Cell Specific Segmentation: HeLa and Glial Cells

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Declaration

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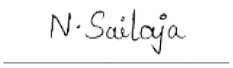

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This Dissertation entitled "Cell specific Segmentation: HeLa and Glial Cells" by Sailaja Nanda is approved for the degree of Masters in Technology from IIT Hyderabad

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Dedication

To my family and friends who have been part of my learning experience

Abstract

Cancer is a disease for which we don't have a medicine till now. Because no one knows their cell structure, and properties of cells. to find those is difficult. so confocal microscopy is used to grab information. Using that cell cultues cells videos are recorded. By observing and quantifying those videos some properties of the cells are identified. Inorder to have maximum information segmentation has to be done. It differentiates the cells from the background. Simultaneously cell shape also notable.

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In this thesis, I attempted to present solutions for the selected biomedical challenges motivated by current clinical significance. In order to find the drug for cervical cancer, first we have to know the differences between the normal and abnormal cells.To find that we approached two method . We now proceed to state each of the challenges that are addressed in this thesis.

2.1 HeLa Cell

2.1.1 History of HeLa Cells

These are the cells taken from a patient called Henrietta Lacks, cervical cancer patient. First human cell line, or group of cells to survive in vitro (test tube). Lacks' tissue samples are grown by a researcher named Dr. George Gey in 1951. He realized that these cells were different from the normal cells. After more than 50 years, these are now billions and trillions of HeLa cells in laboratories all over the world. It's the most commonly used cell line, and its known to be extremely buoyant.

All of the body's normal cells experience the effects of aging over time, known as cellular senescence. Repeated divisions cause the cells DNA to become unstable, and sometimes toxins form. This means that eventually the cells are unable to replicate, or divide, and the cell dies. This is called programmed cell death(PCD), apoptosis or even cellular suicide. It's a part of the normal process for many cells, it varies depending on the type of cell. Generally, PCD occurs about 50 cell divisions. But this is what sets HeLa apart. Even under the right conditions, HeLa cells divide infinitely.

2.1.2 Advantages of HeLa Cells

1. Before HeLa cells, scientists spent more time trying to keep cells alive than performing actual research on the cells. An endless supply of HeLa cells freed up time for discovery.

2. In 1952, the worst year of the polio epidemic, HeLa cells were used to test the vaccine that protected millions.

3. Some cells in Lacks's tissue sample behaved differently than others. Scientists learned to isolate one specific cell, multiply it, and start a cell line. Isolating one cell and keeping it alive is the

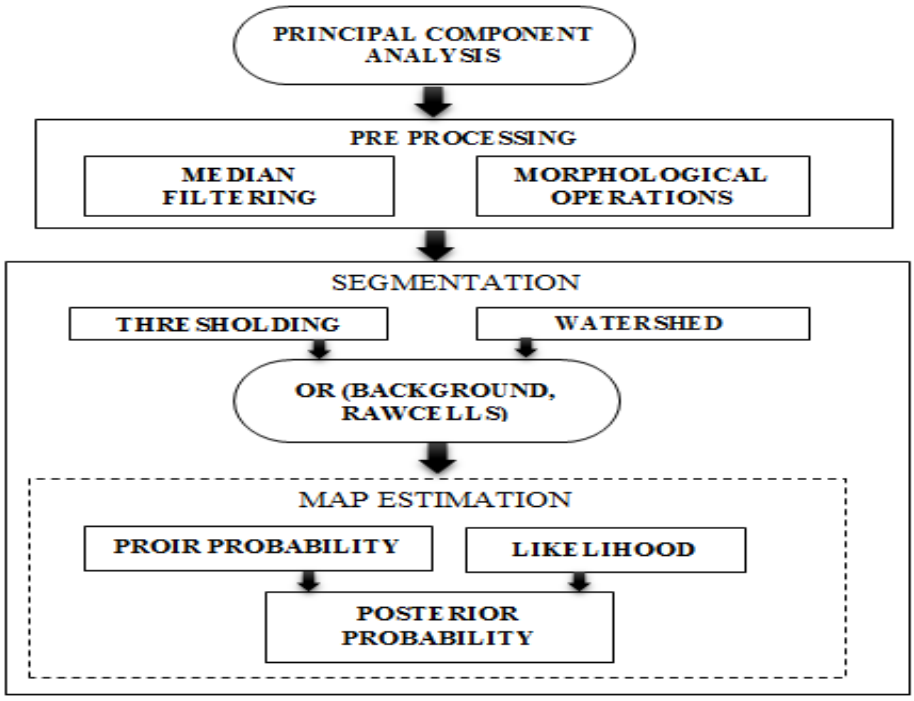


Figure 2.1: Steps involved in this method

basic technique for cloning and in-vitro fertilization.

4. A scientist accidentally poured a chemical on a HeLa cell that spread out its tangled chromosomes. Later on, scientists used this technique to determine that humans have 46 chromosomes 23 pairs not 48, which provided the basis for making several types of genetic diagnoses.

5. It was discovered that Lacks's cancerous cells used an enzyme called telomerase to repair their DNA, allowing them, and other types of cancer cells, to function when normal cells would have died. Anti-cancer drugs that work against this enzyme are currently in early clinical trials.

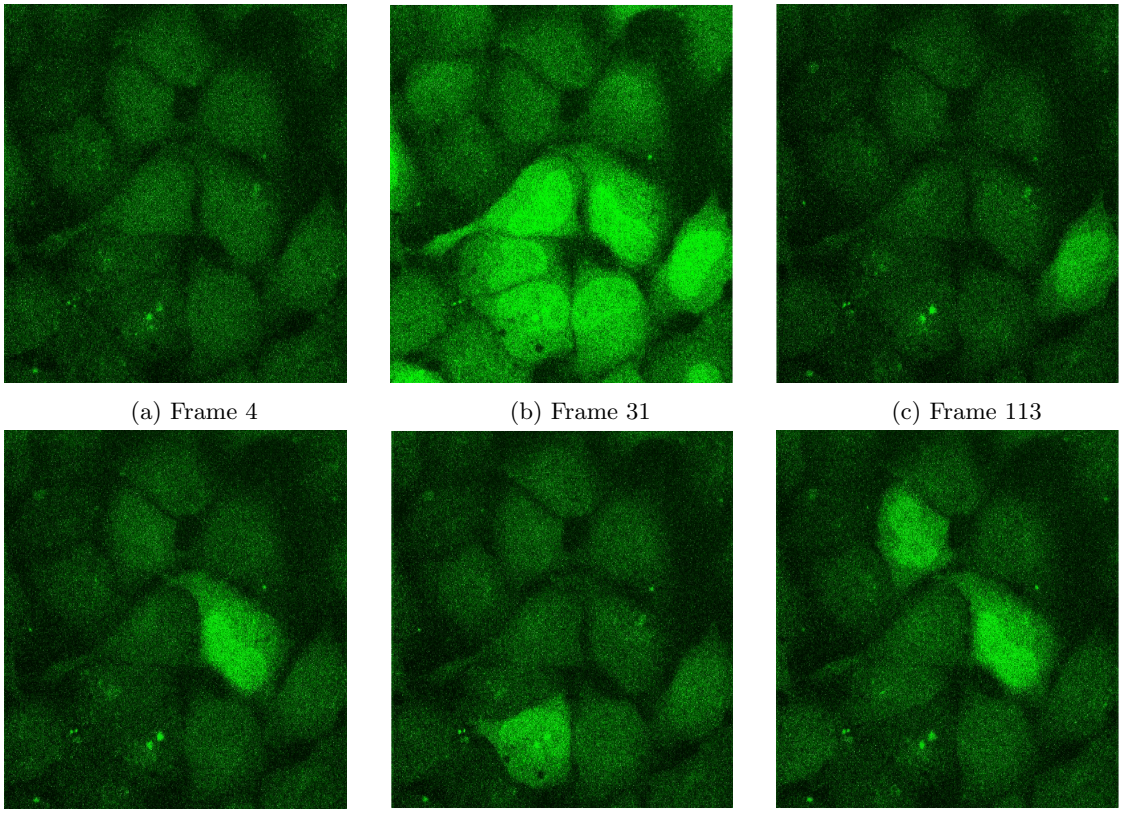
2.2 Segmentation using MAP Estimation

This method includes preprocessing and Segmentatation. Preprocessing includes Median filtering which reomoves noise from the frames. MAP Estimation technique is used to segment the image and results the divided foreground and background.

2.2.1 Finding brightest frame

This HeLa cells video consist RGB frames also known as color images.

Each RGB frame is converted to grayscale image. 8 bit colour format is one of the most famous image formats. It has 256 different shades of colors in it. It is commonly known as Grayscale image. The range of the colors in 8 bit vary from 0-255. Where 0 stands for black, and 255 stands for white, and 127 stands for gray color.



(a) Frame 4 (b) Frame 31 (c) Frame 113

(d) Frame 130 (e) Frame 151 (f) Frame 162

Figure 2.2: Different frames from video

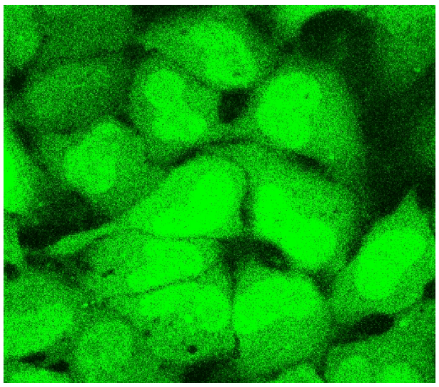
Each frame has of size MxN. Each frame has MN pixels. Pixel is the smallest element of an image. Each pixel corresponds to any one value. In an 8-bit gray scale image, the value of the pixel between 0 and 255. The value of a pixel at any point corresponds to the intensity of the light photons striking at that point. Each pixel store a value proportional to the light intensity at that particular location.. To find the brightest frame in a video, calculated the sum of all pixel intensities. Among all which shows the highest value was the brightest frame.

In the considered video, all HeLa cells are not activated at the same time. So if we consider one of the frames from video, we may lose the information about the non-activated cells. Even if we consider the brightest frame, only some cells are activated. In order to find the brightest and clearer picture of cells Principal Component Analysis is used.

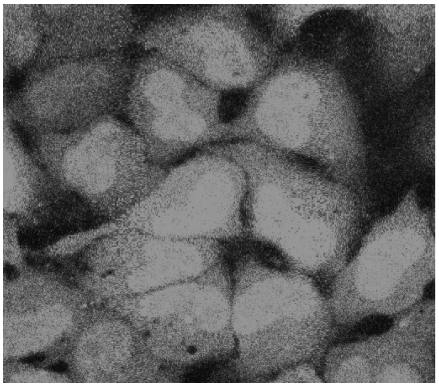
2.2.2 Principal Component Analysis

It is a way of identifying patterns in data, and expressing the data in such a way as to highlight their similarities and differences. Principal Component Analysis is a technique which uses sophisticated underlying mathematical principles to transforms a number of possibly correlated variables into a smaller number of variables called principal components [2].

PCA is a statistical method under the broad title of factor analysis. The purpose of PCA is to reduce the large dimensionality of the data space (observed variables) to the smaller intrinsic dimensionality of feature space (independent variables), which are needed to describe the data



(a) Brightest frame(RGB)



(b) Brightest frame(Grayscale)

Figure 2.3: Hightest intensity frame in RGB and Grayscale

economically. This is the case when there is a strong correlation between observed variables. The jobs which PCA can do are prediction, redundancy removal, feature extraction, data compression, etc. Because PCA is a classical technique which can do something in the linear domain,applications, such as signal processing, image processing, system and control theory, communications, etc.

It is applied along temporal axis. If we consider a video, time to time the properties or characteristics of images are changes. If we concentrate on only one frame then we may lost the information. So, In order to find the brightest and clear perspective of the images PCA is helpful. It gives the maximum information in the first principal component.

It is a statistical procedure that uses an orthogonal transformation to convert a set of possibly correlated variables into a set of values of linearly uncorrelated variables called principal components. The number of principal components is less than or equal to the number of original variables. This transformation is defined in such a way that the first principal component has the largest possible variance (that is, accounts for as much of the variability in the data as possible), and each succeeding component in turn has the highest variance possible under the constraint that it is orthogonal to the preceding components.

Algorithm[3]:

- Get some data
- Subtract the mean or centralize the data
- Calculate the covariance matrix
- Calculate the eigen values and eigen vectors of the covariance matrix
- Choosing components and forming a feature vector
- Multiplication of Data with feature vector.

Method[4]: We are having a video consists of 519 frames. Each image is of size 512x512. [512 rows and 512 columns] Each column vector of image is placed one below the other and form a matrix (vector) of size (512*512) x1. [512x512 rows and 1 column]

$$\text{img\_vec1} = \begin{bmatrix} x_1 \\ x_2 \\ \vdots \\ x_N \end{bmatrix}_{N^2 \times 1}$$

After converting every image to a column vector, each image is placed one adjacent to other and forms a matrix of size (512*512) x519. [512*512 rows and 519 columns]

$$X = \begin{bmatrix} \text{img\_vec}_1 & \text{img\_vec}_2 \cdots & \text{img\_vec}_M \end{bmatrix}_{N^2 \times M}$$

Now we have our data matrix with correlated information among frames. First find the mean of the data matrix.

$$\psi = \frac{1}{M} \sum_{i=1}^{M} \text{img\_vec}_i$$

Subtract mean from each frame (column of data matrix) and form our new data matrix of same size.

$$\bar{X} = X - \psi$$

After that, find the covariance matrix for data which is of size 512x512.

$$C_{XX} = \frac{1}{M} \bar{X}^T \bar{X}$$

Find the Eigen values and vectors for covariance matrix.

The mathematical technique used in PCA is called eigen analysis: we solve for the eigenvalues and eigenvectors of a square symmetric matrix with sums of squares and cross products. The eigenvector associated with the largest eigenvalue has the same direction as the first principal component. The eigenvector associated with the second largest eigenvalue determines the direction of the second principal component. The sum of the eigenvalues equals the trace of the square matrix and the maximum number of eigenvectors equals the number of rows (or columns) of this matrix. Eigen vector matrix has of size 519x519. In those, chose only some Eigen vectors because most of the information lies in first 5 vectors. Our eigne vector matrix V has of size 519x5.

Data matrix is multiplied with obtained eigen vector matrix and form a vector of size (512x512)x5.

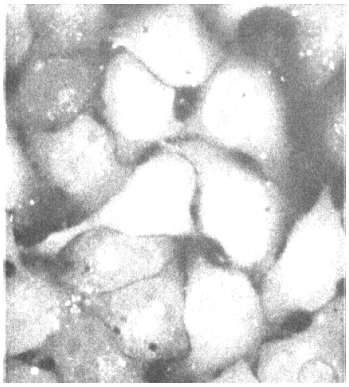
$$Y = X \times V$$

Now we rearrange the data matrix as frames. After rearrangement, We can clearly notice that, First component of PCA has the highest information of data. we know that, all components are uncorrelated. So, 2 nd component has the information which is not there in the first component. The resulted images are shown in the below figure.

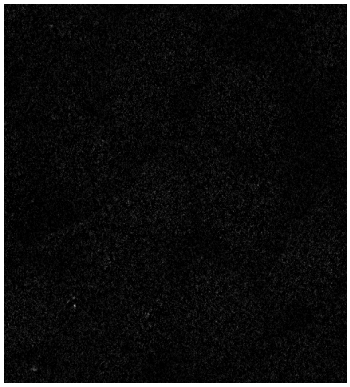
We took first component of PCA image and extracted the background and foreground.

2.2.3 Background extraction:

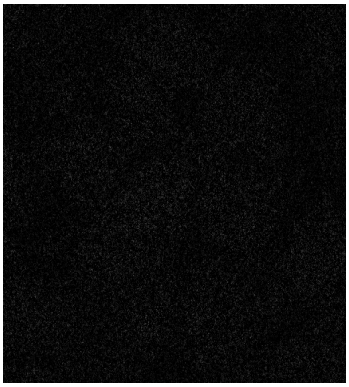
Background is extracted in 2 ways.



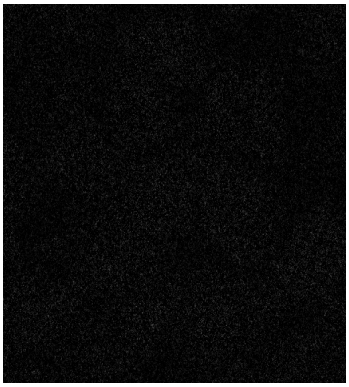
(a) PCA first component



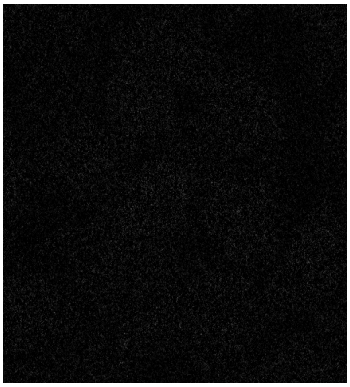
(b) PCA second component



(c) PCA third component



(d) PCA fourth component



(e) PCA fifth component

Figure 2.4: Components of PCA

1. On the first principal component, apply median filter and then thresholded. After some binary morphological operations like erosion, dilation are applied to remove artifacts.

Median Filter:

Median filtering is a nonlinear method used to remove noise from images. In this process, the neighbouring pixels are ranked according to brightness (intensity) and the median value becomes the new value for the central pixel. Median filters[5] can do an excellent job of rejecting certain types of noise, in particular, shot or impulse noise in which some individual pixels have extreme values. In this operation, the pixel values in the neighbourhood window are ranked according to intensity, and the middle value (the median) becomes the output value for the pixel under evaluation.

Replace each pixel value with the median of the gray values in the region of the pixel:

(a) Take a3x3 region centered around pixel(i,j).

(b) Sort the intensity values of the pixels in the region into ascending order.

(c) Select the middle value as the new value of pixel (i,j).

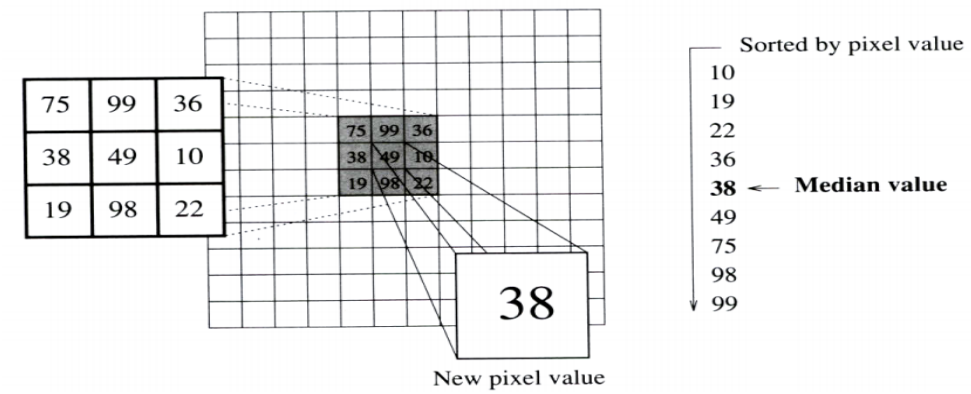


Figure 2.5: Median filtering

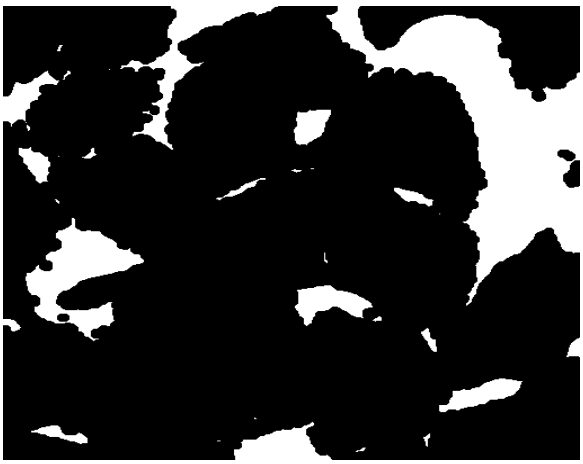


Figure 2.6: Background using Median filtering

After that converted the grayscale image to binary image using thresholding. And some binary morphological operations are used.

Binary morphological operations:

A Structural element or window defines a geometric relationship between a pixel and its neighbours.

Dilation: Logical OR operation of Image and window. It removes small holes r gaps.It increases the size of logical 1 objects.

Erosion: Logical AND operation of Image and window.It removs small objects and narrow peninsulas. It decreases the size of logical 1 objects.

Combination of Erosion and dilation are used to remove the artefacts.

2. First component of PCA which is grayscale image is converted to binary image using threshold. After applied not operation.

Figure 2.7: Background using Threshold

Gray level thresholding:

Pick a threshold T and make a binary decision.

For an image I(i, j) with K levels, pick

$$0 \leq T \leq K - 1$$

then the binary image is represented as

$$J(i,j) = \begin{cases} 1, if I(i,j) \geq T \\ 0, if I(i,j) < T \end{cases}$$

Extracted the brightest part in each cell using Watershed.

2.2.4 Extraction of Rawcells using watershed:

A popular region growing method, which has proved to be very useful in many areas of image segmentation and analysis, is the so-called watershed algorithm. The method was originally suggested by Digabel and Lantujoul, and extended to a more general framework by Beucher Lantujoul (1979). Watershed segmentation has then been refined and used in very many situations (for an overview see Meyer Beucher, 1990; Vincent, 1993).

The main difference between the watershed method and ordinary region growing is that the watershed method works per intensity layer instead of per neighbour layer. If the intensity of the image is interpreted as elevation in a landscape, the watershed algorithm will split the image into regions similar to the drainage regions of this landscape. The watershed borders will be built at the crests in the image.

In a gradient magnitude image, water will start to rise from minima representing areas of low gradient, i.e. the interior of the objects and the background, and the watershed borders will be built at the maxima of the gradient magnitude. However, if watershed segmentation is applied directly

to the gradient magnitude image, it will almost always result in over-segmentation, owing to the intensity variations within both objects and background.

Instead of letting water rise from every minimum in the image, water can be allowed to rise only from places marked as seeds. Seeded watersheds have previously been described (e.g. Meyer Beucher, 1990; Beucher, 1992; Vincent, 1993; Lockett et al., 1998; Landini Othman, 2003).

Fully automatic foreground seeding is tricky, and using morphological filtering, one often ends up with more than one seed per object, or objects containing no seed at all. More than one seed per foreground object will in many methods (i.e. Meyer Beucher, 1990) result in background seeds passing through foreground components, leading to incorrect segmentation results. Many seeded watershed segmentation methods are therefore based on manual seeding (e.g. Lockett et al., 1998), requiring extensive user interaction. In Stoev Straer (2000), a way of using a seeded watershed for extracting a single, manually seeded region of interest is presented. It uses four merging criteria to overcome the over-segmentation problem.

The threshold values needed for the merging step are all calculated from the marked seed in the region of interest. Merging to reduce over-segmentation is also described in Najman Schmitt (1996) and Lemarchal Fjortoft (1998) Edge-based segmentation techniques, which try to connect local maxima of the gradient image, often run into problems when trying to produce closed curves. That is why regionbased methods, such as region growing or watershed, that group similar pixels are often used. These seeds serve as starting points in the watershed algorithm applied to the gradient magnitude image.

The images used in this paper contain bright objects on a darker background. Hence, each object of interest contains at least one local intensity maximum. We define foreground seeds in the original image using the extended h-maxima transform (Soille, 1999). The extended h-maxima transform filters out the relevant maxima using a contrast criterion. All maxima whose heights are smaller than a given threshold level h are suppressed.

The extended h-maxima transformation can be implemented using sorted pixels and searching for local maxima with a given contrast compared with the local neighbourhood. The only parameter h is related to the height of the structures and no size or shape criterion is needed. A low h will result in many seeds, often more than one seed per object. A high h will result in fewer seeds, and some objects may not get a seed at all. Owing to a subsequent merging step based on gradient magnitude (described below), we use a rather low h value to ensure that each object gets at least one seed. The choice of h turns out not to be a critical operation, because a range of values yield satisfactory results, as long as each object gets at least one seed.

All foreground seeds are uniquely labelled using connected component labelling. Just as the objects can be seeded by extended h-maxima in the original image, the background can be seeded by extended h-minima in the original image, i.e. local minima deeper than a certain depth h. This method of seeding the background was used in an earlier version of our method (Whlby Bengtsson, 2003). Owing to generally higher background intensity close to fluorescent objects, this way of seeding hardly generates any background seeds at all close to the objects.

We choose to define our new background seeds in the gradient magnitude image instead, because an uneven background in the original image will not be strongly apparent in the gradient magnitude image. We calculate the gradient magnitude image, as described below, and define our background seeds as the extended h-minima in the gradient magnitude image. Because the interiors of cell nuclei

also will be local minima in the gradient magnitude, we have to discard all connected extended h-minima components smaller than a certain size, s, to make sure that no object pixels are set as background seeds. This way of using the gradient magnitude image to seed the background generates background seeds evenly distributed in the image, even if an image has a non-uniform background.

A foreground seed may overlap with the background seed. If this is the case, the overlap region is set to belong to the foreground seed. It will, however, most likely be merged with the background in the subsequent merging step as its borders will have a very weak gradient magnitude. The seeds are important for the final result of the segmentation. Non-seeded objects will never be found. More than one seed per object does not, however, necessarily lead to over segmentation in the final result. It is therefore better to have too many than too few seeds. A more exact seeding will, by contrast, result in faster segmentation.

Calculating the gradient

The seeds of the objects and the seeds of the background should grow and meet where the gradient magnitude image has a local maximum. The magnitude of the gradient expresses the variation of local contrast in the image, i.e. sharp edges have a high gradient magnitude, whereas more uniform areas in the image have a gradient magnitude close to zero. The local maximum of the gradient amplitude marks the position of the strongest edge between object and background. There are many different approximations of the gradient magnitude of an image in mathematical morphology. Here we used sobel operator, which is

$$\begin{bmatrix} 1 & 2 & 1 \\ 0 & 0 & 0 \\ -1 & -2 & 1 \end{bmatrix}$$

Overview of Watershed

Watershed segmentation can be understood by interpreting the intensity image as a landscape. A hole is drilled in every minima of the landscape, and the landscape is submerged in water. Water will then start to fill the minima, creating catchment basins. As the water rises, water from neighbouring catchment basins will meet. At every point where two catchment basins meet, a dam, or watershed, is built. These watersheds are the segmentation of the image.

Watershed segmentation can be implemented with sorted pixel lists (Vincent Soille, 1991). This implies that the segmentation can be performed very rapidly. In the method described by Vincent Soille (1991), pixels that are located at an equal distance from two catchment basins become part of the watershed lines. This means that we sometimes get thick watershed lines, leading to pixels that are not part of any catchment basin. In our implementation of the watershed algorithm, we keep track of the pixels that are ambiguous, i.e. located at an equal distance from two or more catchment basins, and let water flow around them.

As a last step of the watershed, the most common neighbouring label is assigned to the ambiguous pixel, and every pixel is thereby made part of a catchment basin. This is necessary for the subsequent merging step described below. In our seeded version of the watershed segmentation, water will rise from pixels marked as seeds, as well as from non-seeded regional minima found in the image. Separating dams,or watersheds, are built only between catchment basins associated with different seeds. As soon as the water level of a seeded catchment basin reaches the weakest point of the

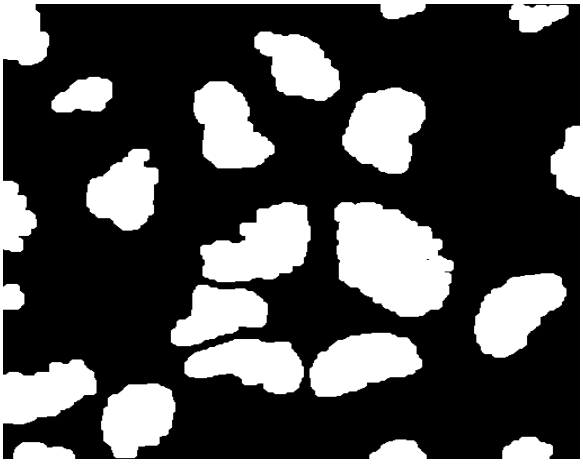


Figure 2.8: Extraction of Rawcells using watershed

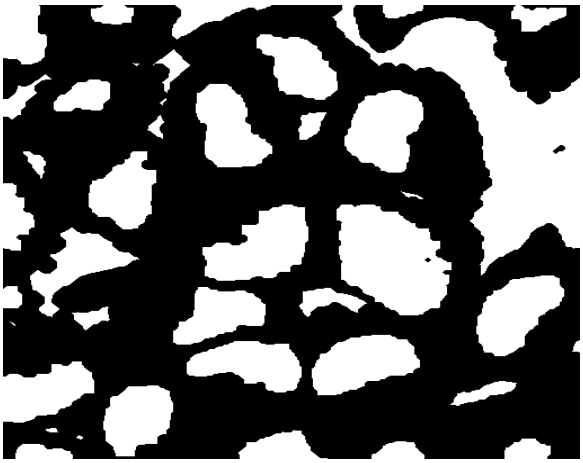


Figure 2.9: Logical OR (Background, Rawcell)

border towards a non-seeded regional minimum, it will be flooded. The water will continue to rise until each seeded catchment basin in the gradient magnitude image meets another seeded catchment basin.

Logical OR operation is applied on Rawcell and Background images which is shown in fig 2.9

2.2.5 MAP Estimation:

Algorithm:

We have a priori information about the physical process whose parameters we want to estimate. Such information can come either from the correct scientific knowledge of the physical process or from previous empirical evidence. We can encode such prior information in terms of a PDF on the parameter to be estimated. Essentially, we treat the parameter

$$\theta$$

as the value of an RV. The associated probabilities

$$P(\theta)$$

are called the prior probabilities. We refer to the inference based on such priors as Bayesian inference. Bayes' theorem shows the way for incorporating prior information in the estimation process:

$$P(\theta|x) = \frac{P(x|\theta)P(\theta)}{P(x)}$$

The term on the left hand side of the equation is called the posterior. On the right hand side, the numerator is the product of the likelihood term and the prior term. The denominator serves as a normalization term so that the posterior PDF integrates to unity. Thus, Bayesian inference produces the maximum a posteriori (MAP) estimate

$$\arg\max_{\theta} P(\theta|x) = \arg\max_{\theta} P(x|\theta)P(\theta)$$

Method:

First consider the raw cell image and label the cells.

Let the raw cell image contains N number of cells from

$$X_1, X_2, ...X_N$$

find out the centroid coordinates for each cell.

$$X_i = \begin{bmatrix} x_i \\ y_i \end{bmatrix}$$

Consider the black pixel of coordinates (x,y) from the Logical OR image and it has Intensity Calculate the prior probability of this pixel using the Gaussian distribution.

$$P_i(x, y) = e^{\frac{-(x-x_i)^2+(y-y_i)^2}{2\sigma^2}}$$

This pixel should be belongs to any of the cells. So sum of the probabilities is 1.

$$\sum_{i=1}^{N} P_i(x, y) = 1$$

So Prior probability of this pixel for particular i th cell is

$$P_i(x, y) = \frac{e^{\frac{-(x-x_i)^2+(y-y_i)^2}{2\sigma^2}}}{\sum\limits_{j=1, j\neq i}^{N} P_j(x, y)}$$

Calculate the histogram for each cell. Histogram gives probability of the intensity values. Likelihood

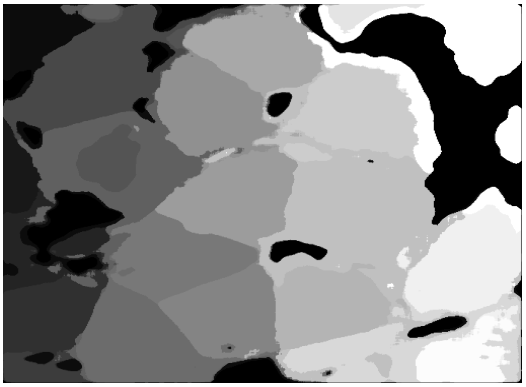


Figure 2.10: Segmented Image

is the pixel probability of intensity value in the given cell.

$$P(I(x,y)|(x_i,y_i))$$

Multiply the prior probability with the likelihood.

$$P((x_i,y_i)|(x,y)) = P_i(x,y) \times P(I(x,y)|(x_i,y_i))$$

For each pixel calculate the prior for each cell and multiply with the likelihood. Continue this from 1 to N number of cells. Find the maximum of all the

$$P((x_i,y_i)|(x,y))$$

. That is

$$\arg\max_i P((x_i,y_i)|(x,y))$$

Then this pixel is belongs to the cell which gives the maximum probability.

2.3 Segmentation using Active Contours

In this method, First we will find the principal components or uncorrelated components of data using PCA. Later, Active contours segmentation is used.

The concept of active contours models was first introduced in 1987 [6] and has later been developed by different researchers. An active contour is an energy minimizing spline that detects specified features within an image. It is a flexible curve (or surface) which can be dynamically adapted to required edges or objects in the image (it can be used to automatic objects segmentation). It consists of a set of control points connected by straight lines.

The active contour is defined by the number of control points as well as sequence of each other. Fitting active contours to shapes in images is an interactive process. The user must suggest an initial contour, which is quite close to the intended shape. The contour will then be attracted to features in the image extracted by internal energy creating an attractor image.

There are 2 basic types of deformable models: parametric and geometric.

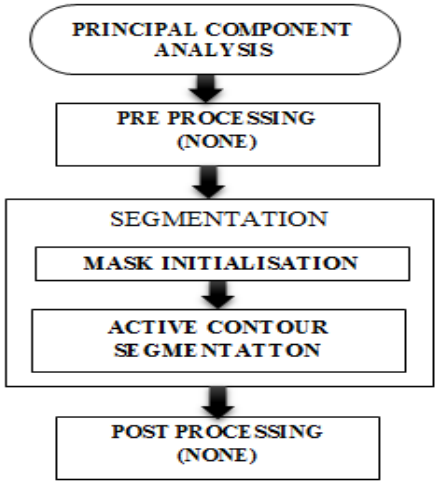


Figure 2.11: Steps in this method 2

The parametric deformable models represent curves and surfaces during deformating explicitly in parametric form. The parametric models can be described with help of some of the following formulations, and so by formulation of energy minimizing or formulation of dynamic force.

The formulation of minimizing energy - the base of deformable models on the basis of energy minimization is searching of parametric curve that minimizes weighted sum of internal energy and potential energy. Internal energy specifies tension or smoothness of contour. The potential energy is defined in the image domain and it usualy has minimal value at the point where it is high intensity gradient in the image what is shown on the object edge. Total energy minimization occures when internal and external energies are equal.

The formulation of dynamic force - it is used in those cases in which it is more confortable to form deformable model straight from dynamic problem with help of force formulation. These formulations facilitate the use of common external forces, even those which are note potential, e.g. forces which cannot be described as a negative gradient of potential energy function.

The geometric deformable models offer elegant solution of the most important limits of parametric deformable models. These models are based on the evolution curve theory and the level set method. At curves and surfaces evolution only geometric criteria are used that leads to the evolution independent from parametrization. As well as at the parametric deformable models, the evolution is connected to image data at objects edge finding. Forasmuch as the evolution is independent from parametrization, the curves and surfaces generating can be represented as the level set of a multidimensional function. The result of this is that topological changes are easy to control. The active contour can be either closed or open curve.

2.4 Calculation of Cell area

Active contour segmentation gives the binary image as output. In which cells are represented using white or logic 1 where background is represented as black or logic 0.

Cell area is calculated by summing all the logic 1 pixels. Percentage of the cell area is calculated

using the sum of all logic 1 pixels divided by the total number of pixels.

If image I has size M X N.

Algorithm:

count=0

for each row i=1 to M

for each colum j=1 to N

if I(i,j)==1

count=count+1

end

end

end

Cell area = count/MN

Background= 1- Cell area

Chapter 3

Segmentation of Glial Cells

3.1 Glial Cells

Glial cells, sometimes called neuroglia or simply glia are non-neuronal cells that maintain homeostasis, form myelin, and provide support and protection for neurons in the central and peripheral nervous systems[].

As the Greek name implies, glia are commonly known as the glue of the nervous system; however, this is not fully accurate. Glia were discovered in 1856, by the pathologist Rudolf Virchow in his search for a "connective tissue" in the brain.[]

Neuroscience currently identifies four main functions of glial cells:

- To surround neurons and hold them in place
- To supply nutrients and oxygen to neurons
- To insulate one neuron from another
- To destroy pathogens and remove dead neurons.

For over a century, it was believed that the neuroglia did not play any role in neurotransmission. However 21st century neuroscience has recognized that glial cells do have some effects on certain physiological processes like breathing,[][] and in assisting the neurons to form synaptic connections between each other[].

3.1.1 Types of glial cells

3.2 Segmentation on Frames using AC/FCM

PCA

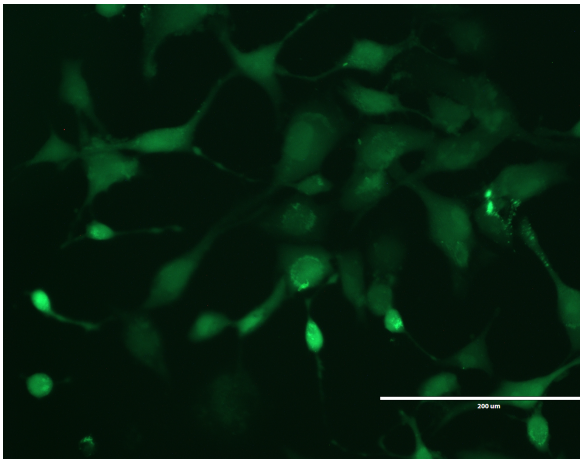


Figure 3.1: Glial cells

3.2.1 segmention using Active contours

3.2.2 segmention using Fuzzy C-Means(FCM)

3.3 Segmention on Frame Difference using AC/FCM

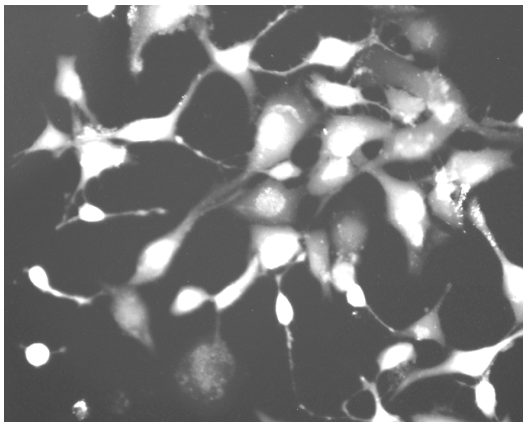
3.3.1 Segmentation of FD PCA using Active contours

3.3.2 segmention of FD PCA using watershed

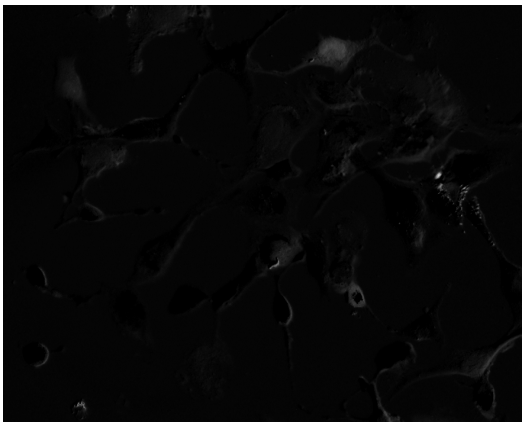
3.4 Labelling of individual cells

3.4.1 Connected Components

3.4.2 Circle Fit algorithm



(a) PCA first component



(b) PCA second component

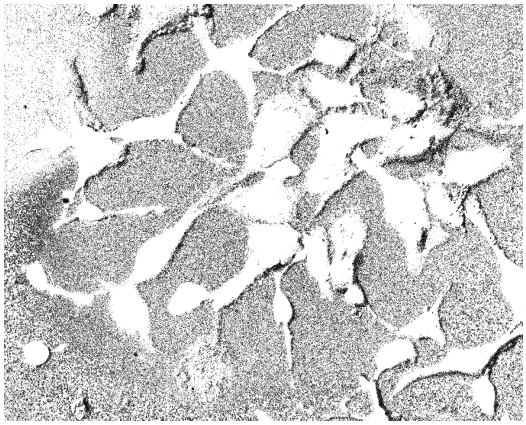
(c) PCA third component

(d) PCA fourth component

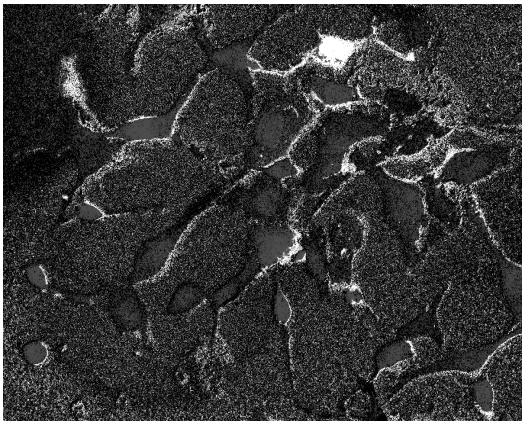
Figure 3.2: Components of PCA

Figure 3.3: Output of Active contours

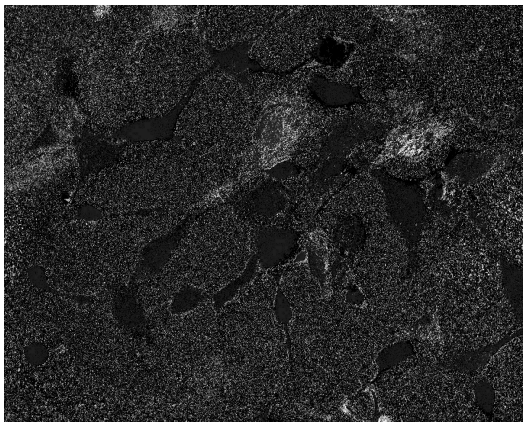
Figure 3.4: Output of Fuzzy C-Means



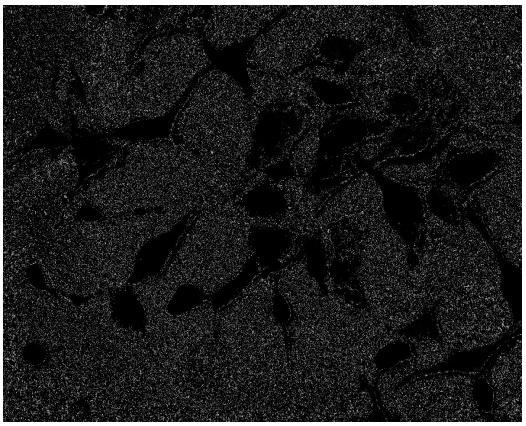
(a) FD PCA first component



(b) FD PCA second component



(c) FD PCA third component



(d) FD PCA 18 th component

Figure 3.5: Components of Frame Difference(FD) PCA

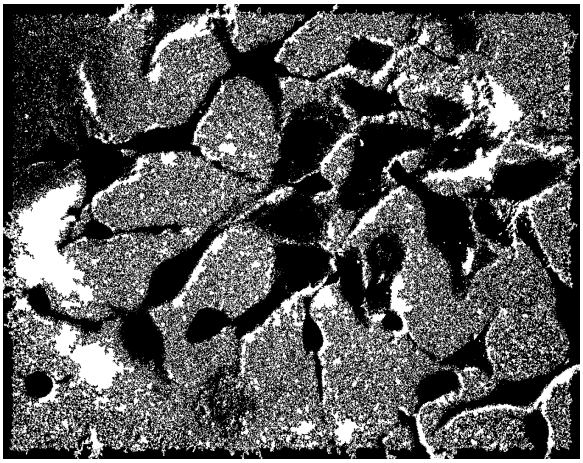


Figure 3.6: Result of AC on FD PCA

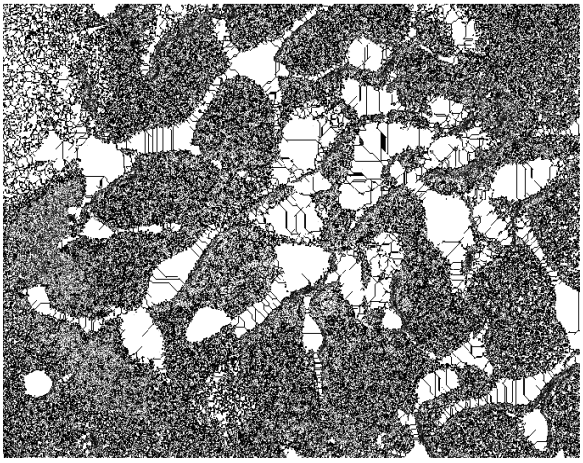


Figure 3.7: Result of Watershed

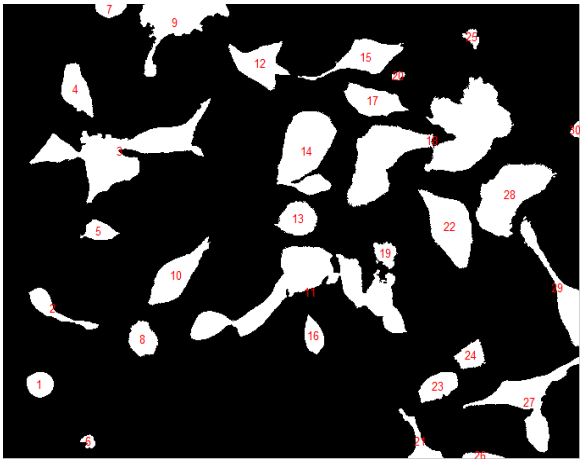


Figure 3.8: Labelled image

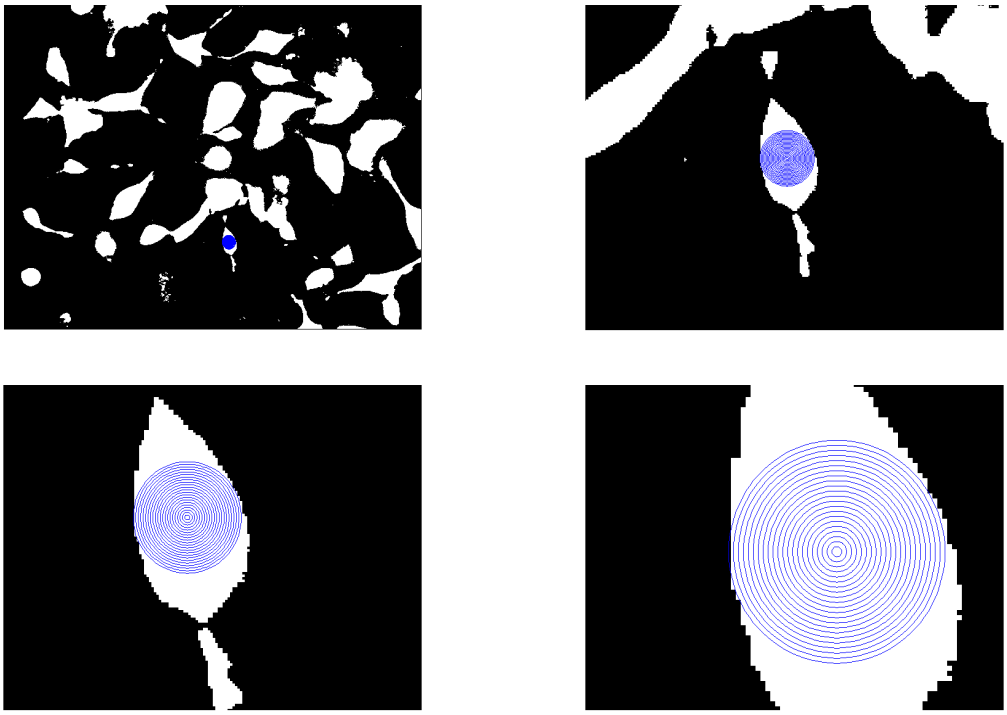


Figure 3.9: Fitting of a Circle at a pixel