up the perimeter and multiplying this number by the pixel width, 'f'. Again, simple arithmetic is all that is required.

In the case of area measurement, once the area of each pixel, 'C', is known, the image analyzer simply counts the pixels within a defined area and multiplies this times the pixel area to produce 'nC', the object's area.

Notice that in each case only simple arithmetic is required to quantify each image feature. Because classical image analyzers only count pixels, is that a drawback? Well, yes and no. If you want a fast and easily produced number for a basic parameter, than pixel counting is certainly a good way to get at it. Certainly this is the way it has been done. However there are a number of desirable image analysis techniques, some requiring the use of such mathematical

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Yet Another Method For Making 2 X 2 Projection Slides Directly From EM Negatives

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Several excellent techniques for the production of high quality projection electron micrographs have been described in past issues of this newsletter. I am surprised, however, that the following method has not been presented. It was taught to me by Dr. Russell Brynes more than 15 year ago.

Our darkroom is outfitted with a Durst S-45 EM photographic enlarger and AGFA Radioprint processor. The AGFA phototypesetting processor is a four bath stabilization system used for making continuous-tone B&W prints on film or paper. The film of choice for this application is AFGA Copyproof RA711p, a very high contrast orthochromatic film. I cut 8" x 10" sheets into 2" x 8" strips and use a fabricated cardboard guide taped to the enlarger baseboard to position the film during exposure.

Condenser configuration for 3 1/4" x 4" film or plates is 240T (inverted) over 240T with 135 mm lens for adequate reduction to 35 mm. 35 mm negatives are printed by condenser configuration 200T over 130T with 100 mm lens. The point light source must be aligned for optimal exposure after changing configurations. I like to run the voltage low and make a 2 second exposure because the film is hand held in place on the guide. Three exposures will fit on each strip. By replacing the stabilizer component of the processor with fixer, archival quality can be attained. Projection slides produced by this method 15 years ago are still clear and have not deteriorated.

I prefer Kodak Ready Mounts to mount projection slides, however, square-hole mounts for slides made from 35 mm negatives are no longer available from Kodak. Recently, I have gotten them from another manufacturer through a local camera shop.

This method is incredibly fast (dry to dry processing in 1 minute) and gives high quality results. In a high volume, quick turnaround laboratory like ours, it is the simplest and most economical way to go.

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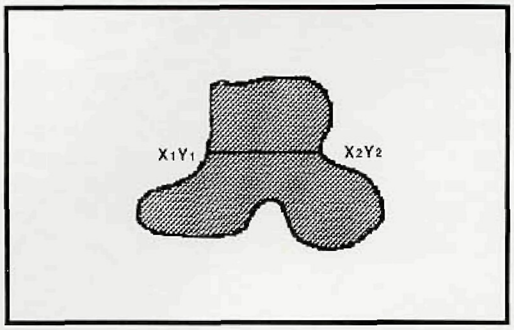

Figure 1: An irregular object

techiques as calculus and differential equations, that cannot be used when data has been acquired using only counting techniques. To apply these sophisticated techniques, more advanced image analysis techniques need to be employed. Additionally, these advanced techniques will result in data more closely approximating the empirical results of human image evaluation.

Future analysis techniques will do more than count pixels. Upgraded image analysis will start with a set of (x,y) coordinate points on image boundaries. From this, the precise functional forms of the image boundaries will be determined. These can then be analyzed using many techniques of modern mathematics and reveal image characteristics that classical image analysis can never show.

Basic Principals of Image Analysis

Four criteria must be met by any advanced image analysis technique in order to be considered effective and of significant value. These criteria are: invariance, information reduction, lack of distortion, and the resulting data must relate to human perception.

- Invariance. To extract useful information from the image descriptors, it is usually necessary to convert it into an invariant form, that is, no variables can exist in the data that can modify the image when mathematically processed.
- Information Reduction. It is vitally important that the process of extracting information from the image will condense the information into a usable and understandable form.
- Lack of Distortion. The process of converting the coordinate points of any boundary or image highlight to invariant descriptors should occur without distorting any data or image feature.
- Relate to Human Perception. The anticipated power of advanced image analysis is that it will include not only common geometric and mathematical data, such as areas, lengths and means, but human observers should be able to relate to the extracted information using verbal descriptors, such as triangular, oval-shaped, etc.

Shape Analysis is Image Analysis

Once the shape (or morphology) of a closed curve is fully characterized, the resulting data will contain not only classical geometric image descriptors but shape and symmetrical information as well. These improved methods of morphological analysis are required to determine the boundary function of an object. Once this function is determined, appropriate equations of mathematical physics can be integrated over the surface (i.e., over the boundary). These geometric boundary values can be used as the boundary conditions of the differential equations useful in analyzing the object.

Using Figure 1 as an example, we can describe this figure as an irregular curvilinear figure. In terms of morphological image analysis terms, the figure is described as having an irregular function. The manipulation of this boundary function by various mathematical techniques allows us to articulate key morphic features of the image that are scientificly rigorous and at the same time are in line with our human experience.

The key to effective description of an object using morphological analysis is to have standards to which the image can be compared. One such standard involves various rules of symmetry. Another standard relates to possible shapes for comparing various samples. These standards will be covered in the next two parts of this series.

This series of articles is extract from a soon-to-be-published *Image Analysis Source Book* written by Dr. Beddow and is featured as part of a one-day short course being offered in September and October, 1995.

Cleaning Osmium Black from Glass Bottles

Michael S. Forbes, Ph.D.

In issue #94-9 of *Microscopy Today*, Angela Welford offers a protocol for cleaning lead citrate-derived precipitate (lead carbonate?) from glass containers and asks for a method for restoring osmium storage bottles to cleanliness.

A number of techniques in neurobiology depend upon some form of silver staining to reveal elements such as degenerating neurons; the glassware for such involved regimens typically goes through a scrupulous cleaning process which ends with immersion for several days in diluted nitric acid (say, a 1:3 dilution of 90% "fuming" nitric acid in water). This nitric acid step was suggested to me by a more neuroanatomically inclined colleague for use on osmium-stained glass containers as well, and I have found it to work nicely (even on the bottle caps, if one is using Wheaton-type bottles).