NUCLEI AND NUCLEOLI SEGMENTATION AND ANALYSIS

A thesis submitted
To Kent State University in partial
Fulfillment of the requirements for the
Degree of Master of Science

By

Adil Lateef Mahmood Albuahhnefis

May 2016

© Copyright
All rights reserved
Except for previously published materials
Thesis written by

Adil Lateef Mahmood Albukhnefis

B.S., University of Al-Qadisiyah, 2009
M.S., Kent State University, 2016

Approved by

Cheng-Chang Lu, Advisor

Javed I. Khan, Chair, Department of Computer Science

James L. Blank, Dean, College of Arts and Sciences
Abstract

In biomedical imaging, segmentation and analysis play an important diagnostic role. Nuclei and nucleoli segmentation and classification have a significant impact on the cancer and tumor diagnostics in biological and medical research studies. Typically, segmentation is difficult in microscopic images because of object shapes and clustering in many samples. In this work we introduce a method that combines simplicity and efficiency. The proposed method utilizes ImageJ framework to automatically segment and classify nuclei and nucleoli after applying some preprocessing techniques to improve the image quality and remove noise. The required preprocessing steps differ based on the kind of segmentation required. Both 2D and 3D segmentation are achieved for the nuclei and nucleoli. The analysis approach provides statistics about volume, area, surface and other properties of the segmented nuclei and nucleoli. The classification process then groups the segmented nuclei and nucleoli based on the previous criteria. Finally, the visualization process shows the results of the proposed method overlaid on the original data set. The proposed method provides a very efficient system for nuclei and nucleoli segmentation and achieves about 98 % accuracy. Furthermore, the plugin is extremely fast when compared to manual segmentation especially with large data sets.
TABLE OF CONTENTS

ABSTRACT ......................................................................................................................... III

LIST OF FIGURES ............................................................................................................... VI

LIST OF TABLES ................................................................................................................ IX

CHAPTER 1 INTRODUCTION ............................................................................................... 10

CHAPTER 2 BACKGROUND AND LITERATURE REVIEW .............................................. 14

2.1 ImageJ ....................................................................................................................... 14

2.2 ImageJ Distributions ............................................................................................... 14

2.3 Image Segmentation ............................................................................................... 15

2.4 Image Segmentation Methods ................................................................................ 16

2.4.1 Region Based Segmentation .............................................................................. 16

2.4.2 Region Growing Method .................................................................................... 17

2.4.3 Edge Based Segmentation Method ................................................................. 17

2.4.4 Thresholding Method ........................................................................................ 18

2.4.5 Feature Based Clustering .................................................................................. 18

2.4.6 K-mean Clustering Segmentation .................................................................... 19

CHAPTER 3 PROPOSED METHOD FOR NUCLEI AND NUCLEOLI

SEGMENTATION AND ANALYSIS (NNSA) ................................................................. 20

3.1 NNSA Definition ..................................................................................................... 20

3.2 Algorithm Steps ....................................................................................................... 21

3.2.1 Two Dimensional Nuclei Preprocessing and Segmentation ......................... 21

3.2.2 Two Dimensional Nucleoli Preprocessing and Segmentation ....................... 24

3.2.3 Three Dimensional Nuclei Preprocessing and Segmentation ....................... 26
LIST OF FIGURES

Figure 2.1. Segmentation Methods................................................................. 16
Figure 2.2. Edge Detection Segmentation Methods........................................ 17
Figure 3.1. NNSA System............................................................................... 20
Figure 3.2. Original Data Set........................................................................... 21
Figure 3.3. Gaussian Blur with Sigma =3.......................................................... 22
Figure 3.4. Nuclei Image with Threshold Value =40 ....................................... 22
Figure 3.5. Nuclei Image after Thresholding Process...................................... 23
Figure 3.6. Segmented Nuclei in 2D................................................................. 23
Figure 3.7. Statistics of the Segmented Nuclei ............................................... 24
Figure 3.8. Gaussian Blur Value=2 for Nucleoli Preprocessing................. 24
Figure 3.9. Thresholding Value =90 for Nucleoli Segmentation.................. 25
Figure 3.10. Segmented Nucleoli in 2D............................................................ 25
Figure 3.11. Nuclei Image after Background Subtraction............................ 26
Figure 3.12. Using of Hilo LUT with Nuclei Image...................................... 27
Figure 3.13. Using Math Subtraction with Nuclei Image.............................. 27
Figure 3.14. 3D Nuclei Segmentation............................................................ 28
Figure 3.15. Statistics of Segmented Nuclei in 3D......................................... 28
Figure 3.16. Image with Gaussian Blur Sigma =1........................................ 29
Figure 3.17. Math Subtraction with Value =90............................................. 29
Figure 3.18. 3D Nucleoli Segmentation

Figure 3.19. 3D Nucleoli Statistics

Figure 3.20. Nucleus 1 Statistics

Figure 3.21. Nucleus 2 Statistics

Figure 3.22. Nucleus 10 Statistics

Figure 3.23. Image Before Visualization

Figure 3.24. Segmented Nuclei Visualization

Figure 3.25. Segmented Nucleoli Visualization

Figure 3.26. Nuclei and Nucleoli Visualization

Figure 3.27. 3D View of Visualization

Figure 3.28. 2D Segmentation with Gaussian Blur Value =1

Figure 3.29. 2D Segmentation with Gaussian Blur Value =2

Figure 3.30. 2D Segmentation with Gaussian Blur Value =3

Figure 3.31. Background Subtraction Value =10

Figure 3.32. Background Subtraction Value =100

Figure 3.33. 3D Nuclei Segmentation with Background Subtraction Value =10

Figure 3.34. 3D Nuclei Segmentation with Background Subtraction Value =100

Figure 3.35. 3D Nucleoli Segmentation with Background Subtraction Value =10

Figure 3.36. 3D Nucleoli Segmentation with Background Subtraction Value =100
Figure 3.37. 3D Nuclei Segmentation with Math Subtraction Value = 50……………38

Figure 3.38. 3D Nuclei Segmentation with Background Subtraction Value = 80……38
LIST OF TABLES

Table 4.1. Deferent Segmentation Results with Deferent Gaussian Blur Values........35
Table 4.2. Deferent Segmentation Results with Deferent Background Subtraction Values ................................................................................................................................................................................37
Table 4.3. Deferent Segmentation Results with Deferent Math Subtraction Values…38
CHAPTER 1

INTRODUCTION

As the brain is a very important part of an organism's body, a nucleus, similar to the brain, is very important part of the cell. All cellular activities in the cell are controlled by the nucleus. The nucleus contains DNA and is organized into chromatin, nucleolus and the nuclear membrane. Some cells have no nucleus such as red blood cells and other have more than one, but generally the cell contains one nucleus. The nucleus shape can be round, oval or like disk. The nucleus performs a number of functions that are very important for the body as well as the cell itself. The functions include storing the necessary genetic information for growth and body metabolism as well as reproductive processes and cell multiplication.

The nucleus contains the nucleolus which is located centrally and consists of DNA, RNA and proteins. The main function is production of the subunits that create ribosomes involved in protein production.

The segmentation and classification of nuclei is a sophisticated topic because of the large number of applications in biomedical imaging. Many methods have been described to facilitate and automated these processes. However, difficulties such as identification, classification, clustering and segmentation of objects in the images have been encountered due to objects’ shape, location and image noise. As a result, nuclei and nucleoli segmentation and analysis is a challenging issue. Many methods have been developed to solve these problems and find an efficient way to keep a good quality and reasonable results.
A model-based segmentation method based on thresholding, extraction of crease segments and polygonal segmentation is presented in [1]. To get a good segmentation results in a huge database, a Viterbi search based on dual active contour algorithm is presented in [2] and a method based on morphological watersheds and other operations is presented in [3]. The pattern recognition based segmentation system (PRS-System) has been used to classify nuclei from more than 100 images of tissues stained with Hematoxylin and Eosin stain (HE-stain) [4]. To distinguish the nuclei from the background, their system used fuzzy logic, and for nuclei classification, their system used different classification methods such as Minimum Distance, Bayesian and Multi-Layer Perceptron (MLP).

Segmentation is a difficult process in biomedical imaging especially in nuclei and nucleoli images because frequently these structures are close to each other and cannot be easily separated. It is essential for many biological studies to find a method to segment not only the nuclei but also the nucleoli within these structures.

Nuclei and nucleoli can be segmented using interactive or automatic methods. Interactive methods are slower than automatic processes and can be used when number of nuclei or nucleoli is limited [5]. There is a method that needs less interaction and is presented in [6] using thresholding followed by 3D visualization, and then the clusters of nuclei are divided interactively using manual marking. In this method, user interaction is still used and the result is not accurate and subjective. Additional methods for segmentation that use automatic algorithms and run faster than interactive methods exist. These procedures can save time and run on large data sets with acceptable results and quality [5]. The automatic algorithms are more convenient and can be modified easily based on the visual results. However, the main drawback of automatic method is inaccuracy especially if nuclei are close to each other. Therefore, it is still a challenge.
to develop a fully automated algorithm that can avoid problems such as nuclei clustering and biological image complexity.[7].

Clustering is another important process that has a big impact in studies of medical and biomedical images since it can help in diagnosing of tumors and diseases. It also can facilitate the statistical analyses of results for better understanding of the relevant images. Different methods have been proposed for classifications in medical imaging field for both grayscale and color images[8].

Visualization is used to evaluate the efficiency and accuracy of segmentation techniques. Usually 3D data visualizations are more complicated than 2D data. Nowadays, visualizations can be performed easily due to the developments in computer hardware, typically in 3D graphic cards. ImageJ has plugins such as image 3D viewer and volume viewer that successfully manipulate and provide the 3D visualization process[9]
CHAPTER 2

BACKGROUND AND LITERATURE REVIEW

2.1 ImageJ

ImageJ is an open source image processing and analysis program written in Java. It can run either online or as a program that can be downloaded on any computer with Java 1.5 or later virtual machine.

Many image formats can be read in ImageJ including TIFF, GIF, JPEG, BMP, DICOM, FITS and ‘raw’. Stacks are also supported by ImageJ where a single window is shared between a series of images. Multithreading is used in ImageJ, so operations that are time consuming such as image file reading can be run together with other operations.[10].

The functions of ImageJ can be extended via Java plugins. Many plugins are used to perform analysis and processing in ImageJ and can be developed using editors and a Java compiler. It is possible to solve almost any problem in image processing and analysis with user-written plugins.[11]

2.2 ImageJ Distributions

There are many distributions that are extensions or based on the original ImageJ. The National Institute of Health website contains the basic package of ImageJ. The ImageJ core distribution is used to develop other different distributions for many scientific fields. Fiji which can be found on the Fiji website (http://fiji.sc/Fiji) is the major distribution in life science. Fiji is basically ImageJ but it comes with many plugins and macros that can be used in life science for image processing. It contains
tools for segmentations, visualization and image registration. The advantages of this distribution are the large number of supplied plugins that come with it as well as a very user-friendly script editor. It also has an extensive update mechanism for both ImageJ as well as some plugins [10].

The capabilities of ImageJ can be extended using plugins, macros or scripts.

Many macros, plugins and scripts are available through the ImageJ web site.

Macros are an easy way to execute a sequence of commands in ImageJ. The macro language is similar to Java language and it contains a set of control structures, operators and built-in functions and can be used to call built-in commands and other macros. Macro code is stored in text files (.txt and .ijm extensions).

Plugins are considerably more influential, flexible and faster than macros (most of ImageJ’s built-in menu commands are actually plugins) but are not easy to write and debug. Java programing language is used to write plugins.

Similarly, to plugins, scripts can access to all ImageJ and Java APIs without compiling process (scripts and macros run interpretively). Scripts is not as simple as macro language and it lacks the full integration in ImageJ [10].

2.3 Image Segmentation

Image segmentation is the process of dividing an image into many segments, in order to change the representation of an image into something that is simpler to analyze. Several techniques have been proposed for image segmentation because it is an important part of image processing [12].
2.4 Image Segmentation Methods

There are many approaches for image segmentations which can be classified into the following: [12]

- Region Based Segmentation
- Edge Based
- Thresholding Method
- Feature Based Clustering

![Segmentation Methods Diagram](image)

**Figure 2.1: Segmentation Methods**

2.4.1 Region Based Segmentation

The pixels that are associated to an object are gathered for segmentation[13]. The thresholding technique is combined with region based segmentation method and the area that is assigned for segmentation should be closed.

One of the most important Region based segmentation methods is the region growing method.
2.4.2 Region Growing Method

In region growing algorithm the pixels are grouped based on some criteria to get sub regions in the image. Firstly, the seed point or number of seed points in original image should be selected. Secondly, select similar criteria for segmentation then expand the region by adding to each seed the close pixel with properties similar to seed pixel and finally if there is no pixel has the required criterion, region growing should stop.

2.4.3 Edge Based Segmentation Method

Edge detection technique can be used for image segmentation where pixels or edges between variants regions are detected. Edges are distinguished to find the cutoffs in the image. Edges on the region are outlined by discovering the pixel value and it is compared with the neighboring pixels. In this edge based segmentation, it is not necessary for the detected edges to be closed.

![Figure 2.2: Edge Detection Segmentation Methods](image)
2.4.4 Thresholding Method

Image segmentation using thresholding technique is an easy and efficient way for image segmentation especially for images that have dark background.

In thresholding method, a multilevel image is converted into a binary image. The objects are separated from the background depending on specific threshold that determines the division of the image into regions. If the pixel value is greater than or equal to threshold value, then it is considered as a part of the object. If the pixel value is less than or equal to threshold value, then it is a part of the background. When the threshold is a constant value it is called global thresholding otherwise it is called local thresholding. When the background illumination is uneven, the global thresholding method is inefficient. In local thresholding, various thresholds are used to compensate for uneven illumination.[14]

The thresholding method has many drawbacks, including no more than two classes can be generated, and multichannel image thresholding is difficult. In addition, some images have much noise and it will affect the thresholding process.

2.4.5 Feature Based Clustering

Clustering can be used for segmentation. In some cases, an image is converted to a histogram and then clustering is applied on it. Many procedures may be applied before clustering, but it is also possible to apply it directly to the image based on the histogram[15]. An unsupervised technique called fuzzy C is used to cluster pixels on the color images for segmentation if the image does not contain noise.
2.4.6 K-Means Clustering Segmentation

One of the popular segmentation methods is k-means clustering. In k-means clustering, a collection of data is partitioned into a k number of groups of data. The data are classified into k disjoint clusters. There are two main steps in k-mean clustering algorithm. The k centroid is calculated in the first step. In the second step, the points are taken to the cluster with the nearest centroid to the data points. One of the most used methods to calculate the distance between nearest centroid and the data point is Euclidean Distance. After the points are grouped, the Euclidian distance is calculated again between the points, and the center, the points is then assigned to the center that has the minimum Euclidean distance.[16]
CHAPTER 3

PROPOSED METHOD FOR NUCLEI AND NUCLEOLI SEGMENTATION AND ANALYSIS (NNSA)

In this chapter the NNSA proposed system is presented with the structure and main algorithm.

3.1 NNSA Definition

The NNSA is a system for segmentation and analysis of nuclei and nucleoli based on the ImageJ framework. This system combines simplicity and efficiency. The main structure of the system is shown in the following figure.

![Figure 3.1: NNSA System](image)

In this chapter the NNSA proposed system is presented with the structure and main algorithm.
3.2 Algorithm Steps

The process starts by reading the image stack which may contain many slides of nuclei and nucleoli that are not segmented yet.

![Figure 3.2: Original Data Set](image)

3.2.1 Two Dimensional Nuclei Preprocessing and Segmentation

The data are 3D confocal image stacks of human brain tissue stained for nuclei using the stain DAPI(4’,6-diamindino-2-phenylinole). The original data sets cannot be segmented directly because they have significant amounts of noise. The preprocessing steps are necessary for later processing and the result of the segmentation depends on the preprocessing efficiency. One of the popular filters used to remove noise and process images before the segmentation is Gaussian Blur. It is one of the image preprocessing methods which can be useful in the detection of nuclei. Running a Gaussian Blur over the image will reduce the amount of noise and details present in the image.

Gaussian Blur is achieved by convolving the image with a Gaussian function for all
After Gaussian Blur function is applied to the image stack then the Thresholding process is ready to be used. The threshold value can be modified manually to get the better image quality. The following figure shows the result of the stack after applying the thresholding process.

Figure 3.3: Gaussian Blur with Sigma =3

Figure 3.4: Nuclei Image with Threshold Value =40
The final step in 2D Nuclei segmentation is running a particle analyzer to find statistics and information about the segmented object.

Figure 3.5: Nuclei Image after Thresholding Process

Figure 3.6: Segmented Nuclei in 2D
3.2.2 Two Dimensional Nucleoli Preprocessing and Segmentation:

The preprocessing and segmentation of the nucleoli is similar to preprocessing and segmentation of the nuclei, but the main difference is in the value of the Gaussian Blur. Smaller values are used to enable the nucleoli to be detected by the next step (segmentation). The other difference is in the value of the thresholding process to segment the nucleoli, the value is greater than the value which is used in nuclei segmentation.
Figure 3.9: Thresholding Value = 90 for Nucleoli Segmentation

Figure 3.10: Segmented Nucleoli in 2D
3.2.3 Three Dimensional Nuclei Preprocessing and Segmentation

3D preprocessing and segmentation are more complex than in 2D, and they are not limited to the Gaussian Blur Filter. To get a good result from 3D segmentation, additional steps are required. After applying the Gaussian Blur Filter, background of the images (stack) should be subtracted with a reasonable value. This process will fix the issue of uneven background. The radius should be set to be equal to at least to the size of the largest object that is not part of the background.

![Image of Nuclei Image after Background Subtraction]

**Figure 3.11: Nuclei Image after Background Subtraction**

The next step will be background correction which can be done in multiple ways. The simplest method is to use Hilo LUT. This method will display zero values as blue, and pixels with value 255 (white values) as red.
Now the image stack is ready for the final step of preprocessing before 3D segmentation. This step is achieved using Math subtraction from ImageJ.

The 3D segmentation of the nuclei is performed through the 3D simple segmentation [17] Plugin with previous preprocessing. This plugin allows manipulation of 3D objects threshold to get better segmentation results.
Finally, the statistics of segmented nuclei can be shown as in the table below.

![Figure 3.14: 3D Nuclei Segmentation](image)

<table>
<thead>
<tr>
<th>Label</th>
<th>Volume (pix^3)</th>
<th>Surface (pix^2)</th>
<th>No of obj.</th>
<th>No of wnt.</th>
<th>Index</th>
<th>Mean</th>
<th>StdDev</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>Mean dist to surf</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Objects map of Seg_Object_1</td>
<td>3056</td>
<td>1730</td>
<td>3069</td>
<td>920</td>
<td>47612</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>14</td>
<td>165.042</td>
<td>220.376</td>
<td>12.105</td>
<td>9.114</td>
</tr>
<tr>
<td>2 Objects map of Seg_Object_2</td>
<td>1019</td>
<td>1200</td>
<td>1019</td>
<td>761</td>
<td>38920</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>17</td>
<td>147.051</td>
<td>262.000</td>
<td>10.000</td>
<td>9.000</td>
</tr>
<tr>
<td>3 Objects map of Seg_Object_3</td>
<td>3934</td>
<td>1798</td>
<td>3934</td>
<td>954</td>
<td>68012</td>
<td>19</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>18</td>
<td>180.593</td>
<td>156.814</td>
<td>21.029</td>
<td>9.056</td>
</tr>
<tr>
<td>4 Objects map of Seg_Object_4</td>
<td>3774</td>
<td>1945</td>
<td>3774</td>
<td>987</td>
<td>67906</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>266.960</td>
<td>194.298</td>
<td>29.526</td>
<td>9.402</td>
</tr>
<tr>
<td>5 Objects map of Seg_Object_5</td>
<td>1772</td>
<td>1542</td>
<td>7774</td>
<td>1826</td>
<td>154480</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>20</td>
<td>41.946</td>
<td>153.760</td>
<td>95.648</td>
<td>17.032</td>
</tr>
<tr>
<td>6 Objects map of Seg_Object_6</td>
<td>16</td>
<td>52</td>
<td>16</td>
<td>16</td>
<td>304</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>24</td>
<td>83.562</td>
<td>116.120</td>
<td>22.720</td>
<td>1.230</td>
</tr>
<tr>
<td>7 Objects map of Seg_Object_7</td>
<td>95</td>
<td>192</td>
<td>95</td>
<td>82</td>
<td>2105</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>23</td>
<td>155.094</td>
<td>83.480</td>
<td>27.716</td>
<td>2.023</td>
</tr>
<tr>
<td>8 Objects map of Seg_Object_8</td>
<td>4536</td>
<td>2395</td>
<td>4538</td>
<td>1325</td>
<td>66600</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>15</td>
<td>116.076</td>
<td>104.540</td>
<td>41.227</td>
<td>11.501</td>
</tr>
<tr>
<td>9 Objects map of Seg_Object_9</td>
<td>3617</td>
<td>2209</td>
<td>3617</td>
<td>1270</td>
<td>54272</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>16</td>
<td>106.159</td>
<td>104.910</td>
<td>43.515</td>
<td>12.321</td>
</tr>
<tr>
<td>10 Objects map of Seg_Object_10</td>
<td>3138</td>
<td>1604</td>
<td>3138</td>
<td>692</td>
<td>48164</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>19</td>
<td>187.200</td>
<td>148.092</td>
<td>49.607</td>
<td>8.606</td>
</tr>
<tr>
<td>11 Objects map of Seg_Object_11</td>
<td>59</td>
<td>129</td>
<td>59</td>
<td>59</td>
<td>1180</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>33</td>
<td>165.630</td>
<td>220.530</td>
<td>63.120</td>
<td>1.886</td>
</tr>
<tr>
<td>12 Objects map of Seg_Object_12</td>
<td>4</td>
<td>16</td>
<td>6</td>
<td>6</td>
<td>84</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>21</td>
<td>165.508</td>
<td>221.680</td>
<td>45.000</td>
<td>3.707</td>
</tr>
</tbody>
</table>

![Figure 3.15: Statistics of Segmented Nuclei in 3D](image)
3.2.4 Three Dimensional Nucleoli Preprocessing and Segmentation

The same steps can be applied in the 3D nucleoli segmentation, preprocessing. However, in the preprocessing, the value of Gaussian Blur filter should be $\sigma = 1$ to remove noise and keep the small details of nucleoli for later segmentation.

![Figure 3.16: Image with Gaussian Blur Sigma $\sigma = 1$](image1)

There is another important difference where the value in math subtraction should be increased to segment the nucleoli.

![Figure 3.17: Math Subtraction with Value $= 90$](image2)
After that the 3D simple segmentation is applied to get the segmented nucleoli.

Figure 3.18: 3D Nucleoli Segmentation

<table>
<thead>
<tr>
<th>Label</th>
<th>Volume (pixel^3)</th>
<th>Surface (pixel^2)</th>
<th>No of obj. voxels</th>
<th>No of surf. voxels</th>
<th>IntDen</th>
<th>Mean</th>
<th>StdDev</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
<th>X</th>
<th>Y</th>
<th>Z</th>
<th>Mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Objects map of Seg_Object_1</td>
<td>13</td>
<td>44</td>
<td>13</td>
<td>13</td>
<td>468</td>
<td>86</td>
<td>0</td>
<td>36</td>
<td>36</td>
<td>96</td>
<td>206154</td>
<td>105462</td>
<td>27530</td>
</tr>
<tr>
<td>2</td>
<td>Objects map of Seg_Object_2</td>
<td>2</td>
<td>12</td>
<td>2</td>
<td>2</td>
<td>74</td>
<td>37</td>
<td>0</td>
<td>37</td>
<td>37</td>
<td>37</td>
<td>178950</td>
<td>162000</td>
<td>162000</td>
</tr>
<tr>
<td>3</td>
<td>Objects map of Seg_Object_3</td>
<td>200</td>
<td>364</td>
<td>200</td>
<td>158</td>
<td>7000</td>
<td>35</td>
<td>0</td>
<td>35</td>
<td>35</td>
<td>35</td>
<td>206760</td>
<td>190800</td>
<td>32960</td>
</tr>
<tr>
<td>4</td>
<td>Objects map of Seg_Object_4</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>38</td>
<td>38</td>
<td>0</td>
<td>38</td>
<td>38</td>
<td>38</td>
<td>202000</td>
<td>810000</td>
<td>350000</td>
</tr>
<tr>
<td>5</td>
<td>Objects map of Seg_Object_5</td>
<td>906</td>
<td>542</td>
<td>306</td>
<td>232</td>
<td>11934</td>
<td>39</td>
<td>0</td>
<td>39</td>
<td>39</td>
<td>39</td>
<td>41732</td>
<td>150144</td>
<td>58788</td>
</tr>
<tr>
<td>6</td>
<td>Objects map of Seg_Object_6</td>
<td>124</td>
<td>222</td>
<td>124</td>
<td>89</td>
<td>5332</td>
<td>43</td>
<td>0</td>
<td>43</td>
<td>43</td>
<td>43</td>
<td>117290</td>
<td>183048</td>
<td>300000</td>
</tr>
<tr>
<td>7</td>
<td>Objects map of Seg_Object_7</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>42</td>
<td>42</td>
<td>0</td>
<td>42</td>
<td>42</td>
<td>42</td>
<td>200000</td>
<td>410000</td>
<td>370000</td>
</tr>
<tr>
<td>8</td>
<td>Objects map of Seg_Object_8</td>
<td>96</td>
<td>194</td>
<td>96</td>
<td>77</td>
<td>3810</td>
<td>40</td>
<td>0</td>
<td>40</td>
<td>40</td>
<td>40</td>
<td>42604</td>
<td>160956</td>
<td>41177</td>
</tr>
<tr>
<td>9</td>
<td>Objects map of Seg_Object_9</td>
<td>12</td>
<td>40</td>
<td>12</td>
<td>11</td>
<td>492</td>
<td>41</td>
<td>0</td>
<td>41</td>
<td>41</td>
<td>41</td>
<td>191838</td>
<td>148388</td>
<td>38917</td>
</tr>
<tr>
<td>10</td>
<td>Objects map of Seg_Object_10</td>
<td>4</td>
<td>20</td>
<td>4</td>
<td>4</td>
<td>184</td>
<td>46</td>
<td>0</td>
<td>46</td>
<td>46</td>
<td>46</td>
<td>120000</td>
<td>187000</td>
<td>39500</td>
</tr>
<tr>
<td>11</td>
<td>Objects map of Seg_Object_11</td>
<td>480</td>
<td>820</td>
<td>480</td>
<td>369</td>
<td>21800</td>
<td>45</td>
<td>0</td>
<td>45</td>
<td>45</td>
<td>45</td>
<td>108685</td>
<td>135668</td>
<td>44610</td>
</tr>
<tr>
<td>12</td>
<td>Objects map of Seg_Object_12</td>
<td>82</td>
<td>160</td>
<td>82</td>
<td>71</td>
<td>9008</td>
<td>44</td>
<td>0</td>
<td>44</td>
<td>44</td>
<td>44</td>
<td>112256</td>
<td>189954</td>
<td>42195</td>
</tr>
<tr>
<td>13</td>
<td>Objects map of Seg_Object_13</td>
<td>185</td>
<td>370</td>
<td>185</td>
<td>153</td>
<td>9250</td>
<td>50</td>
<td>0</td>
<td>50</td>
<td>50</td>
<td>50</td>
<td>221130</td>
<td>216964</td>
<td>44103</td>
</tr>
<tr>
<td>14</td>
<td>Objects map of Seg_Object_14</td>
<td>148</td>
<td>212</td>
<td>148</td>
<td>97</td>
<td>7752</td>
<td>49</td>
<td>0</td>
<td>49</td>
<td>49</td>
<td>49</td>
<td>169821</td>
<td>145189</td>
<td>44500</td>
</tr>
<tr>
<td>15</td>
<td>Objects map of Seg_Object_15</td>
<td>35</td>
<td>88</td>
<td>35</td>
<td>29</td>
<td>1645</td>
<td>47</td>
<td>0</td>
<td>47</td>
<td>47</td>
<td>47</td>
<td>46457</td>
<td>157845</td>
<td>45229</td>
</tr>
<tr>
<td>16</td>
<td>Objects map of Seg_Object_16</td>
<td>22</td>
<td>60</td>
<td>22</td>
<td>19</td>
<td>1006</td>
<td>46</td>
<td>0</td>
<td>46</td>
<td>46</td>
<td>46</td>
<td>111591</td>
<td>184273</td>
<td>44081</td>
</tr>
<tr>
<td>17</td>
<td>Objects map of Seg_Object_17</td>
<td>11</td>
<td>30</td>
<td>11</td>
<td>11</td>
<td>606</td>
<td>55</td>
<td>0</td>
<td>55</td>
<td>55</td>
<td>55</td>
<td>120000</td>
<td>109545</td>
<td>43909</td>
</tr>
<tr>
<td>18</td>
<td>Objects map of Seg_Object_18</td>
<td>94</td>
<td>150</td>
<td>94</td>
<td>67</td>
<td>4992</td>
<td>53</td>
<td>0</td>
<td>53</td>
<td>53</td>
<td>53</td>
<td>182170</td>
<td>144277</td>
<td>46021</td>
</tr>
<tr>
<td>19</td>
<td>Objects map of Seg_Object_19</td>
<td>2</td>
<td>10</td>
<td>2</td>
<td>2</td>
<td>108</td>
<td>54</td>
<td>0</td>
<td>54</td>
<td>54</td>
<td>54</td>
<td>45000</td>
<td>147000</td>
<td>44500</td>
</tr>
<tr>
<td>20</td>
<td>Objects map of Seg_Object_20</td>
<td>34</td>
<td>80</td>
<td>34</td>
<td>30</td>
<td>1734</td>
<td>51</td>
<td>0</td>
<td>51</td>
<td>51</td>
<td>51</td>
<td>43502</td>
<td>150471</td>
<td>45971</td>
</tr>
<tr>
<td>21</td>
<td>Objects map of Seg_Object_21</td>
<td>25</td>
<td>56</td>
<td>25</td>
<td>24</td>
<td>1800</td>
<td>52</td>
<td>0</td>
<td>52</td>
<td>52</td>
<td>52</td>
<td>120800</td>
<td>181590</td>
<td>44800</td>
</tr>
<tr>
<td>22</td>
<td>Objects map of Seg_Object_22</td>
<td>1</td>
<td>6</td>
<td>1</td>
<td>1</td>
<td>56</td>
<td>56</td>
<td>0</td>
<td>56</td>
<td>56</td>
<td>56</td>
<td>191000</td>
<td>142000</td>
<td>45000</td>
</tr>
</tbody>
</table>

Figure 3.19: 3D Nucleoli Statistics
3.2.5 Nucleus -Nucleolus Clustering:

To increase the functionality of the proposed algorithm, Nucleus -Nucleolus Clustering is performed to link the nucleoli to the nucleus that they belongs to. Each nucleus will be considered as a cluster for the small objects (nucleoli) and we can classify them as one group. In the following table the window represents nucleus, and the objects in the window are the nucleoli.

<table>
<thead>
<tr>
<th>Label</th>
<th>Volume (pixel^3)</th>
<th>Surface (pixel^2)</th>
<th>No of obj. voxels</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Objects map of Nucleolus of NUC 1 - c.tif_Object_1</td>
<td>25</td>
<td>70</td>
</tr>
<tr>
<td>2</td>
<td>Objects map of Nucleolus of NUC 1 - c.tif_Object_2</td>
<td>13</td>
<td>38</td>
</tr>
<tr>
<td>3</td>
<td>Objects map of Nucleolus of NUC 1 - c.tif_Object_3</td>
<td>3</td>
<td>14</td>
</tr>
<tr>
<td>4</td>
<td>Objects map of Nucleolus of NUC 1 - c.tif_Object_4</td>
<td>12</td>
<td>40</td>
</tr>
<tr>
<td>5</td>
<td>Objects map of Nucleolus of NUC 1 - c.tif_Object_5</td>
<td>13</td>
<td>42</td>
</tr>
<tr>
<td>6</td>
<td>Objects map of Nucleolus of NUC 1 - c.tif_Object_6</td>
<td>2</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>Objects map of Nucleolus of NUC 1 - c.tif_Object_7</td>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>8</td>
<td>Objects map of Nucleolus of NUC 1 - c.tif_Object_8</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>9</td>
<td>Objects map of Nucleolus of NUC 1 - c.tif_Object_9</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>10</td>
<td>Objects map of Nucleolus of NUC 1 - c.tif_Object_10</td>
<td>50</td>
<td>122</td>
</tr>
<tr>
<td>11</td>
<td>Objects map of Nucleolus of NUC 1 - c.tif_Object_11</td>
<td>11</td>
<td>40</td>
</tr>
<tr>
<td>12</td>
<td>Objects map of Nucleolus of NUC 1 - c.tif_Object_12</td>
<td>10</td>
<td>34</td>
</tr>
</tbody>
</table>

Figure 3.20: Nucleus 1 Statistics

<table>
<thead>
<tr>
<th>Label</th>
<th>Volume (pixel^3)</th>
<th>Surface (pixel^2)</th>
<th>Nb of obj. voxels</th>
<th>Nb of surf. voxels</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Objects map of Nucleolus of NUC 2 - c.tif_Object_1</td>
<td>980</td>
<td>1402</td>
<td>980</td>
</tr>
<tr>
<td>2</td>
<td>Objects map of Nucleolus of NUC 2 - c.tif_Object_2</td>
<td>1</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>Objects map of Nucleolus of NUC 2 - c.tif_Object_3</td>
<td>3</td>
<td>14</td>
<td>3</td>
</tr>
</tbody>
</table>

Figure 3.21: Nucleus 2 Statistics
3.2.6 Results Visualization:

Visualization is a very important step in nuclei and nucleoli segmentation and analysis because it can show the results of the system visually and the user can easily analyze the data and evaluate the results. The visualization process uses the images of segmented nuclei, images of segmented nucleoli and the original image. Each data set can be merged by taking the data that contain a good detail from each image and merge the rest of data set with them.

Figure 3.22: Nucleus 10 Statistics

<table>
<thead>
<tr>
<th>Label</th>
<th>Volume (pixel(^3))</th>
<th>Surface (pixel(^2))</th>
<th>Nb of obj. voxels</th>
<th>Nb of surf. voxels</th>
</tr>
</thead>
<tbody>
<tr>
<td>1  Objects map of Nucleolus of NUC 10 - c.tif_Object_1</td>
<td>387</td>
<td>387</td>
<td>335</td>
<td></td>
</tr>
<tr>
<td>2  Objects map of Nucleolus of NUC 10 - c.tif_Object_2</td>
<td>2</td>
<td>10</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3  Objects map of Nucleolus of NUC 10 - c.tif_Object_3</td>
<td>7</td>
<td>26</td>
<td>7</td>
<td>7</td>
</tr>
</tbody>
</table>

Figure 3.23: Image before Visualization

Figure 3.24: Segmented Nuclei Visualization
Figure 3.25: Segmented Nucleoli Visualization

Figure 3.26: Nuclei and Nucleoli Visualization

Figure 3.27: 3D View Visualization
CHAPTER 4

EXPERIMENTAL RESULTS AND ANALYSIS

The proposed NNSA system performs efficiently the segmentation and analysis of nuclei and nucleoli in the data sets. The system is fast and can save a significant amount of time. When using the manual segmentation, the time needed to segment all the nuclei and nucleoli in the data set - which contains 60 slide in one stack- is about 34 minutes and 37 minutes respectively. However, the NNSA can achieves the segmentation with less than 10 seconds. In the large data sets, the system can significantly facilitate the process of segmentation. The manual segmentation results are not always accurate (70% to 87%), but the efficiency of the NNSA system is considerably high and can achieve 98% accuracy. The accuracy of the system depends on the preprocessing that removes the noise and prepares the image for the segmentation. Gaussian Blur plays an important role in the preprocessing and its value can affect the result of segmentation for nuclei and nucleoli.
<table>
<thead>
<tr>
<th>Filter type</th>
<th>Value</th>
<th>Deference in Nuclei segmentation</th>
<th>Deference in Nucleoli segmentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaussian Blur</td>
<td>1</td>
<td>60%</td>
<td>0.1%</td>
</tr>
<tr>
<td>Gaussian Blur</td>
<td>2</td>
<td>30%</td>
<td>28%</td>
</tr>
<tr>
<td>Gaussian Blur</td>
<td>3</td>
<td>0.1%</td>
<td>73%</td>
</tr>
</tbody>
</table>

Table 4.1 Deferent Segmentation Results with Deferent Gaussian Blur Values
Additional noise removal processes such as background subtraction, math subtraction and Hilo LUT also can change the results of segmentation especially in nucleoli segmentation because the nucleoli are smaller than nuclei.

Figure 3.31: Background Subtraction value =10

Figure 3.32: Background Subtraction value =100

Figure 3.33: 3D Nuclei Segmentation with Background Subtraction Value =10

Figure 3.34: 3D Nuclei Segmentation with Background Subtraction Value =100
<table>
<thead>
<tr>
<th>Filter type</th>
<th>Value</th>
<th>Deference in Nuclei segmentation</th>
<th>Deference in Nucleoli segmentation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Background subtraction</td>
<td>10</td>
<td>24%</td>
<td>26%</td>
</tr>
<tr>
<td>Background subtraction</td>
<td>100</td>
<td>20%</td>
<td>21%</td>
</tr>
<tr>
<td>Background subtraction</td>
<td>50</td>
<td>0.1%</td>
<td>0.1%</td>
</tr>
</tbody>
</table>

Table 4.2 Deferent Segmentation Results with Deferent Background Subtraction values
Table 4.3. Deferent Segmentation Results with Deferent Math Subtraction Values
CHAPTER 5

CONCLUSION AND FUTURE WORK

The proposed system provides an efficient method for nuclei and nucleoli segmentation and analysis. This system facilitates complex processes and achieves very good results and save significant amount of time. However, there are some developments that can be added to the system for more functionality including the following: -

1- Use additional filters that can remove noise efficiently such as median filter, or mean filter with Gaussian blur to increase the preprocessing strength.

2- Clustering can be performed using nearest neighbor method that depends on the Euclidean distance among the objects.

3- Use the k-mean clustering for nuclei and nucleoli segmentation and classification to cluster the objects based on the color.

4- Generalize the system to segment and analyze additional objects and not to be limited to nuclei and nucleoli segmentation.
REFERENCES


