Mathematical morphology techniques for image processing applications in biomedical imaging

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ABSTRACT

Mathematical morphology operations allow object identification based on shape and are useful for grouping a cluster of small objects into one object. Because of these capabilities, we have implemented and evaluated this technique for our study of Alzheimer's disease. The microscopic hallmark of Alzheimer's disease is the presence of brain lesions known as neurofibrillary tangles and senile plaques. These lesions have distinct shapes compared to normal brain tissue. Neurofibrillary tangles appear as flame-shaped structures, whereas senile plaques appear as circular clusters of small objects. In order to quantitatively analyze the distribution of these lesions, we have developed and applied the tools of mathematical morphology on the Pixar Image Computer. As a preliminary test of the accuracy of the automatic detection algorithm, a study comparing computer and human detection of senile plaques was performed by evaluating 50 images from 5 different patients. The results of this comparison demonstrates that the computer counts correlate very well with the human counts (correlation coefficient = .81). Now that the basic algorithm has been shown to work, optimization of the software will be performed to improve its speed. Also future improvements such as local adaptive thresholding will be made to the image analysis routine to further improve the systems accuracy.

1. INTRODUCTION

Since the development of digital image processing in the 1960's, the main tools have been convolution filtering, look up table (LUT) manipulation, and Fourier analysis. These techniques permit the manipulation of image contrast to reveal objects of interest. An emerging image processing technique is that of mathematical morphology. For object identification typically required in biological applications, especially microscopy, the operations of mathematical morphology are more useful than the convolution operations because the morphological operators deal directly with shape. Although the operations of mathematical morphology are non-linear, it is the appropriate processing approach for identification of objects, such as cells.

The biological application areas where mathematical morphology has been used are quantitative microscopy and gel electrophoresis. In these areas, a common problem is that the background of the image varies in gray scale due usually to uneven illumination and/or staining. If the objects of interest are white, a gray scale opening with a large ball will result in an image of just the background. By subtracting this background image from the original image one has a background normalized image. Sternberg has applied this technique to 2-D gel electrophoresis analysis1. This technique can also been applied to Magnetic Resonance Imaging (MRI) to normalize the uneven background created by surface coils. Once the background has been normalized, morphological operators can be used again to detect objects of interest within the image.

This paper will describe the use of morphological image processing in the microscopic analysis of brain tissue in Alzheimer's disease. First we will describe the disease in general and the microscopic changes that we are looking for in brain tissue. Then we will give a brief review of the definitions and important properties of mathematical morphology. We will next describe the software implementation for the morphological operations and the automatic detection algorithm on the Pixar Image Computer. Finally we will describe the results of a comparison test between computer and manual detection of senile plaques in Alzheimer's disease.

1.1. ALZHEIMER'S DISEASE

Alzheimer's disease is characterized by degeneration of the brain, primarily the cerebral cortex, including the hippocampus. The predominant symptom of this disease is progressive dementia. Dementia is a syndrome
characterized by intellectual deterioration in an adult that usually involves memory, language use, perception, the ability to learn, solve problems, think in abstract terms and judgement. Alzheimer's disease primarily affects the elderly, accounting for 90% of the demented people in this group. Currently, it is estimated that 5% of people over 65 and 25% of people over 75 are affected by the disease. The disease currently affects over 2 million people in the United States and since the life span of the population is increasing, it is evident that this disease is posing an increasingly major health problem.

The diagnosis of Alzheimer's disease is based on the presence of microscopic lesions known as neurofibrillary tangles (NFT) and senile plaques (SP). These changes may also be found in normal aged individuals, but in them they are much less numerous. Neurofibrillary tangles, which are flame or globoid shaped, are accumulation of abnormal neurofilaments within neurons that are arranged as paired helical filaments (PHF). If modelled as a disk, the typical size of the NFT is 25 to 100 μm in diameter. Senile plaques are circular clusters of degenerating nerve terminals whose cores contain amyloid protein. These degenerating nerve terminals also contain paired helical filaments. The size of senile plaques range from 50 to 250 μm in diameter. Figure 1 illustrates typical NFT and SP.

![Figure 1. Senile plaques (●) and neurofibrillary tangles (▲) are illustrated in a photomicrograph of brain tissue stained with thioflavin-S (200x).](image)

Because these lesions are more numerous in Alzheimer's disease than in normal aging, it is desirable to study their frequency and distribution in both diseased and normal patients. To date, very few quantitative studies have been completed because of the time consuming labor required. Since the tangles and plaques are microscopic in size and very numerous, it is impractical to systematically go through even a small area of brain tissue and manually count the number of plaques and tangles. For example, Figure 1 shows a typical image seen at 200x magnification. Since this image is approximately 1 mm² one needs to count hundreds or thousands of fields for each section. There is clearly a need to automate this procedure to reduce the time spent on laborious tasks such as counting, and that is where an image analysis tool is needed. This tool automatically counts the number of plaques and tangles within a brain section of variable size (usually 4 cm²). In addition, the system must also retain the NFT and SP locations so that the spatial distribution and density of these lesions in the various layers of the cerebral cortex may be determined.

### 2. MATERIALS AND METHODS

This section will first review the basic definitions of binary mathematical morphology. More detailed treatments of binary and gray scale mathematical morphology theory are given by Serra and by Haralick et al. Once the basic definitions have been reviewed, the software implementation of these algorithms will be discussed. Finally we will detail the methods used to evaluate the accuracy of the computer algorithm to detect senile plaques.

study involves capturing the images, having a neuropathologist manually determine the number of senile plaques in the image, and then having the Pixar Image Computer automatically determine the number of senile plaques in the image.

2.1. Binary Mathematical Morphology

The language of mathematical morphology is that of set theory. For binary mathematical morphology, the image is assumed to consist only of white and black pixels. In this review, white pixels are the foreground (objects of interest) and black pixels are the background. Likewise, the structuring element is also a set where the white is the foreground and black is the background. An example of a binary image and a structuring element are shown in Figures 2a and 2b. Mathematical morphological transformations apply to sets of any dimensions, those like Euclidean N-space, or those like its discrete or digitized equivalent, the set of N-tuple of integers, $Z^N$. For the sake of simplicity we will refer to either of these sets as $E^N$. For binary images $N = 2$.

![Figure 2](image_url)  
**Figure 2.** Dilation. a. Original binary image ($I$). b. Disk structuring element with radius 10 ($A$). c. Result of the dilation $I \oplus A$.

2.1.1. Dilation and Erosion

The basic operations of mathematical morphology are called dilation and erosion, and can be thought of as "growing" and "shrinking" the objects within an image. The amount and character of the growing or shrinking is determined by a set known as the structuring element, which can be thought of as a shape such as a disk or square. This structuring element is similar to the kernel in convolution filtering in that it is usually a small window positioned at each pixel in the image. Dilation as an image processing operation was employed by several early investigators in image processing as smoothing operations. Dilation as an image operator for shape extraction and estimation of image parameters was explored by Serra and Matheron. All of these early applications dealt with binary images only.

Dilation is the morphological transformation which combines two sets using vector addition of set elements. Let $I$ and $A$ be sets in N-space ($E^N$) with elements $i$ and $a$ respectively, $i = (i_1, \ldots, i_N)$ and $a = (a_1, \ldots, a_N)$ being N-tuples of element coordinates. For the binary case, these coordinates are (row, column) and specify the location of the foreground pixel. The dilation of the image $I$ by the structuring element $A$ is the set of all possible vector sums of pairs of elements, one coming from $I$ and one coming from $A$. Denoting dilation by $\oplus$,

$$I \oplus A = \{b \in E^N | b = i + a \text{ for some } i \in I \text{ and } a \in A\}$$

Dilation by disk structuring elements corresponds to isotropic swelling or expansion algorithms common to binary image processing. Figure 2c shows an image that has been dilated by a disk of radius 10.
A common implementation of this operation, which we use in our system, involves considering dilations in terms of image translations. The translation of $I$ by $z$ is denoted by $(I)_z$ and is defined by

$$(I)_z = \{ c \in E^N \mid c = i + z \text{ for some } i \in I \}$$

The dilation of $I$ by $A$ can be computed as the union of translations of the image by the points in the structuring element. That is,

$$I \oplus A = \bigcup_{a \in A} (I)_a$$

Erosion is the morphological dual to dilation. It is the morphological transformation which combines two sets using the vector subtraction of set elements. Erosion is denoted by $\ominus$ and is defined by

$$I \ominus A = \{ z \in E^N \mid z + a \in I \text{ for every } a \in A \}$$

The utility of the erosion transformation is better appreciated when the erosion is expressed in a different form. The erosion of an image $I$ by a structuring element $A$ is the set of all points in the image $(E^N)$ for which $A$ translated to $z$ is contained in $I$. Thus the structuring element $A$ may be visualized as a probe which slides across the image $I$, testing the spatial nature of $I$ at every point. Where $A$ translated to $z$ can be contained in $I$ (by placing the origin of $A$ at $z$), then $z$ belongs to the erosion. An example of the erosion transformation using the same image and structuring element in Figure 2 is shown in Figure 3.

![Figure 3. Erosion. a. Original binary image (I). b. Result of the erosion $I \ominus A$. c. Disk structuring element of radius 10 ($A$).](image)

### 2.1.2. Openings and Closings

In practice, dilations and erosions are usually employed in pairs, either dilation of an image followed by the erosion of the dilated result (closing), or image erosion followed by dilation (opening). In either case, the result of iteratively applied dilations and erosions is an elimination of specific image detail smaller than the structuring element without the global geometric distortion of unsuppressed features. For example, Figure 4c shows that closing an image smooths the contours, fuses narrow breaks and long thin gulls, eliminates small holes, fills gaps on the contours, and groups together clusters of small objects. Figure 4d shows that opening an image with a disk structuring element smooths the contour, breaks narrow isthmuses, and eliminates small islands and sharp peaks or capes. The opening of image $I$ by structuring element $A$ is denoted by $I \circ A$ and is defined as

$$I \circ A = (I \ominus A) \oplus A$$
The closing of image $I$ by structuring element $A$ is denoted by $I \ast A$ and is defined by

$$I \ast A = (I \oplus A) \ominus A$$

2.1.3. Duality

The dilation and erosion transformations bear a marked similarity, in that what one does to the image foreground the other does to the image background. The opening and closing operations also have this similarity. Indeed, their similarity can be formalized as a duality relationship. Recall that two operators are dual when the negation of a formulation employing the first operator is equal to that formulation employing the second operator on the negated variables.

In morphology, the negation of a set is considered in a geometrical sense: that of reversing the orientation of the set with respect to its coordinate axes. Such reversing is called reflection. The reflection of $A$ occurs about the origin and is denoted by $\bar{A}$ and is defined by

$$\bar{A} = \{x \mid \text{for some } a \in A, x = -a\}$$

The duality of dilation and erosion employs both logical and geometric negation because of the different roles of the image and the structuring element in an expression employing these morphological operators. Using duality, erosion can be expressed by

$$I \ominus A = (I^c \ominus \bar{A})^c$$

The duality of dilation and erosion, and thus opening and closing in binary morphology has great significance in our software implementation of morphological operators. Only dilation of a white foreground and black background needs to be implemented since erosion is merely the dual operation. Table 1 shows how to compute the different morphological operations given a black or white foreground using only dilation, complementation, and reflection operators.

<table>
<thead>
<tr>
<th>Operation</th>
<th>White Object</th>
<th>Black Object</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dilation</td>
<td>Dilate with $A$</td>
<td>Complement Dilate with $\bar{A}$ Complement</td>
</tr>
<tr>
<td>Erosion</td>
<td>Complement Dilate with $\bar{A}$ Complement</td>
<td>Dilate with $A$</td>
</tr>
<tr>
<td>Opening</td>
<td>Complement Dilate with $\bar{A}$ Complement Dilate with $A$</td>
<td>Complement</td>
</tr>
<tr>
<td>Closing</td>
<td>Dilate with $A$ Complement Dilate with $\bar{A}$ Complement</td>
<td>Complement Dilate with $A$</td>
</tr>
</tbody>
</table>

Table 1. This table illustrates how to compute all the binary morphological transformations with the structuring element $A$. The three operations that must be implemented are dilation for a white foreground, complementation, and reflection.

2.2. Histological preparation and image acquisition

The brain tissues used in this study were coronal sections of the hippocampus or amygdala. A total of five sections from different retrospective cases were used and ten images were taken from each section. In order to
differentiate the NFT and the SP from background, it is necessary to have an image with high contrast. Ideally the NFT and SP in the digitized images are white and everything else is black. Conventional silver stains for NFT and SP will not suffice since the normal neurons and neuropil may appear just as darkly as the objects of interest on the screen.

Our sections were stained with thioflavin-S, a fluorescent stain that illuminates the amyloid protein and NFT. This stain is commonly used to diagnose Alzheimer’s disease because it stains NFT, SP, and blood vessels which contain amyloid (amyloid angiopathy). Lipofuscin particles which accumulate in normal aged neurons are autofluorescent and appear as bright objects on the screen. When viewed through the microscope with this stain, the NFT and SP appear yellow whereas the lipofuscin particles appear orange. The background is dark green. As shown in Figure 1, this stain gives good image contrast and has fairly good specificity for NFT and SP. Unfortunately, if a normal neuron has many lipofuscin particles it can be mistaken for a NFT on the screen since the camera cannot differentiate colors.

It is also possible to use various antibody stains which are specific to either the amyloid protein, or paired helical filaments. Actually this stain may be preferred because the lipofuscin particles, which may be confused with neurofibrillary tangles, will not appear in the image. In this case the objects of interest will be black on a white background. However, for quantitative analysis, thioflavin is more accurate than the antibody stains because the number of objects stained with the antibody stains vary with the concentration of, and the length of exposure to, the antibody.

The images were acquired using an Olympus BH-2 microscope with a fluorescent attachment. The exciting wavelength was at 490 nm and the barrier filter was at 455 nm. The objective lens was 20x and the camera attachment had a 10x eyepiece, so the resulting magnification was 200x. The images were captured using an RS170 standard video camera, the DAGE-MTI, and the PC-Vision digitizer on an IBM PC-compatible personal computer. The spatial resolution of the image was 512 x 512 pixels and the gray scale resolution was 8 bits.

2.3. Image Analysis Hardware

Once the images were acquired, they were transferred to the image analysis computer. We have implemented the morphological operations on two different systems, first on the Gould IP8400 and subsequently on the Pixar Image Computer. We have chosen the Pixar to succeed the IP8400 for our studies because of its increased capabilities such as speed and frame buffer size. Speed is crucial for our application since thousands of images from each section must be examined. Speed is attained from the Pixar’s high-speed channel processor (Chap). Actually the Chap contains four parallel processors organized in a single-instruction-multiple-data (SIMD) architecture. Because of this architecture, the Chap can handle 40 million instructions per second (40 MIPS).

The other key feature of the Pixar is a large frame buffer (24 Mbytes). The frame buffer can be as large as 2048 x 2048 with four channels, each 12 bits deep. By contrast, the frame buffer of the IP8400 is only 1 Mbyte with four channels at 512 x 512, each 8 bits deep. Because of this large frame buffer one can compose sixteen 512 x 512 48-bit images into the Pixar frame buffer and operate on them all simultaneously. Typically one 12-bit image channel is used to store the original image and the other three channels are used to store intermediate results as well as the final image.

2.4. Software Implementation

There are three levels of programming capabilities on the PIXAR: the Pirl, Chad, and Chap levels. The Pirl level is a set of C-callable routines to perform many image processing and graphics functions such as image addition or annotation. This is the simplest of the programming levels, but is frequently too restrictive. This level may be suitable for end users when composing the different routines into an application. The next level of software programming is the Chad level which is a set of C-callable routines used to run routines developed at the Chap level. At this level one has a large amount of flexibility, but must sacrifice some speed for having the C language interface to the Chap routines. The lowest level of software programming is microcoding. In the Pixar environment, this is known as the Chap level. Only at this level can one explicitly control the ALUs and multipliers, and take advantage of the pipelining capabilities of the Pixar architecture. We have implemented the morphological operations at the Chap level in order to maximize the performance.
2.4.1. Morphological Operations

The dilation operation is implemented using the “shift and or” computation described earlier. That is,
\[ I \oplus A = \bigcup_{a \in A} (I)_a \]

According to this method, the image is shifted by each point in the structuring element set. The union of all these shifted images is the resultant dilated image. As shown in Table 1, only dilation using a white foreground actually needs to be implemented because of the duality relationship.

In our implementation of this method, the scratchpad memory was used to hold two arrays, each the size of a scanline. To begin with, one array \( S \) stores the top scanline of the image translated by the first point of the structuring element, and the other array \( R \) is filled with zeros. The result of \( S \cup R \) is placed back into \( R \). Next \( S \) is filled with the top scanline of the image translated by the second point of the structuring element. The result of \( S \cup R \) is placed back into \( R \). This process continues until all the points in the structuring element have been used. At this point the array \( R \) holds the resultant top scanline and is placed in the output image. In our implementation, the output image is stored in a different image plane so as not to corrupt the original image scanline which may be needed for computation of a later scanline. Once the top scanline is finished, \( R \) is filled again with zeros and the process is repeated. In this fashion, our algorithm works on one scanline at a time from the top to the bottom of the image.

2.4.2. Automatic detection algorithm

Gray scale images acquired from the microscope are transformed into binary images by use of global binary thresholding. Currently, the threshold point is determined manually, but eventually an automatic thresholding routine will be implemented. An example of such a transformation is shown in Figures 4a and 4b. Once the image has been transformed into binary, the automatic detection algorithm can work. The first goal of the algorithm is to detect the senile plaques. In order to do this one must first join together the objects within a senile plaque to form just one large region and then remove all the remaining small objects from the image. Once the SP have been detected, they are removed from the original image via subtraction. The NFT can now be detected by removing all the non-NFT objects from the image.

In order to join the objects within a senile plaque, the closing operation is used with a structuring element similar to that shown in Figure 5. We shall call this shape a “gamma”. We have recently developed a routine based on the Bayesian decision making method to determine the optimal height and length of the gamma for each image. We have found that this structuring element works better than a disk in that it reduces the occurrence of grouping together objects that are not part of an SP. For example, the gamma performs better at keeping two senile plaques that are close together as separate objects. We first close the image with the appropriate sized gamma, and then close the resultant image with a small disk (radius 2) to fill in the remaining holes and gaps. This result is shown in Figure 4c. Next the image is opened with a disk of radius 7 to remove all the objects on the image that are too small to be a SP. The disk size of 7 was determined experimentally by examining many different images. The result of this step is shown in Figure 4d.

Once the SP have been detected, they are subtracted from the original binary image. In another example, Figures 6a and 6b show the original gray scale and corresponding binary images. Figure 6c shows the result of subtracting the detected SP from the image in Figure 6b. This image is opened with a disk to remove all the objects that are too small to be a NFT. The resulting image contains the detected NFT and is shown in Figure 6d.

2.4.3. Comparison Study

As a preliminary test of the accuracy of the automatic detection algorithm, we compared the number of senile plaques detected independently by a neuropathologist with the number detected by the computer for the same microscope fields. As was mentioned earlier, we used a total of five sections from random retrospective cases. The only criterion used for selection were adequate staining of the slides for image contrast and that there were some SP in the section. Ten images from each section were chosen by the neuropathologist. For each image acquired, the neuropathologist examined the corresponding field in the microscope and recorded the number of SP that he detected. While counting, he also looked at the digitized image on the screen to make sure that he was counting within the same boundaries. These counts are the basis of comparison for the automated image analysis algorithm. After the digitized images were analyzed, the results were compared with the number of SP and NFT obtained by manual counting.

3. Results and Discussion

The results are quite encouraging. In comparing the total number of SP, including partial ones at the borders of the image, the sample correlation coefficient was .81. The data is plotted in Figure 7. Using the one sample
t-test for the correlation coefficient, the null hypothesis of $\rho = 0$ is rejected with $p < .0005$. We also performed a one sample z test for $\rho = 1$ and found that we cannot reject the hypothesis that $\rho = 1$.

![Plot of correlation between human and computer counts of senile plaques.](image)

**Figure 7.** Plot of correlation between human and computer counts of senile plaques.

Although the results are encouraging, there are many areas where the automatic detection algorithm can be improved. For example, we have found that the detection algorithm is fairly sensitive to the binary threshold that was chosen. Choosing a sufficiently high threshold to reduce the noise in the image will also eliminate some of the smaller and darker SP. We are currently investigating local adaptive binary thresholding algorithms.

Another problem is that of counting SP at the edges of the image since many times they are cut off and thus not recognizable as SP. To solve this problem we will define a border around the frame buffer. This border will be slightly larger than the largest SP. A detected SP will only be counted if its centroid falls medial to this border. When moving to the next frame to be imaged, we will move one full frame minus the border distance. In this way a SP that was not counted in the previous frame will certainly fall within the border and be counted.

The fact that there is overlap in the sizes of the NFT and SP causes another problem. A large globoid NFT can be mistaken for a very small SP. To be sure we are counting the SP we are forced to choose a small disk (radius 7) in our opening operation. By doing this we have increased the number of false detections.

Finally, the last major problem is the thioflavin-S stain we have chosen to use. As was mentioned before, lipofuscin particles in normal neurons will autofluoresce and appear on the digitized image with the same intensity as the NFT and SP. Therefore, these normal neurons can very easily be mistaken for NFT since they are the same shape and size. Solutions to this problem may include special barrier filters for the microscope or multispectral imaging and image analysis techniques. Another problem with this stain is that it fades relatively quickly. For example, one would not be able to analyze the sections a year later and obtain the same results. A solution to these problems is to use the antibody stains discussed earlier and we are currently investigating using these stains. Although we have not yet tested these stains on our sections, they should eliminate the lipofuscin artifact.

**4. Conclusions**

This comparison study has shown that we have succeeded in using mathematical morphology techniques as an image analysis tool to automatically detect the senile plaques present in Alzheimer's disease. The morphological operator of closing allows the system to group the objects within a senile plaque, thereby allowing the detection of these lesions. The opening operation works as a shape and size filter to remove objects on the screen that are not senile plaques. Once the senile plaques are counted and removed from the image the opening operation can be used again to remove objects on the screen that are not neurofibrillary tangles, thereby allowing detection of these lesions.
Further improvements such as local adaptive thresholding, border count correction, and perhaps different staining techniques are currently being investigated. We are also optimizing the software to improve the speed. It currently takes about one minute to analyze each image. It is hoped that we can improve the performance greatly by using the parallel and pipelining capabilities of the Pixar and by using some of the chaining properties of mathematical morphology.

Once we have optimized the automatic detection routines, we will implement some standard morphometry routines such as area measurement and manual drawing. This will allow us to measure the density of the lesions in different areas of the brain as well as within different regions in the same section. Also, we will implement mapping routines to place the lesions detected at a higher magnification on the same registered section taken at a lower magnification. This will allow visualization of the spatial distribution of these lesions over a large area.

In conclusion, this paper has shown that mathematical morphology can be applied for pattern recognition and object detection on binary images. For gray scale images, mathematical morphology is useful for background normalization. In our case, we used the binary morphological operators to detect senile plaques and neurofibrillary tangles. Although there is still much work to do in optimizing the automatic detection routine, our comparison study has shown that computer and human detection are well correlated and that this image analysis tool can indeed be useful in studying Alzheimer's disease.

5. ACKNOWLEDGEMENTS

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6. REFERENCES