Extraction of data from digital images of microorganisms
by Paul Andrew Shope

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Computer Science
Montana State University

Abstract:
A method is proposed for the extraction of data from digital images of microorganisms. Digital images of microorganisms have certain characteristics which make information extraction difficult. These characteristics are identified, and four steps are suggested to effectively overcome the obstacles inherent in these images. The steps are image enhancement, microbe feature extraction, microbe feature representation, and microbe recognition and enumeration. Examples of the techniques which comprise these steps are shown and each technique's effectiveness is described.

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Paul Andrew Shope

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4/26/93
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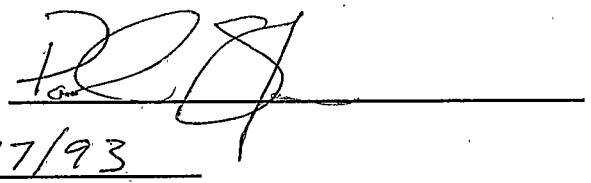
Signature 

Date 4/27/93

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ABSTRACT

A method is proposed for the extraction of data from digital images of microorganisms. Digital images of microorganisms have certain characteristics which make information extraction difficult. These characteristics are identified, and four steps are suggested to effectively overcome the obstacles inherent in these images. The steps are image enhancement, microbe feature extraction, microbe feature representation, and microbe recognition and enumeration. Examples of the techniques which comprise these steps are shown and each technique's effectiveness is described.

CHAPTER 1

INTRODUCTION

Image Processing is a field which has grown rapidly over the last decade. Digital image processing can be defined as the procedures used to manipulate digital images to:

1) have improved pictorial information for human interpretation; [Gonzalez]

2) extract data autonomously.

There have been many advances in image processing techniques and new techniques are being devised all the time. Yet, even with these advances, little is known about how to apply these techniques to many specific problems. Almost all problems require more than one image processing step in order to attain the desired result. This is due to the complex nature of imaging problems. Therefore, the formulation of systematic approaches is vital to solve these problems efficiently. These formulations could be compared and blended into one image processing strategy which would more closely resemble the human visual system. It is toward this goal that this paper tries to better define one specific image processing problem and the corresponding systems.

The problem which will be addressed involves collecting information from digital images of microorganisms. This type of information is useful for researchers in microbiology, environmental engineering, health, and many other related fields. The problem is to develop procedures for manipulating images and extracting information in a structured and robust manner, so that they can be applied to many applications. This means that the process must not only be effective for solving a given imaging problem, but also consistent over a range of problems involving microorganisms.

The goal is to extract data from images. There are two categories of data that need to be collected for this problem. These are 1) quantitative data and 2) qualitative data. These two categories are not independent of each other, but are simply used as a means to entirely describe the data being extracted. Under the category of quantitative data fall such things as the width, height, number, and position of microbes in the image. Shape, texture and classification of microbes are qualitative in nature. These two data categories comprise the goal of image processing as applied to images of microorganisms.

It is beneficial to understand the hindrances or obstacles which may present themselves during the data extraction process. The images may vary in many different ways; microorganisms are often different sizes and shapes. This makes finding one effective process more difficult. Images

can be captured at any number of different magnifications. Thus, a single variety of microorganism can appear at almost any size, depending on the scale of the image. Image collection factors also add to the inconsistent condition of images. In many cases, images contain one or more of the following image collection problems:

1. Resolution

If the image resolution is not great enough, important detail will be lost, making data retrieval more difficult. Increasing resolution through magnification may be necessary if certain types of data are to be collected.

2. Lighting

Areas with too much or too little light may fade out or hide detail, whereas areas with uneven lighting may hinder feature extraction. Images should be collected with as even lighting and as much contrast as possible.

3. Blur

Blur can be caused by a combination of the two problems listed above (Resolution and Lighting) or by movement of some part of the image collection mechanism. This movement might be the

microscope, the slide, or the microorganisms themselves. These parts of the image collection mechanism should be kept as uniform as possible to enable the greatest amount of information to be transferred to the image.

4. Noise

Noise is extraneous image data which has polluted the image collection process at some point. For example, noise can be introduced by the image capturing camera, or in the process of digitization.

5. Experimental Conditions

Experimental Conditions, such as warping of the media to be imaged and varying surface characteristics, cause unwanted visual effects to occur in the image.

These are the elements (size, shape, scale, and collection factors) which make images vary greatly. If the effect of these elements can be reduced, either before or after processing, the problem of extracting data becomes increasingly simple.

There are other obstacles besides image variation which can complicate the solution. One of these involves the use of processing

resources. Processing time and space will often need to be minimized. The method used to solve this problem needs to be independent of processing time and space available as much as possible. Having a certain limitation of processing time or space should not restrict one from applying this approach. The last obstacle which should be mentioned is that of human interaction. The interaction of humans at various points in the process of data extraction can be very useful. For instance, a human might better be able to adjust a certain parameter of an imaging function so that separation of cells is the most apparent. This interaction may not be available or desired, but also could be necessary in some cases. Thus, the process for obtaining microorganism data should not depend on, nor rule out the use of human interaction.

The last step in defining the problem, is to analyze the tools one has available to build a solution. Even though tools are definitely part of the solution, they are also part of the problem. The main tool used to implement this process will be a serial-based algorithmic language (in this case the C language). This makes certain aspects of image processing more difficult since visual data seems better suited for parallel processing. However, the approach which will be presented in this paper will hopefully allow for any implementation of the steps involved, whether they be serial-based, parallel-based, or some combination of the two.

The problem can be summarized as consisting of three components.

These are:

1) The Goal

- Extract the desired quantitative and qualitative data from digital images.

2) The Obstacles

- Varying image data including characteristics of microorganisms(size, shape, etc.), scale of the images, and image collection factors;
- Utilizing processing resources efficiently;
- Allowing for but not depending on human interaction.

3) The Tool

- A serial-based algorithmic programming language.

Given these basic components, an approach can be formulated. The methods developed in this approach are required to be both systematic and flexible. In order to be systematic the approach must attack the problem in a logical manner, and have ways of overcoming each obstacle. To do this, the desired output should be well defined. The image processing steps will depend on what kind of data is to be extracted. For example, the steps for finding the positions of microorganisms will differ from the steps for enumerating the microorganisms. Secondly, the problem must be broken down into smaller more manageable components. Each step should correspond to a certain sub-goal, each being fairly simple in nature. Lastly, the steps involved in the process must make measured strides toward the goal without the loss of significant data. One step in an image processing

problem might be to find the edges of the objects in an image. If this function is performed carelessly, valuable feature information, which may be needed in a upcoming step, is lost.

The second requirement is flexibility. This means that the process should allow for different types of input data and not be effective only on specific images. This requirement should not be difficult to meet if the range of images is chosen in a reasonable manner. If the input space is too large, the approach will lose its effectiveness.

CHAPTER 2

OUTLINE OF APPROACH

The data extraction process consists of a series of steps, where each step consists of certain types of operations which may be performed on the input images. Note that the process may end after any step, depending on the type of data to be extracted. For example, visual clarity problems can normally be solved by applying the techniques in the first step.

A formal definition of a gray scale image is an N x M array where each element in the array contains an integer value which approximates the continuous image f(x,y). [Gonzalez]

$$\begin{bmatrix} f(0,0) & f(0,1) & \cdots & f(0, M-1) \\ f(1,0) & f(1,1) & \cdots & f(1, M-1) \\ \vdots & & & \\ f(N-1, 0) & f(N-1, 1) & \cdots & f(N-1,M-1) \end{bmatrix}$$

This definition of an image will be assumed in all processing examples that follow.

Image enhancement is the first step in the approach. Image enhancement consists of all operations which globally reduce unwanted image information and emphasize the pertinent image data. Image enhancement differs from later steps in that it is more preparation than manipulation. The main goal of enhancement techniques is to process an image so that the result is more suitable for a specific application. [Gonzalez] Specific image enhancement operations include:

A. Histogram Processing and Contrast Adjustment

- These operations affect how light or dark the image appears.

B. Image Thresholding

- Thresholding tries to separate the foreground data (objects) from the background data.

C. Smoothing Filters

- These filters tend to blur the image and reduce the amount of noise in the image.

D. Sharpening Filters

- These filters highlight detail and emphasize object separation from background.

The second step in the approach is feature extraction. The microorganisms in images have certain features which can be differentiated from the surrounding image data by means of image processing operations. The operation used to find these features is:

Edge Detection

- Edges between objects and background are found. Edges can be defined as places in an image where pixels differ in some specified way (intensity, texture, etc.)

Feature Representation is the next step in the approach. Feature Representation takes the features gathered in the last step and processes them so they are normalized and easier to manipulate. The success of this step is dependent on how effective the feature extraction step was. This step dependency may be found at each level of the approach. The operations involved in this step include:

A. Edge Reconstruction

- Edges Shapes are built as closed forms so that they may be more easily represented.

B. Shape Number Formulation

- The shape of an object is recorded in a normalized way through the formulation of chain codes and shape numbers.

C. Signature Formulation

- The shape of a candidate microbe is represented by its signature.

The last step in the process is microbe recognition. In this step, the information collected in the previous steps is examined and the objects are classified as microorganisms, undesired objects, or noise data. The operations which make up this step are:

A. Classification of Microbes

- Objects are identified and placed in the appropriate category.

B. Enumeration of Microbes

- Objects of the same classification type are counted.

C. Position Identification

- The x and y coordinates of the microbes are identified.

In summary, the steps which comprise the data-collecting process are:

1. **Image Enhancement;**
2. **Microbe Feature Extraction;**
3. **Microbe Feature Representation;**
4. **Microbe Recognition.**

The work which has been done in these areas will be presented in the next chapters. This will provide a framework by which many of the microbial imaging problems can be solved.

CHAPTER 3

IMAGE ENHANCEMENT

Histogram Processing and Contrast Adjustment

The first component of image enhancement which will be discussed is histogram processing. The histogram gives information about the probability of an occurrence of a certain gray-level. [Gonzalez] Histogram equalization attempts to use these probabilities to produce an enhanced image with groups of highly probable pixels spread out among the available pixel levels. It does this by using a transformation function equal to the cumulative distribution of the original probability density function which is derived from the histogram.

For instance, an image where the background and microbe gray-levels are similar is shown in Figure 1. Notice that many of upper gray levels are being wasted, that is, they could be used to emphasize differences in lightness and darkness in the image. After equalization, the entire pixel value range is represented (Figure 2). The histograms which correspond to these images are shown in Figures 3 and 4. The process of histogram

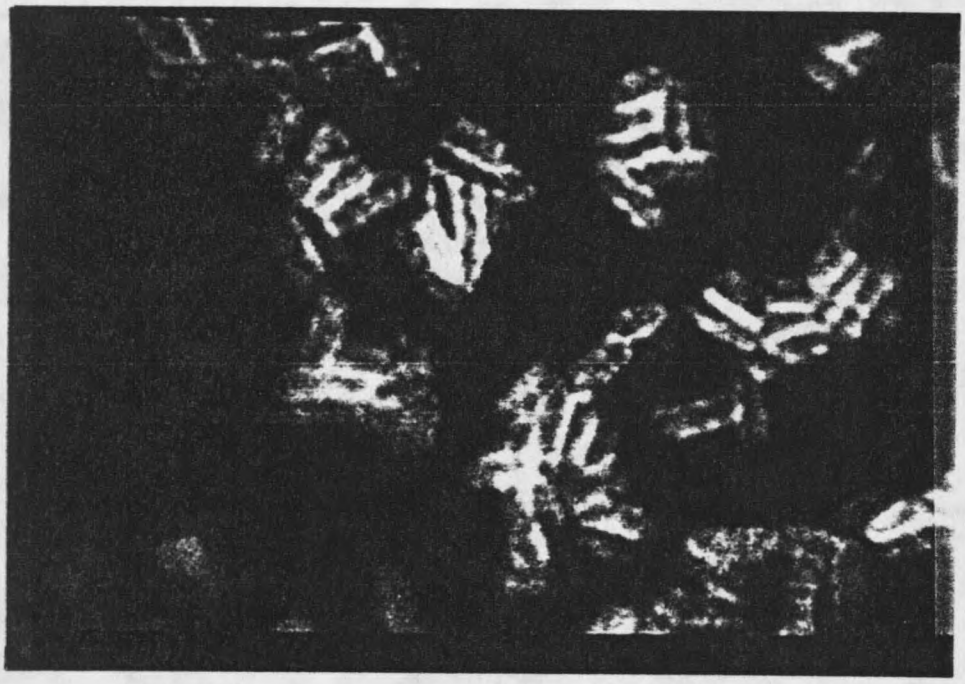


Figure 1 • Image Before Equalization

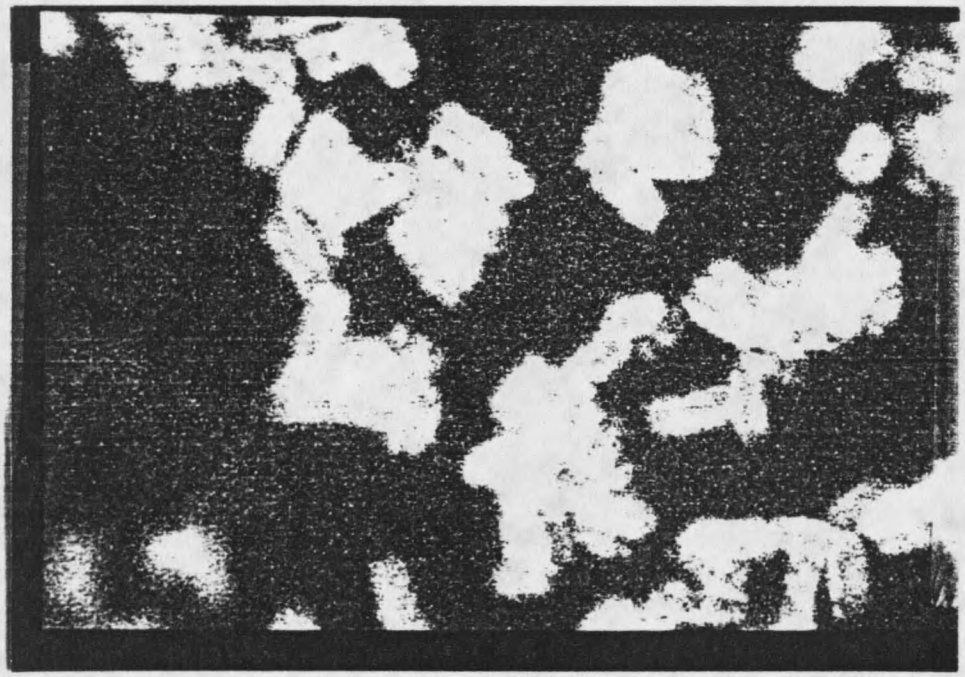


Figure 2 • Image After Equalization

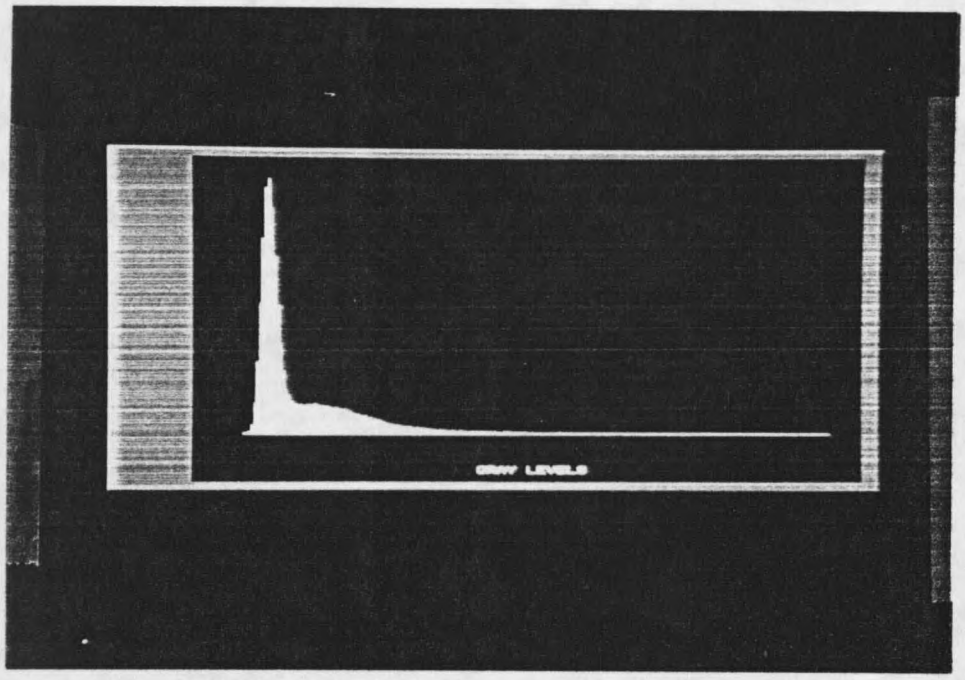


Figure 3 • Histogram of Low Contrast Image

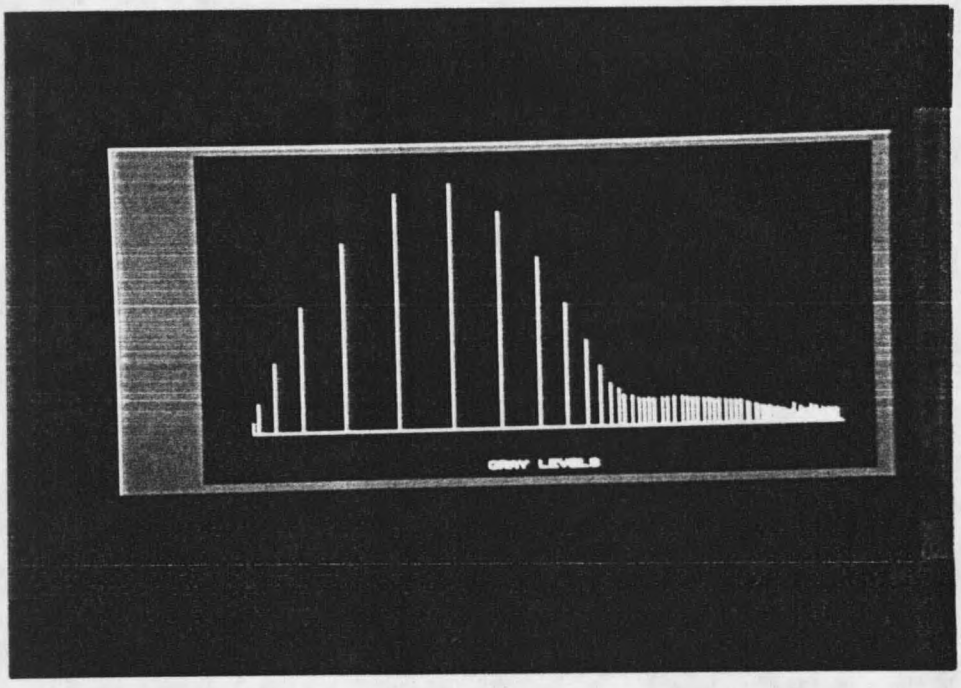


Figure 4 • Histogram of Image After Equalization

equalization may be described in the following way:

1. Obtain the histogram by enumerating each pixel which falls in a given gray-level.

2. Calculate the probability density function based on this histogram. Values will now be between 0 and 1.

3. Calculate the cumulative density function by computing the accumulated probability for each level.

4. Create a transformation function based on this cumulative density function by scaling it to the range of possible pixel values.

5. Apply the transformation function to each pixel in the image.

Histogram equalization is effective for increasing contrast without human interaction. Other methods allow a person to specify a desired histogram modification and have it applied. This would be useful if one knew the gray-level range of features which needed to be emphasized. The easiest way to do this is to:

1. Pick the gray level range to be emphasized;

2. Create a transformation function which adjusts the pixels in this range so they are highly contrasting with the levels which are not to be emphasized;

3. Apply the transformation function.

This process is known as *gray level slicing.* An example of a slicing transformation function and its resulting image are shown in Figures 5 and 6.

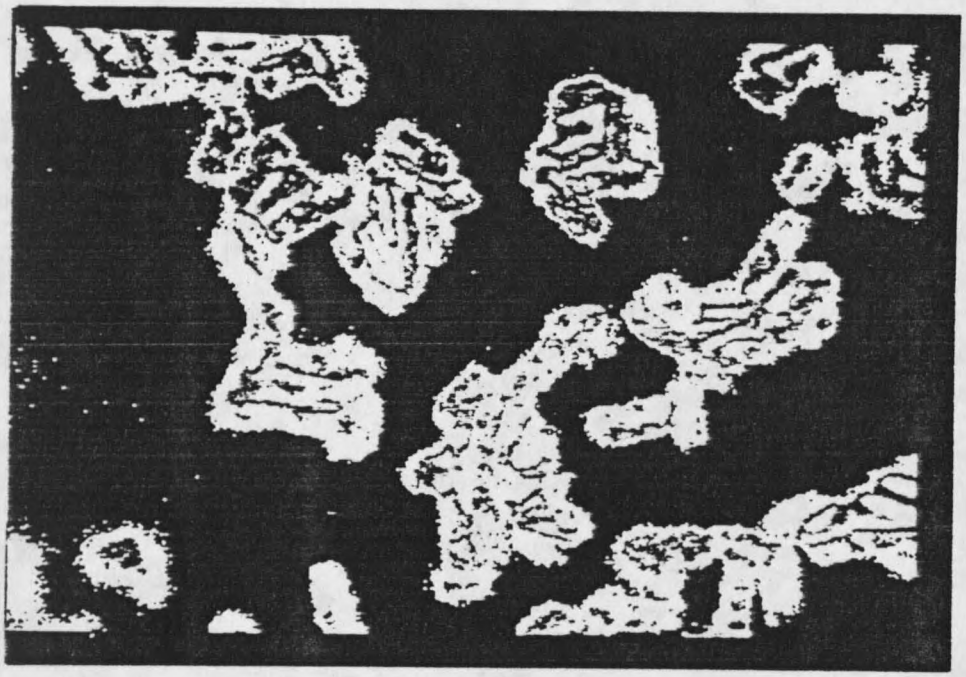


Figure 5 • Gray Level Slicing Image

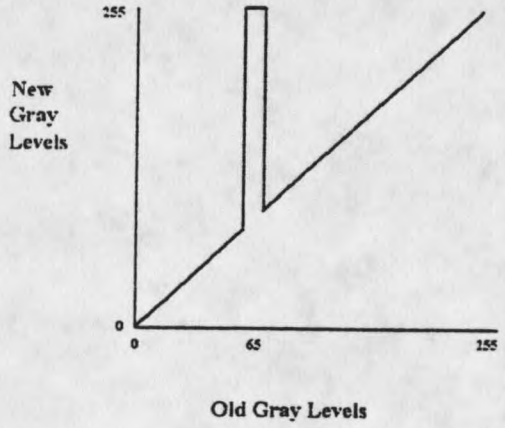


Figure 6 • Gray Level Slicing Transformation Function

A more advanced technique for emphasizing certain gray-levels is histogram specification. In this process:

1. The levels of the original image are equalized as described earlier.

2. A new histogram which has a desirable shape is constructed interactively.

3. The transformation function to equalize an image based on this new histogram is computed.

4. The inverse of this transformation function is applied to the image equalized in step 1 to create an image with the desired histogram.

This process is useful if equalization does not contrast effectively or if there are localities in the histogram which need contrast enhancement.

Thresholding

The techniques described above are all examples of contrast adjustment. Each of them involved taking the original pixel value and assigning it a new value which will hopefully emphasize certain desired characteristics. Taking this idea to the extreme, one could simply divide the gray levels into two or more groups and assign each pixel to one of the groups. This is the concept behind *thresholding*. Thresholding distinguishes pixels that have higher gray values from pixels that have lower gray values. [Haralick] This process is very useful in microbial image processing since it is often the case that there are only two or three major

gray-level ranges in these images. The background is often in one range (and usually the largest), and the microbes are in another range. Thus, if these two ranges can be effectively separated, further processing will be much easier. The difficult part of thresholding an image is picking the point or points which will be used to divide up the image. This can be done interactively by viewing thresholded results using various threshold values and choosing the best. This technique can be effective since a threshold which seems to best emphasize the microbes without losing valuable edge information can be chosen. If human interaction is not available, a threshold can be automatically chosen by algorithmic methods. One such method involves minimizing the within-group variance. [Otsu] If the histogram is bimodal, the histogram thresholding problem is to determine a best threshold separating the two modes of the histogram from each other. [Haralick] One method is to pick a threshold for which the weighted sum of group variances is minimized, where the weights are the probabilities of the respective groups.

This technique is described in the following steps:

1. At each possible threshold value, sequentially compute the within-group variance based on the equation:

$$\sigma^2{}_w(t) = q_1(t)\,\sigma^2{}_1(t) + q_2(t)\,\sigma^2{}_2(t)$$

where t = the threshold value being considered;
$q_1(t)$ = the probability for the group with values less than or equal to t;
$q_2(t)$= the probability for the group with values greater than t;
$\sigma_2 w(t)$= the weighted sum of group variances;
$\sigma^2_1(t)$= the variance for the group with values less than or equal to t;
$\sigma^2_2(t)$= the variance for the group with values greater than t.

[Otsu]

2. Choose the threshold value which produces the smallest within-group variance value;

3. Apply a transformation function based on this threshold.

Another way of computing this thresholding value is to minimize the *Kullback Information Distance* [Kittler, Illingsworth]. The procedure is similar to minimizing the within-group variance except the following equation is used:

$$H=\frac{1+\log(2\pi)}{2}-q_1\log(q_1)-q_2\log(q_2)+\frac{1}{2}(q_1\log(\sigma_1^2)+q_2\log(\sigma_2^2))$$

where q and σ have the same meaning as in the within-group variance equation and H is the variable to be minimized.

Both of these techniques can be effective in choosing a threshold if the modes which comprise the image are Gaussian in nature, and are not disrupted by interfering distributions. Examples of microbial images thresholded using these two techniques are shown in Figures 8 and 9.

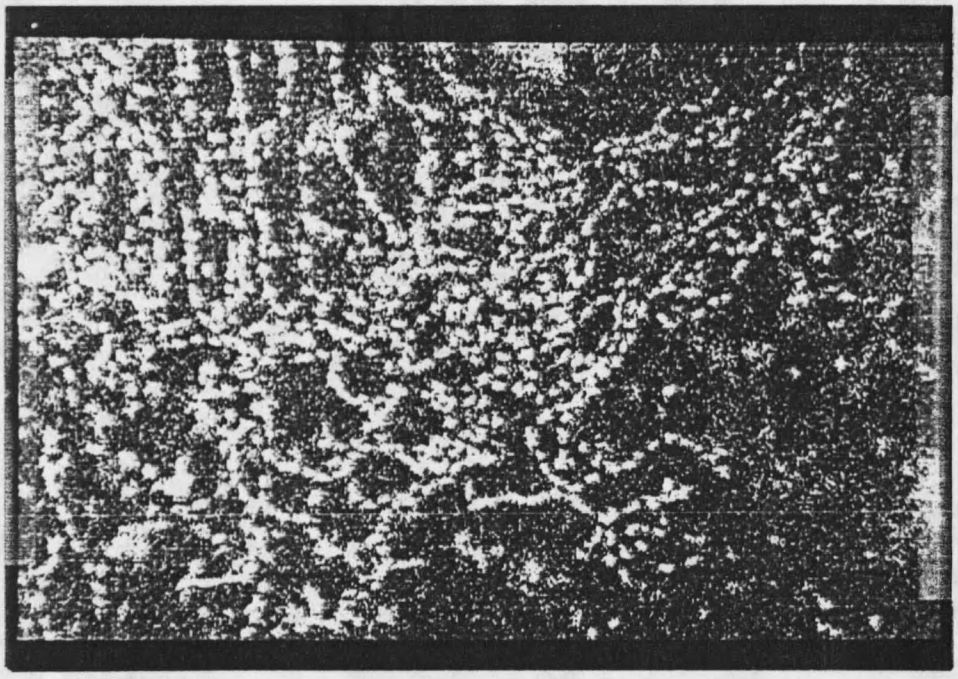


Figure 7 • Image Before Thresholding

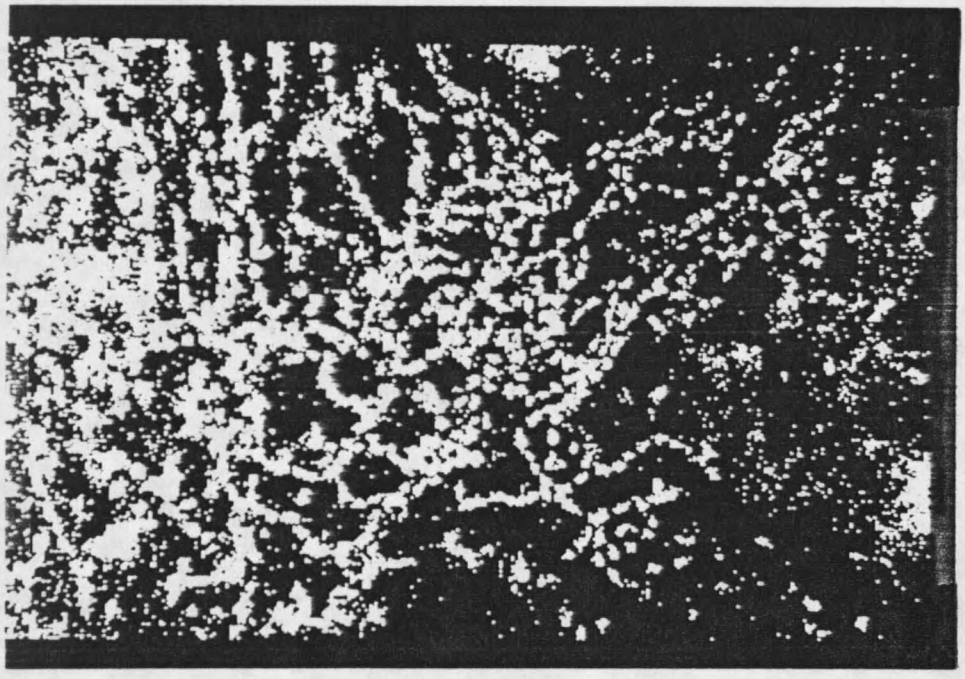


Figure 8 • Image Thresholded with Otsu Threshold

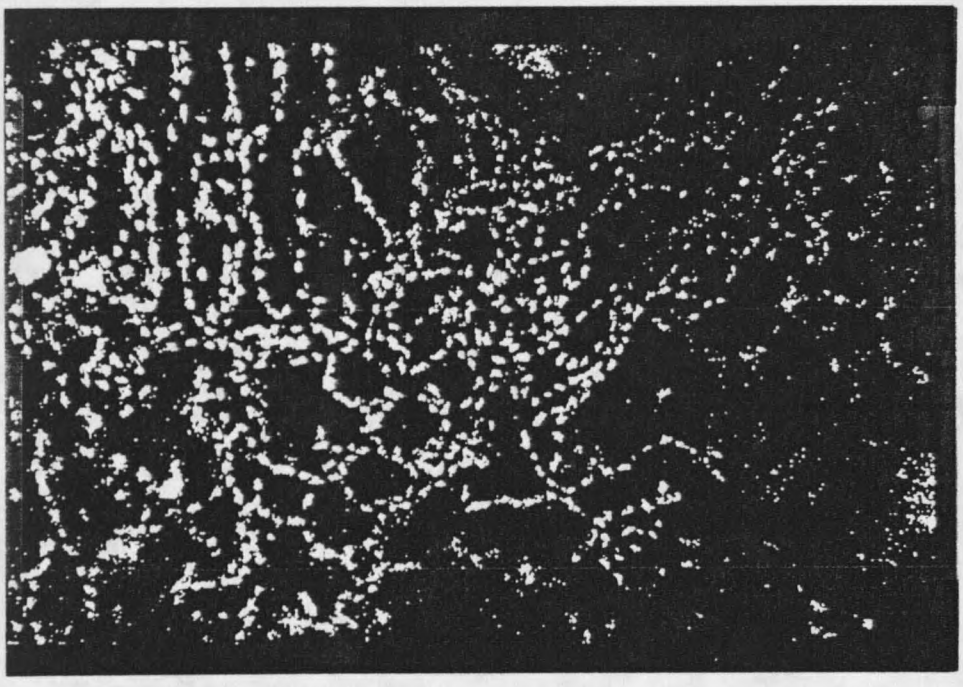


Figure 9 • Image Thresholded with Kittler Threshold

Another thresholding method is the valley-seeking threshold selection technique. [Sahasrabudhe, Das Gupta] In this technique, the image is divided into sub-images and local thresholds are computed. These thresholds are then examined to obtain a threshold surface for the entire image.

Valley Seeking Method:

1. Divide the entire image into square blocks;

2. Compute a histogram for the image and locate every peak and valley.

3. A figure of merit is computed for all the valleys using the following valley features:

   A_1 = depth of the valley with respect to adjoining peaks.

   A_2 = separation of the two peaks - not necessarily just the gray level distance.

   A_3 = location of the valley in the histogram.

   Q (figure of merit) = $A_1 * A_2 * A_3$;

4. The valley corresponding to the highest figure of merit is chosen as the valid threshold for that block;

5. The local thresholds are used to fit a smooth threshold surface over the entire image.

This technique is beneficial when there are areas of poor contrast or lighting throughout the image. This is due to the localized nature of the algorithm.

Smoothing Filters

An effective method for removing noise is to apply a spatial filter or mask to the image. The mask is positioned at each pixel and a result is computed based on the pixel and its neighborhood as well as the values in the mask. Often this computation is the product of the mask and the neighborhood being evaluated. The equation for this general condition is:

$$\mathbf{R = w_1z_1 + w_2z_2 + \ldots + w_nz_n.}$$

where z_i represents image pixel values, w_i represents mask values and R represents the resulting value.

One such spatial filter is known as *neighborhood averaging* or a Mean Filter [Gonzalez]. In a three-by-three mask, each position in the mask (w_i) contains a 1, and the result is multiplied by 1/9 to arrive at the averaged value. This averaged value then replaces the center pixel in the neighborhood being considered. In general, for an n x n averaging mask, each position in the mask contains a 1 and the result is multiplied by 1/(n x n).

The neighborhood averaging filter reduces noise data by moving each noise pixel value closer to an actual image pixel value. However, actual image pixel values are also skewed towards the noise values. If this effect

is unacceptable, a Median Filter may be applied. This filter finds the median of a neighborhood of pixels instead of the average of them. Small pieces of noise can be effectively removed using this technique, but the computational complexity is more than the averaging filter. Figures 10 and 11 show microbial images after processing with the smoothing filters.

Sharpening Filters

These spatial filters or masks are used to emphasize edges and points of detail. The masks are similar to smoothing masks in that they have the same multiplicative format for evaluation. A basic Highpass Filter [Gonzalez] is shown below:

1/9 x

-1	-1	-1
-1	8	-1
-1	-1	-1

Using this mask, the equation for finding a resulting pixel is the sharpened image is:

$$R = 1/9\,((-1 * z_1) + (-1 * z_2) + (-1 * z_3) + (-1 * z_4) + (+8 * z_5) + (-1 * z_6) + (-1 * z_7) + (-1 * z_8) + (-1 * z_9)).$$

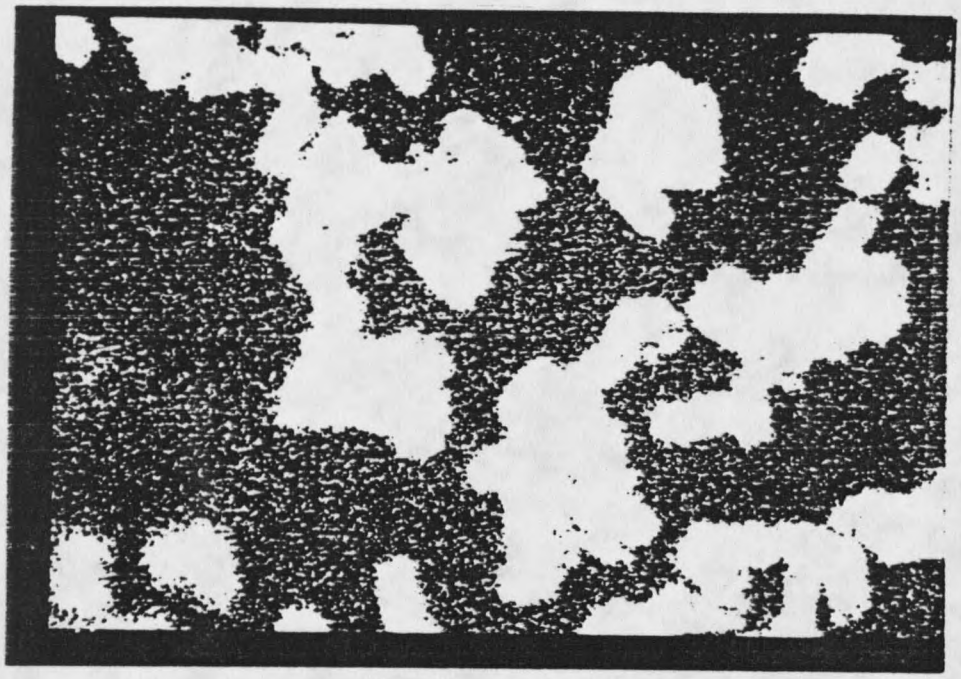


Figure 10 • Image After Mean Filter Application

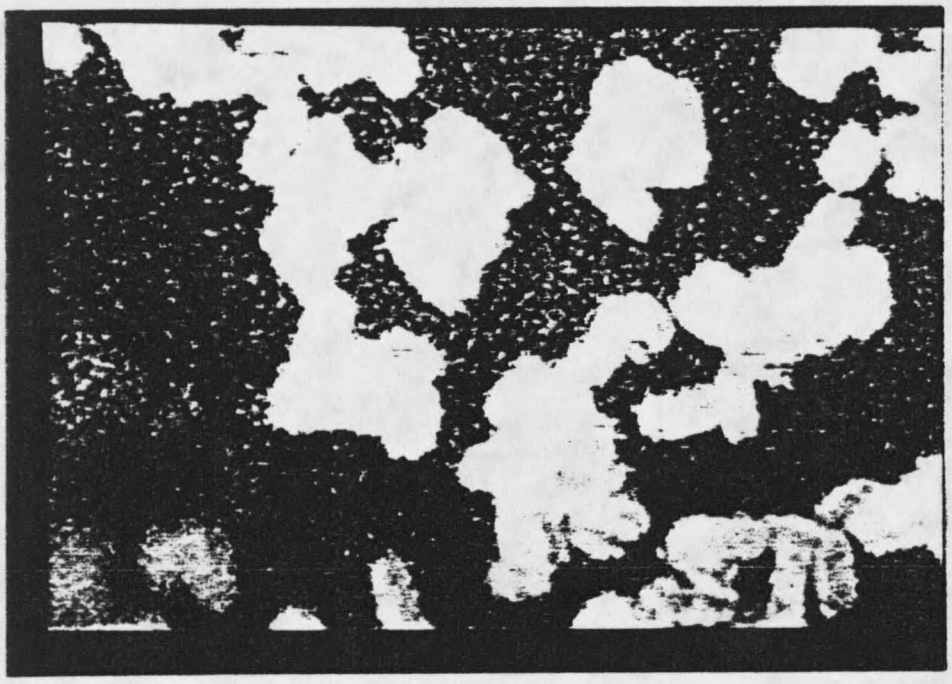


Figure 11 • Image After Median Filter Application

This filter can be used to help restore microbial images which have suffered blurring effects as described in Chapter 1. A slightly modified filter is the Highboost Filter which adds a percentage of the original image to the results obtained through a Highpass Filter. Examples of microbial images which have been processed with sharpening filters are shown in Figures 13 and 14. The original image is shown in Figure 12.

After applying the image enhancement techniques described in this chapter, the microbial image should be prepared for information extraction.

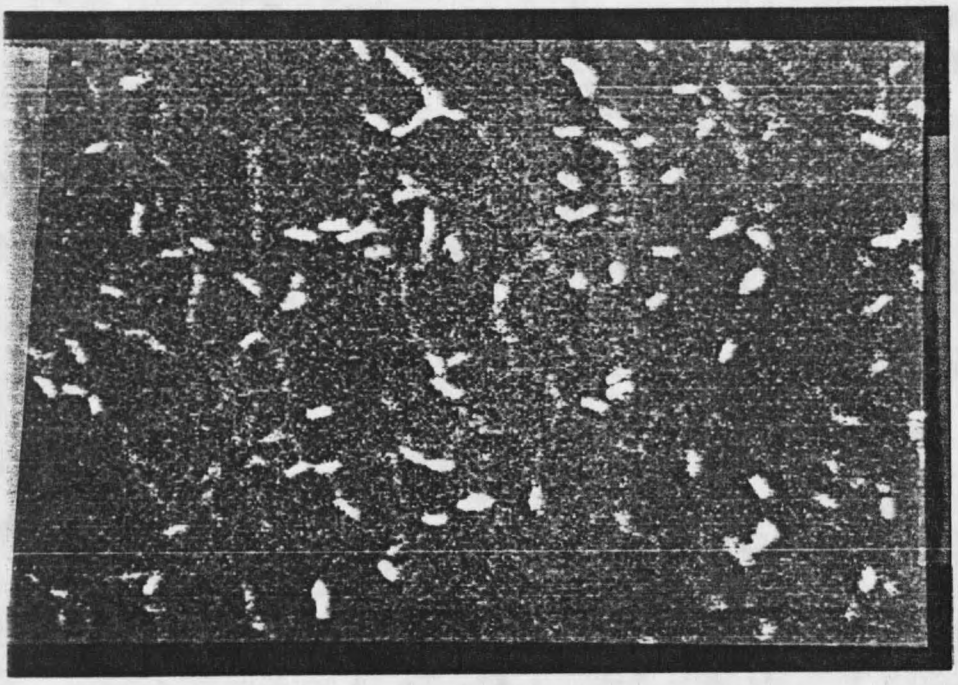


Figure 12 • Image Before Sharpening Filter Application

Figure 13 • Highpass Filtered Image

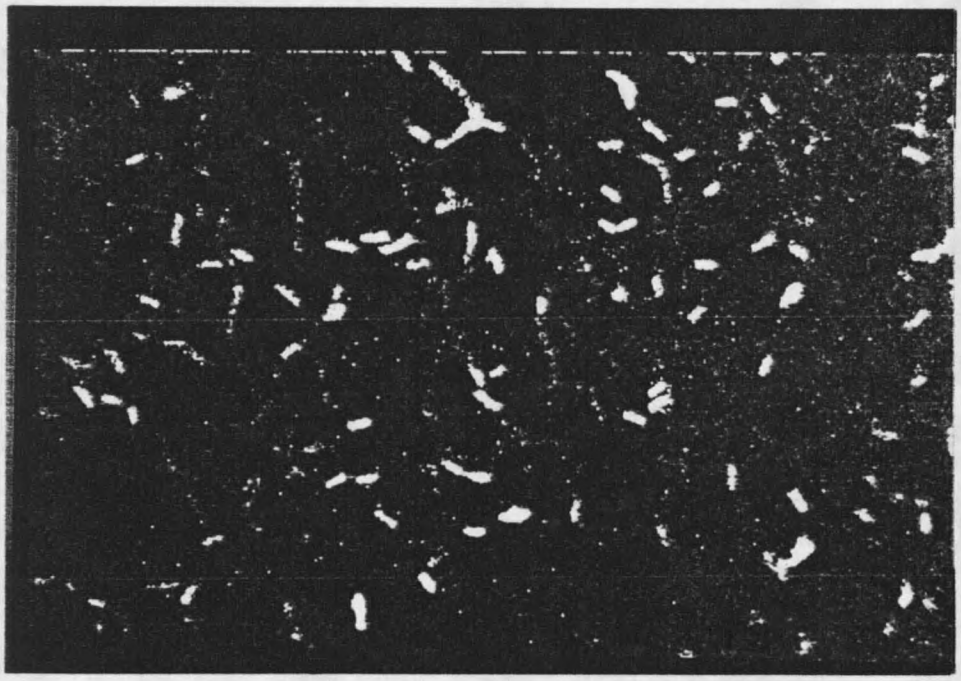


Figure 14 • Highboost Filtered Image

CHAPTER 4

MICROBE FEATURE EXTRACTION

Edge Detection

An *edge* can be defined as a boundary in an image that separates two regions that have significantly dissimilar characteristics. [Tan, Gelfand, Delp] The dissimilarity may be due to the geometry of the object, illumination, viewpoint or a combination of these. In images of microorganisms, the edges are those contrasted by microbes on a background, or microbes overlapping. Several methods can be effectively used to detect the edges in an image. A simple edge detection method is to apply a gradient operator to the image. The *gradient* of an image f(x, y) at location (x, y) is the vector:

$$\nabla f = \begin{bmatrix} G_x \\ G_y \end{bmatrix} = \begin{bmatrix} \frac{\delta f}{\delta x} \\ \frac{\delta f}{\delta y} \end{bmatrix}$$

This vector can be approximated by adding the absolute values of the gradients in the x and y directions. Two masks are constructed to achieve

this result:

$$Gx = (z_7 + 2z_8 + z_9) - (z_1 + 2z_2 + z_3);$$

$$Gy = (z_3 + 2z_6 + z_9) - (z_1 + 2z_4 + z_7);$$

$$\text{Gradient} = | G_x | + | G_y |$$

This is a standard method of edge detection which can be used to judge the effectiveness of other methods.

The gradient operator is applied as a simple spatial mask. There are other, more complex methods which may be more effective when applied to noisier microbial images. One such method is to derive a cost function based on certain factors (number of edge points, fragmentation, edge thickness, region dissimilarity and curvature) and minimize this function at all points in the image. [Tan, Gelfand, Delp] This method can be described in the following way:

1. Locate edge pixels by applying an appropriate dissimilarity function. The dissimilarity function can be of any type (Gradient, Laplacian, texture sensitive operators) and is left undefined for flexibility.

2. The cost function is defined in terms of the enhanced image and is the weighted sum of five cost factors (number of edge points, fragmentation, edge thickness, region dissimilarity and curvature). These costs are weighted to suit the specific application.

3. A defined group of edge structures are applied to the image at each point. The most suitable edge structure is chosen and a cost for the pixel using this structure is computed. If a transformation of

the edge structure produces a smaller cost, this is chosen to represent the point instead of the original structure. Each cost takes into account the edge structures chosen for the surrounding pixels. Figure 15 shows some of the transformations which may be used to produce a lower cost edge configuration.

4. The minimization of the cost function at each pixel produces the final edge detected image.

Examples of images processed with the gradient and cost minimization approaches are shown in Figures 16 and 17.

Another approach which can be used to effectively find edges in images of microorganisms is the Multiple-Threshold Approach. This approach can be described with the following steps:

1. Make a determination of the number of threshold points necessary. This can be done by enumerating the peaks or valleys in the histogram.

2. Choose the threshold points using any one of the following methods or any other effective thresholding algorithm.

A. Automatic Thresholding Methods mentioned in Chapter III.

B. Local Thresholding Method - 1) locate a small image area containing an edge; 2) register average pixel values on either side of the edge; 3) choose a threshold between these two values.

3. For each threshold point, apply the thresholding operator and record the position of pixels with values of 255 which are neighbors of pixels with values of 0.

4. To produce the final enhanced image, take each list of edge pixels from each thresholding operation and combine them.

This method has the desirable effect of always producing edges that

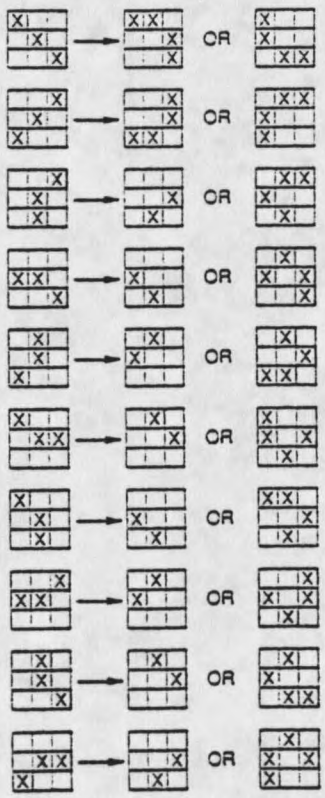


Figure 15 • Edge Transformation Structures

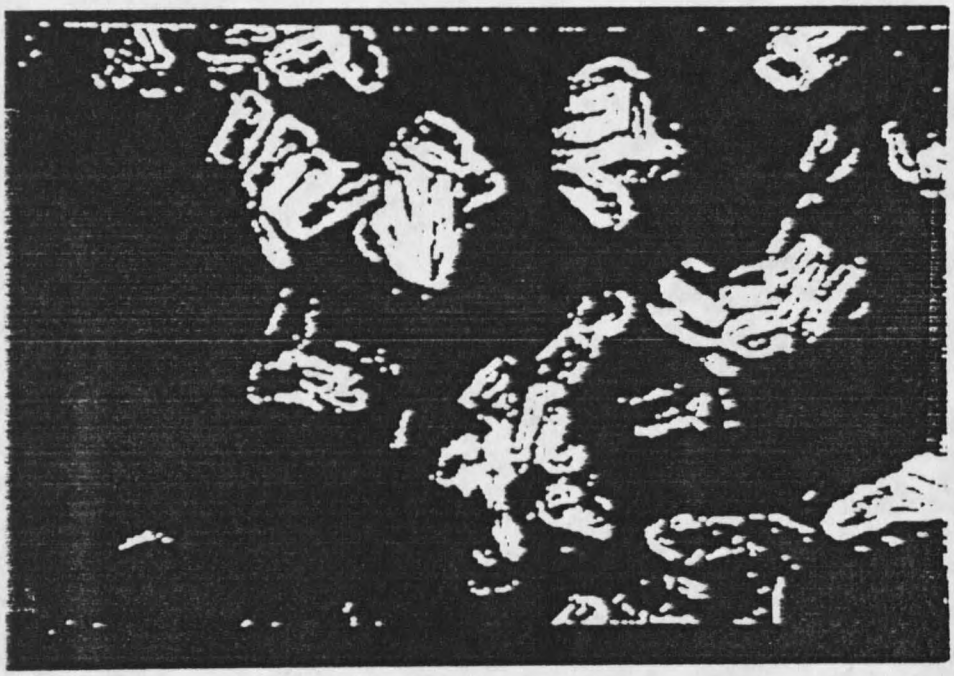


Figure 16 • Image with Gradient Application

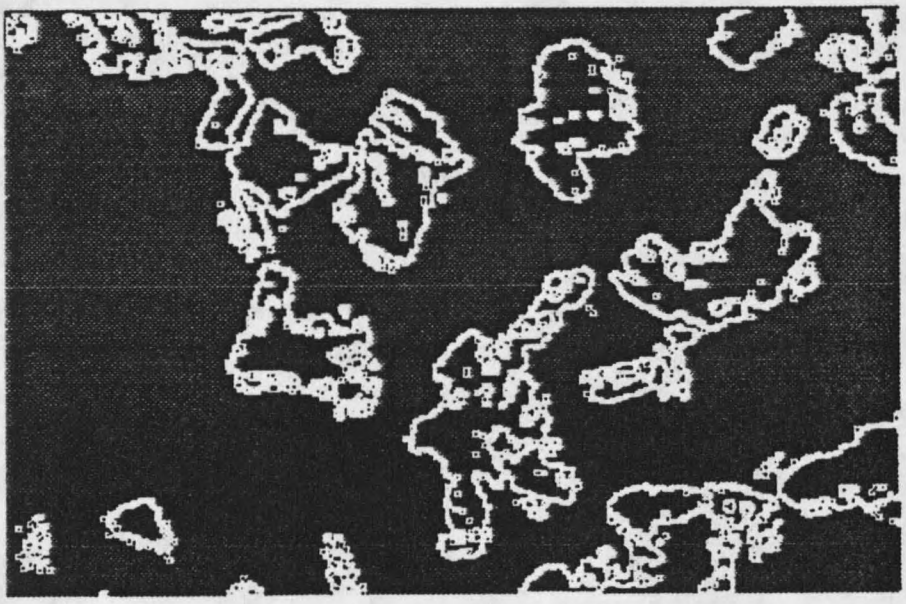


Figure 17 • Cost Minimization Edge Detection Example

are closed shapes. Since the shape of microorganisms are typically elliptical in nature, this method works effectively on microbial images. An example of a microbial image which has been processed with a Multiple Thresholding edge detector is shown in Figure 18.

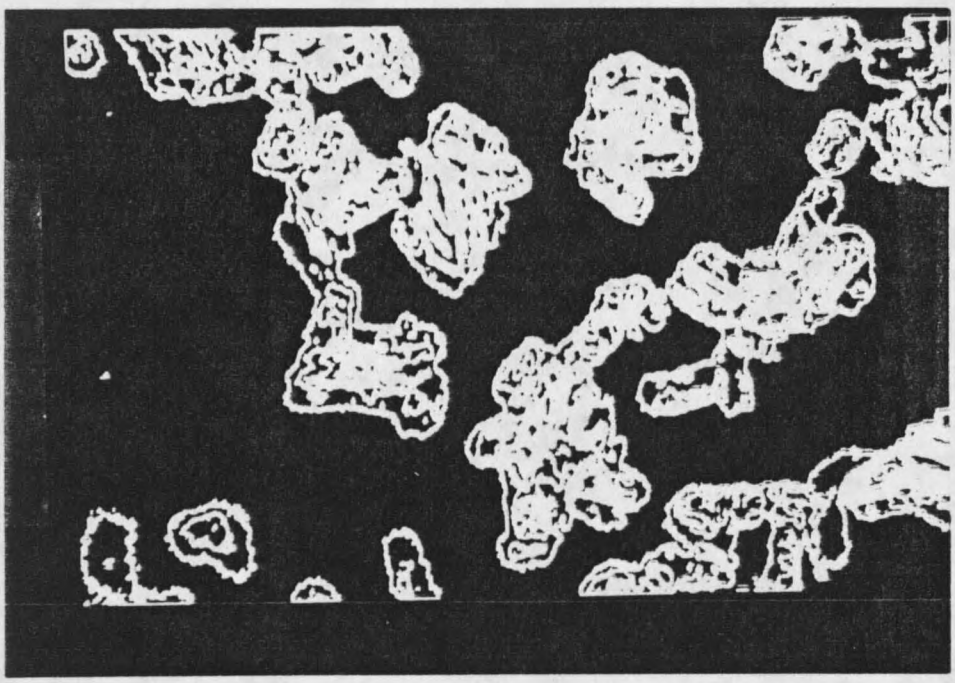


Figure 18 • Multiple Threshold Edge Detection Example

CHAPTER 5

MICROBE FEATURE REPRESENTATION

Once the microbial edge data has been gathered, there are many ways in which it can be further processed in order to arrive at meaningful data. If the microbial edges are incomplete, functions can be performed to 'fill in' the missing pieces. If the edges are complete and closed, size and shape information can be gathered and normalized. These are the primary goals of feature analysis as it is applied to microbial images:

1. Complete Microbe Edges - this operation is based on size and shape and goes beyond any edge linking that may have occurred in the edge detection phase.

2. Compute a Normalized Shape Number or Signature for each closed object - This value is computed for all microbe candidates.

Reconstructing Microbial Objects

The first task is to locate incomplete edge segments and attempt to complete them by making assumptions about the size and shape of the objects which they represent. One technique is to try to fit ellipses to the two dimensional edge data which has been gathered. Since microbes are usually elliptical in shape, this method may be useful in many microbial applications [Porrill]. This technique can be described in the following way:

Use the equation describing the general conic -

$$ax^2 + 2bxy + cy^2 + 2dx + 2ey + f = 0$$

to fit against the edge data. Since a + c can never be zero for an ellipse, the normalization a + c = 1 can be used and all ellipses can then be described by the vector:

$$x = (a, b, d, e, f)'$$

which will be estimated given the image edge observations.

A more simple method is to estimate the microbe by symmetry. This method involves choosing maximum and minimum slope change points. To use this technique:

1.) Start at any place on the edge data and calculate where the slope change is the slowest. (This can be accomplished by using the direction formulations described in the next section).

2.) From this point, proceed in one direction along the edge data and find the point in which the slope change is the largest.

3.) These two points (and the pixels in between) define a quadrant of the microbe candidate.

4.) Since microbes are usually symmetric, recreate the rest of the microbe candidate using this quadrant as a guide.

An example of this technique is shown in Figure 19.

Normalization of Microbial Objects

After curve fitting has been applied to each incomplete edge segment, it is necessary to describe the shapes of the closed objects in a normalized

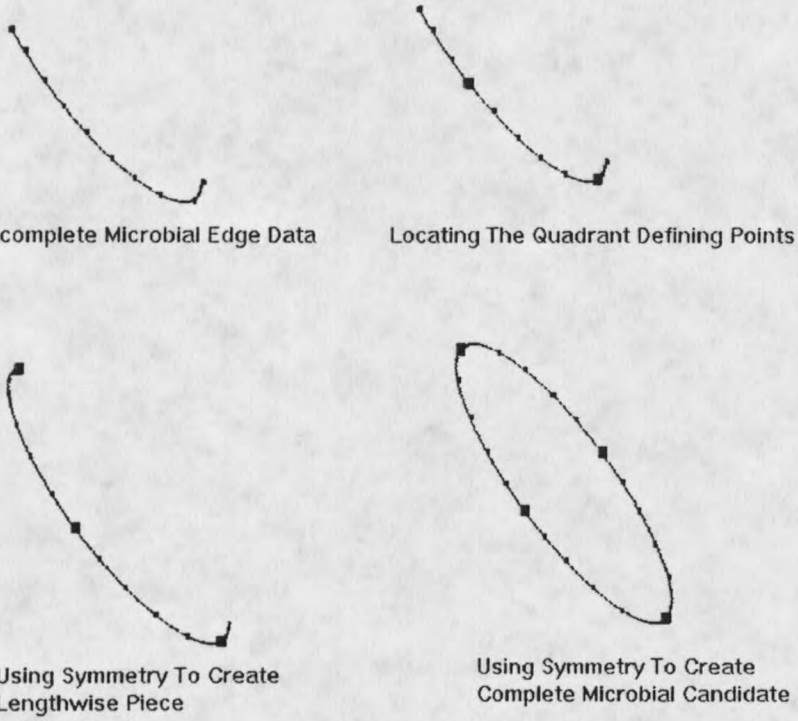


Figure 19 • Symmetric Microbe Estimation Example

way. A technique which can be used successfully is to compute the chain code and from that create a normalized shape number [Gonzalez].

Computation of Shape Number

1. Starting at any position in a closed edge, record the direction of the next edge pixel in the edge loop. Each direction can be represented by a number between zero and seven as shown in Figure 20. The combination of all directions is a list of numbers known as the chain code.

2. Compute the differences between each direction in the chain code. This is not numerical difference, but the difference in direction (either clockwise or counterclockwise).

3. Create the Shape Number by normalizing the list of differences. To do this, reorganize the difference list so that the Shape Number has the smallest possible value.

A contrasting approach to representing and describing the microbial edge data is computing the signature of the object.

Computation of Signature [Gonzalez]

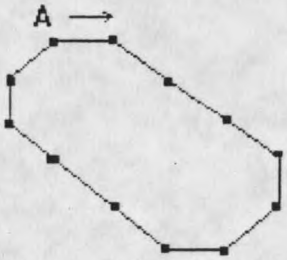
1. Calculate the centroid of the edge loop. Averaging the x and y coordinates of each edge pixel is an effective way to do this.

2. Starting at the edge pixel which is farthest from the centroid, record the distance from the centroid.

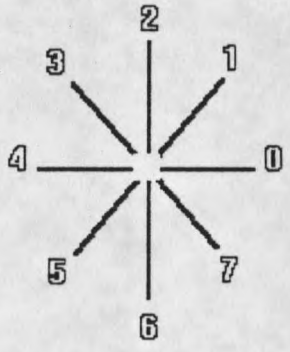
3. Continue recording the distance from the centroid for each angle in a predetermined direction (clockwise or counter-clockwise).

An example of how the signature is computed is shown in Figure 21.

Microbe Outline



Chain Code (Starting from point A)	077765433321
Difference Between Directions in Code	770077770077
Shape Number	007777007777



Directions

Figure 20 • Shape Number Computation Example

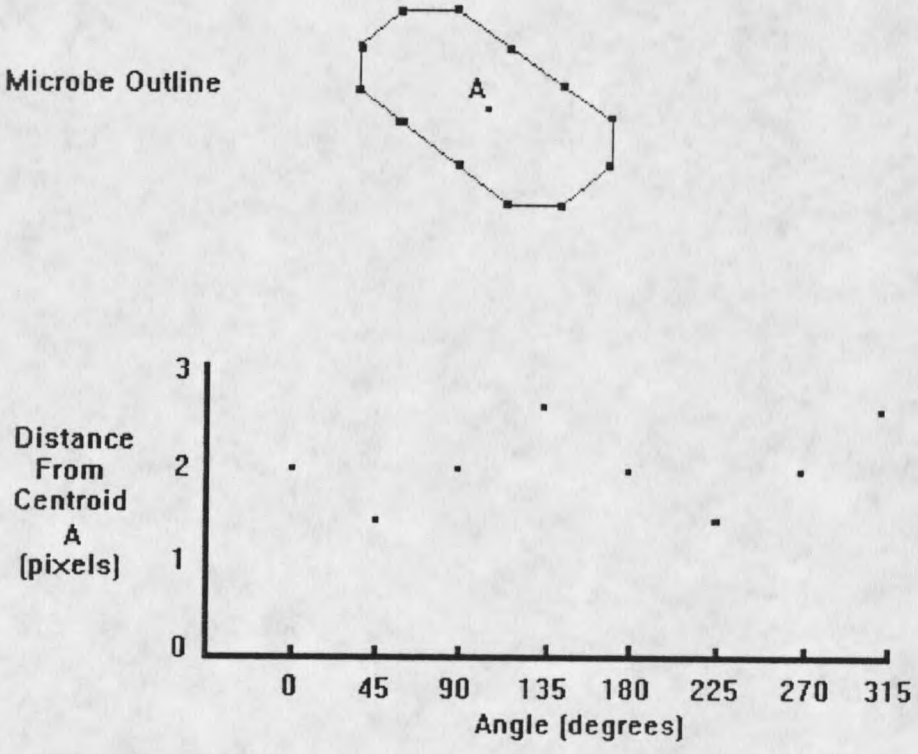


Figure 21 • Signature Computation Example

These methods, shape number and signature, differ in their approach to representing the edge loops found in the microbial image. However, they are similar in that they both uniquely represent the edge data in a normalized manner.

Other properties such as length and width can be gathered easily once these representations have been computed. Since the microbes being examined are primarily elliptical and symmetric in shape, the two peaks in the signature can be used to compute the length of the microbe. These two peaks correspond to the two edge pixels which are farthest apart in the object. Therefore, calculating the distance between these two points will yield the length of the microbe candidate. If the two pixels are (x_1, y_1) and (x_2, y_2), then the distance (in pixels) would be:

$$d=\sqrt{(x_2-x_1)^2-(y_2-y_1)^2}$$

Using the two lowest points in the signature instead of the peaks will yield the width in pixels of the microbe candidate.

The actual length and width of the candidate microbes should be computed before any microbe recognition is attempted. To do this, each length and width value should be multiplied by a scaling factor which represents the micron to pixel ratio.

CHAPTER 6

MICROBE RECOGNITION AND ENUMERATION

The final step in the data extraction process is microbe recognition and enumeration. In this step, the features which were extracted in the previous steps (width height, shape number, signature) are analyzed and compared with the features of known microorganism types. After the microbes have been typed, they can be counted and their coordinates can be found.

Bayes Classifier

The techniques of statistical pattern recognition may be used to classify microbial data samples. If the distributions of the possible microbe classes are known, the best choice for a classifier is the Bayes Classifier. [Fukanaga] The Bayes Classifier contributes the least amount of error achievable from the given distributions. This classifier is defined as:

$$\frac{1}{2}(X-M_1)^T\Sigma_1^{-1}(X-M_1) - \frac{1}{2}(X-M_2)^T\Sigma_2^{-1}(X-M_2) + \frac{1}{2}\ln\frac{|\Sigma_1|}{|\Sigma_2|} \underset{\omega_2}{\overset{\omega_1}{\gtrless}} \ln\frac{P_1}{P_2}$$

where X is the observed vector of feature values, M_i is the mean vector for the class distributions, Σ_i is the variance vector for the distributions, and P_i is the a priori probability of ϖ_i (Class i). [Fukanaga] If the left side of the equation is greater, given the values for the mean vectors and the variance vector for the two microbe distributions, then the microbe will be classified as belonging to group one, otherwise, it will be placed in group two.

Clustering

If the distributions of the possible microbe types in the image are not known, the technique of clustering is effective for distinguishing objects with similar characteristics. [Fukanaga] Through the minimization of a clustering criterion (nearest mean, nearest neighbor, etc.), each microbe candidate is assigned to a given class. These classes can then be identified as microbes, noise, or other objects.

Microbe Enumeration

If edge shapes have been successfully extracted from the image, and each shape has been typed, the enumeration process is a simple one. All that needs to be done is to total up the number of edge shapes for a given type of object. However, if the feature extraction steps of edge detection and ellipse fitting are not feasible, the number of microbes (or objects) can still be computed.

An effective method for enumerating microbes in images where it is not desirable to use feature extraction techniques is the Blob Coloring Method. [Bovik] This technique requires that the image be thresholded. Then, white pixels which are connected (either 4-connected or 8-connected) are grouped together. After all the pixels have been grouped, each group is counted as a separate object. If the size of the object is reasonable for the type of microbe in the image, it can be counted as one microbe. Otherwise, the object must be discounted as noise (object is too small) or split into separate objects (object is too large). If x and y positions of the microbes are not needed, an approximation of the number of microbes can be computed from the size of the large object.

$$Number\ of\ Microbes\ in\ Object = \frac{Size\ of\ Object\ (in\ pixels)}{Size\ of\ Microbe\ (in\ pixels)}$$

A more precise approximation to the number of microbes in a large

object and their positions can be computed by considering certain characteristics of the large object. Discontinuities in the edge of the object most likely represent places where two microbes are intersecting. Using these discontinuity coordinates and the average size of the microbe in the image, approximations can be made about where each edge microbe is situated. These microbes are then enumerated. Any remaining area in the object is estimated, and an approximation is made concerning the number of additional microbes.

Finding Coordinates

After identifying microbes through edge detection and classification, or by blob coloring, finding the x and y coordinates of the microbes is simply a matter of averaging the x and y coordinates of each pixel comprising the microbe. This value is the centroid of the microbe. A different method for computing the coordinates of the microbe is to find where the major and minor axis of the microbial shape intersect.

CHAPTER 7

CONCLUSION

A process has been presented for extracting information from digital images of microorganisms. This process is comprised of four basic steps:

1. Image Enhancement
2. Microbe Feature Extraction
3 Microbe Feature Representation
4. Microbe Recognition and Enumeration

Using these four steps, the following data pertaining to each candidate microbe can be extracted:

1. Width and Height
2. Area (Number of pixels)
3. Shape (shape number and signature)
4. X and Y coordinates
5. Classification (Microbe Type)
6. Count (Enumeration of each object type)

Examples of how each type of information is extracted were given, and the effectiveness of each technique was discussed.

There are many techniques which can be effectively applied to images of microorganisms. In this paper, a subset of those were presented having these desirable characteristics:

1. Each technique can be autonomously applied, but none rule the use of human intervention.

2. No technique requires an unreasonable amount of processing time

or space.

3. Each technique can be implemented in a serial-based programming language.

Given this subset of effective techniques, as well as the process by which they are applied, many imaging problems involving microorganisms may be solved. This results in the successful extraction of data from images of microorganisms.

Future Research

Each of the four steps described in this paper are areas which can be improved through further research. Some possibilities might include advanced feature extraction techniques involving color and three dimensional processing of microbial images. Information gained from these techniques could be used to more easily type the microbes, as well as provide three dimensional models of microbe shape and position.

Another area which would benefit from further research is approximating missing edge and shape information in images with incomplete microbial data. If each type of microbe was mathematically representable as a combination of conic sections, the incomplete microbes could be reconstructed more accurately given this conic configuration. In

addition, shape abnormalities (bending, twisting, etc.) and grouping characteristics which occur in certain types of microbes could be registered and used to help identify microbes as well as to complete microbial images.

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