CHAPTER 1

INTRODUCTION

1.1 BRIEF BACKGROUND ON TUBERCULOSIS

Tuberculosis (TB) is an infectious disease characterized by the growth of nodules (tubercles) in the tissues especially the lungs. It has the largest mortality ratio for an illness caused by a single type of microorganism. TB is a major health concern today as it is a disease with deep social and economical roots. Due to its worldwide incidence and prevalence, it has been referred as the white plague. Though TB is a predominant public health issue it is a treatable disease (Lumb 2005, Elveren and Yumusak 2011).

The etiologic agent of tuberculosis in human is *Mycobacterium tuberculosis* which is a rod-shaped, non-spore forming aerobic bacterium. The organism is also known as tubercle bacillus as it causes lesions called tubercles. Humans are the only reservoir for the bacterium (Todar 2009, Knechel 2009).

The risk of being infected by tubercle bacilli depends on the duration and intensiveness to the exposure of bacteria. The bacteria are expelled into the air when an infected person coughs, sneezes, talks or spits (Elveren and Yumusak 2011). The tubercle bacilli enter the lung and cause a focal infection in the site where it is deposited after inhalation. If the infection cannot be contained at the local level, bacilli dissemination occurs initially by hematogenic route inside phagocytic cells, towards different organs and to the
contiguous pleura. It reaches hilar lymph nodes via the lymphatic route thereby leading to a second systemic dissemination. It develops through the thoracic duct and superior vena cava, with the advancement of local foci in the lungs (Palomino et al 2007).

The infection developed with lung as the local foci is called Pulmonary Tuberculosis (PTB). The typical symptoms of PTB are chronic cough, weight loss, intermittent fever, loss of appetite, night sweats, coughing out with blood, chest pain and breathlessness. The disease develops in two stages in the human body. Having been acquired from an infected individual, the infection sets in the first stage and is referred to as the tuberculosis infection stage and the disease develops as tuberculosis itself in the second stage.

The clinical manifestations of TB depend on the local organic defenses on the sites of bacilli multiplication. The bacilli can also spread to other organs or tissues such as brain, kidneys, skin, lymphatics, pleura, bones and joints or meninges causing Extra-Pulmonary Tuberculosis (EPTB). EPTB can also be produced by hematogenic and lymphatic dissemination. It has been emphasized that the use of Bacille Calmette-Guerin vaccine may play a role in avoiding dissemination and occurrence of EPTB (Forero et al 2006, Palomino et al 2007, Knechel 2009).

1.2 METHODS OF TUBERCULOSIS DIAGNOSIS

The diagnostic techniques for TB include both invasive and non-invasive methods. Invasive techniques, such as QuantiFERON and T-SPOT blood tests, promise to be more precise and reliable than other commonly used non-invasive methods. However, according to World Health Organization (WHO) the invasive methods are too expensive for poor resource countries.
The non-invasive methods play an important role as repeated examinations are considered necessary to learn more about the cause, treatment and early detection of the airway disease. Chest radiography, culture and smear microscopy are the less expensive methods among the non-invasive techniques (Crncevik-Urek et al 2002).

1.2.1 Chest Radiography

Radiography is sensitive but not highly specific diagnostic technique and works effectively only during severe infection and hence cannot be used for an early detection of TB (Sotaquira et al 2009).

Lesions of PTB can take almost any form in a radiographic image. Also apart from TB many other lung diseases show similar appearance in the radiographic image. Consequently radiographic examination is not specific to TB but may be used as an auxiliary diagnostic procedure before smears and culture. In any case, individuals with abnormal chest radiographs are recommended to proffer several sputum samples for smear examination before PTB is diagnosed (Toman 2004, Filho et al 2012).

1.2.2 Culture Test

The *Mycobacterium tuberculosis* can be cultured to detect PTB as well as EPTB. Culture is not a priority test for systematic detection of TB cases. A person who is TB positive only on culture is less infectious than those who are positive to both culture and smear microscopy. It is also more expensive and requires complicated processing than microscopy and there is relatively a long delay until the result is available (Toman 2004).
Clinicians must wait for culture results as long as two months, as this bacillus takes 5 - 20 hours to duplicate itself (Forero et al 2003). Thus culture examination is not used to guide a clinical decision when there are much faster techniques such as the sputum smear microscopy.

1.2.3 Sputum Smear Microscopy

The WHO developed the Directly Observed Treatment, Short course (DOTS) strategy for TB control which has been adopted by many National Tuberculosis Programmes (NTPs). The DOTS strategy recommends sputum smear microscopy as the most effective tool for diagnosis of TB and for monitoring patients’ response to treatment (Lumb 2005, Steingart et al 2006).

Smear examination is an important initial test in diagnosing infectious PTB. The operational advantages of smear examination are: the results can be obtained faster and associate better with infection. It is the most efficient means for identifying sources of tuberculosis infection since repeated tests can be performed on the patient to assure early detection of the disease. It recognizes patients with severe infection in their initial treatment routine and helps to monitor the drug requirement (Toman 2004, Sotaquira et al 2009).

1.3 SPUTUM SMEAR MICROSCOPY TECHNIQUES

Fluorescence and conventional microscopy are the two microscopic diagnostic techniques used for TB screening.

1.3.1 Bright Field or Conventional Microscopy

The conventional microscopy uses a conventional artificial light source. The smears are stained with carbolfuchsin Ziehl-Neelsen (ZN) or
Kinyoun acid-fast stains which cause the TB bacilli to appear magenta against a light blue background. The bacilli may also take different colors varying from light fuchsia to dark purple. These bacilli are called Acid-Fast Bacilli (AFB) as they retain the dye even after washing with acid and alcohol (Chang et al 2012, Filho et al 2012).

Conventional microscopy is inexpensive, rapid, and highly specific but has poor sensitivity, particularly in patients co-infected with Human Immunodeficiency Virus (HIV). In addition, examination of ZN-stained smears takes more time than fluorochrome-stained smears (Steingart et al 2006, Cattamanchi et al 2009). Also the bacilli are not clearly separated from the background in the image obtained by a conventional light microscope (Costa et al 2008).

1.3.2 Fluorescence Microscopy

Fluorescence microscopy is a critical imaging modality for biomedical analysis at the object level as it provides resolutions in the order of micrometer. It has spurred the development of more sophisticated microscopes (Tasdizen et al 2008).

Fluorescence microscopy renders improved sensitivity in the diagnosis of PTB. The technique uses an acid-fast fluorochrome dye such as auramine O or auramine-rhodamine and high intense light source such as a halogen or high pressure mercury vapor lamp. The bacilli assume different colors such as reddish yellow, reddish golden yellow or bright orange yellow fluorescence in a dark background depending on the staining procedures. The bacilli when stained with auramine O and excited by blue light (450–480 nm) emits in the green-reddish yellow range (500–600 nm) (Marais et al 2008, Filho et al 2012).
It has the following advantages over conventional microscopy: Fluorescence microscopy uses low power objective lens (25x), while conventional microscopy uses high power objective lens (100x). As a consequence with fluorescence microscopy the same area of a smear is scanned in a much shorter time than with conventional microscopy. Also fluorescence microscopy is approximately 10% more sensitive than conventional microscopy.

The most common method for diagnosing patients with TB is by visually screening for rod-shaped objects (tubercle bacilli) in the stained smears prepared from sputum (Chang et al 2012). In manual screening the specialist analyzes between 20 and 100 fields in the microscopic image to achieve correct diagnosis. The positivity of smears depends on the number of tubercle bacilli present in a field or image. Visual evaluation of fluorescence microscopy images are quite often tedious because of inter and intra observer variability (Wahlby et al 2002, Toman 2004, Sotaquira et al 2009).

The manual procedure is time consuming and labor intensive with high false-negative results that questions the sensitivity of the procedure. Also fatigue and visual strain limits the volume of slides handled per day (Sadaphal et al 2008, Sotaquira et al 2009, Raof et al 2011). Hence an automated TB diagnosis is required to handle large number of cases with enhanced accuracy to speed up the screening process, minimize its reliance on technicians, enhance quantitative classification and reduce errors. Attempts have already been made to digitize image captured by a camera attached to the microscope to overcome the manual procedure.

1.4 AUTOMATION OF SPUTUM SMEAR MICROSCOPY

The design and development of automation in fluorescence microscopic technique permits collection of large, high dimensional cell

There is a need for digital processing with the advent of automation to extract quantitative information about the specimen from a microscope image. Digital processing of microscope images has opened up new realms of medical research and brought about the possibility of advanced clinical diagnostic procedures. Digital imaging has come into prominence with the advent of affordable, high performance computer and image sensor technologies. The microscopic imaging has replaced traditional film-based photomicrography which was once the most widely used method for microscope image acquisition and storage. Microscopic imaging is nearly ubiquitous in several medical informatics disciplines, including but not limited to clinical decision support systems (Wu et al 2008).

1.5 ARTIFACTS PRESENT IN DIGITAL TB IMAGES

Microscopic image segmentation is challenging due to the complex morphological cells, illuminant reflection and inherent microscopy noises. The characteristic ill-effects include poor contrast between cell gray levels and background, a high number of occluding cells in a single view, and excess homogeneity in cell images due to irregular staining among cells and tissues (Du and Dua 2010).

Objects different from bacilli such as food rests, fibres, pollen and sometimes scratched slides may appear as an artifact in the sputum smear images and are dependent on the illumination level (Makkapati et al 2009).
Depending on the source of illumination the sample’s background may vary from image to image and may take colors such as white, cream and beige. Also depending on the level of infection there can be many bacilli and clusters that are not always easily recognizable and make the diagnostic process a difficult and inaccurate task. Acid-fast artifacts may be present in a smear and hence it is necessary to review the cell morphology carefully (Forero et al 2006, Sotaquira et al 2009).

The algorithm has to satisfy different artifacts present in the digital smear images which are the prime issues. The algorithm also needs to correctly detect bacilli and reduce the level of false positives efficiently irrespective of different levels of illumination (Sotaquira et al 2009). Hence the procedure for bacilli identification should be robust with respect to above variations.

1.5.1 Sources of Non-uniform Illumination

There are several sources for image degradation when digitizing images acquired by a microscope. The sources of noise include camera noise, variation in focal length, background illumination intensity provided by the microscope light source optics, aging filament, temperature of the light source, faulty reference voltages and contaminated aperture. The illumination is most often not homogeneous throughout the view field and hence it is common to have a bright spot in the middle of the field (Tasdizen et al 2008, Lumb 2005).

1.5.2 Illumination Correction

Image illumination correction is needed to be done in order to overcome the non-homogeneity issue (Lumb 2005). Illumination correction methods are classified into two major groups, first being performed while
acquiring images (a priori methods or calibration-based methods) and second after acquisition (retrospective or a posteriori methods).

A priori-based correction methods are fast, simple to implement, and may efficiently minimize most of the illumination effects that originate during the acquisition process. A drawback is that they cannot deal with object-dependent correction caused by variation in specimen thickness in transmission imaging or due to non-planar surface in reflection imaging. Since these methods require additional a priori acquisitions, they cannot be applied retroactively (Likar et al 2000). Also they use additional images obtained at the time of image capture. In a posteriori (retrospective) correction, the additional images are not available and therefore an ideal illumination model has to be assumed. The retrospective methods have the advantage of not requiring additional hardware devices for making the illumination more uniform (Lee and Kim 2009).

The taxonomy of retrospective illumination correction algorithms has been distinguished as parametric and non-parametric methods. Parametric methods assume some mathematical model of the illumination issue whereas non-parametric methods do not assume any model but enforce spatial resolutions. The non-parametric methods are computer intensive and require huge memory in some cases (Fernandez et al 2004, Zheng et al 2009).

The parametric approach that is often used is a retrospective method which relies solely on the information content of the acquired image. This method can be of spatial filtering and Surface Fitting Methods (SFM). Illumination variations mainly lie in the low frequency band and can be reduced by removing low frequency components (Likar et al 2000, Chen et al 2006). The smoothly varying illumination components can be modelled by second order polynomial. The SFM technique models the change of illumination as a polynomial function of spatial image coordinates. Such
models can handle global change due to camera gain or ambient lighting and local illumination changes due to change in position of illumination sources (Likar et al 2000, Paruchuri et al 2011).

The illumination correction algorithm namely Multiple Regression Method (MRM) simulate the illumination identity density plane. This parametric method enables to correct the non-uniform illumination as it models the change of illumination as a regression function of spatial image coordinates (Guo et al 2005).

A non-parametric method, Bidirectional Empirical Mode Decomposition (BEMD) decomposes the image into low and high frequency information. It makes use of extracting a number of Intrinsic Mode Function (IMF) from the original image (Qin et al 2008). The illumination component is basically a low pass component as the illumination presents no high spatial changes. BEMD isolates these slowly changing lower spatial frequencies in the last few IMFs which can be subtracted from the original image to get the corrected image (Teoh et al 2008, Yuehui and Minghui 2008).

1.5.3 Validation of Non-uniform Illumination Correction Methods

Qualitative and quantitative tests can be performed to demonstrate the effect of non-uniform illumination correction techniques. Qualitative test include intensity histograms of the image and intensity profiles along a line in the original and corrected image (Likar and Pernus 2000). The quantitative test consists of performance measures such as Mean Square Error (MSE), Peak Signal to Noise Ratio (PSNR), Maximum Error (MAX ERR) and Root Mean Square Error (RMSE) (Lindblad and Bengtsson 2001, Babaloukas et al 2011). The variation of global or local statistical features such as kurtosis, contrast, Eigenvalue and entropy can also be evaluated from the original and

Further to determine whether the illumination correction algorithms meet the specified requirements, the process of validation is conducted. This finds the suitable illumination correction method by using the probability distribution of pixels in the image. Histogram is a common approach that determines the probability distribution. Recently fractal analysis has been proposed as an additional to histogram analysis which can capture the spatial distribution in a better way (Xu et al 2010).

Fractal is a tool that describes the fractal geometry and allows capturing heterogeneity of irregular shapes that is not available in traditional geometrical representation (Lopes and Betrouni 2009). An extension of fractal is the multifractal approach that offers appropriate tools to overcome limitations arising from the fractal geometry. The multifractal analysis reveals distinct distributions of pixels and detects variations within the image. It is capable of describing image features both from local and global point of view (Stojic et al 2006).

The multifractal spectrum analysis is an effective approach which could be used for statistical characterization of the image (Stosic and Stosic 2006). It reflects the real features of the image more accurately as it is associated with the probability of pixel distribution. Recently, multifractal analysis has found wide applications in the field of medical diagnosis such as characterization of electroencephalogram, electrocardiogram signals, brain images, mammography and microscopic tissue images (Lopes and Betrouni 2009, Soares et al 2009, Hemsley and Mukundan 2009).
1.6 SEGMENTATION OF DIGITAL IMAGES

Image segmentation is the process of identifying or delineating objects in images. Segmentation is often required as the first processing step to geometric analysis and it is the most crucial among all computerized operations done on acquired images. In spite of several decades of research, segmentation remains a challenging concern in image processing and computer vision (Udupa et al 2006, Du and Dua 2010).

The level of subdividing an image depends on the issues being solved, which means that the segmentation would stop when the delineation process is complete. Further, occluded objects have to be separated from each other and segmented from the image background before features are extracted (Wahlby et al 2002, Khare and Srivastava 2012).

Image segmentation technique helps to discriminate between the TB bacilli and outliers which may be due to dust, food rests, cell macrophages, bacilli destroyed by macrophages, overlapping bacilli, remnants of cells, food particles and improper staining or preparation of the slide (Khutlang et al 2010 a, Filho and Costa 2012).

The bacilli in these images measure approximately 1 to 10 μm in length and 0.2 to 0.6 μm in width and they exhibit a straight, curved or bent shape. This information is important in the segmentation and identification processes (Forero et al 2003).

The approaches to image segmentation can be roughly classified into boundary-based (or edge-based) segmentation which relies on the generation of prominent edges, and region-based segmentation which relies on the homogeneity of spatially localized features and properties (Song 2003).
1.6.1 Edge-based Segmentation

Early edge-based segmentation and thresholding use local filtering techniques. They have less computational burden and are stable under noise, but have difficulty in establishing the connected edge (Song 2003).

Edge-based segmentation looks for discontinuities in the intensity of an image. An edge can be defined as the border between two regions with relatively separate properties. The assumption of edge-based segmentation is that every sub-region in an image is sufficiently uniform so that the transition between two sub-regions is determined based on discontinuities alone. When this condition is not satisfied, region-based segmentation can be attempted which provides more reasonable segmentation outcome (Baswaraj et al 2012).

1.6.2 Region-based Segmentation

Region-based segmentation looks for equality inside a sub-region, based on a desired property such as intensity, color and texture (Baswaraj et al 2012). These traditional segmentation approaches need further processing to be performed to link the discontinued object boundaries. To address this difficulty, deformable model approach has been identified as one of the promising methods for medical image segmentation.

Deformable models are curves or surfaces that can be defined within an image which can move under the influence of internal and external forces. The internal forces are defined within the curve or surface, whereas the external forces are computed from the image data. The internal forces are designed to keep the model smooth during the deformation. The external forces are defined to move the model toward an object boundary or other desired features within an image (McInerney and Terzopoulos 1996). Snakes
or active contour methods work on deformable models and has attracted the attention for segmenting objects in a wide range of applications.

In active contour method, a curve is typically associated with an energy functional which is normally defined based on the smoothness of the boundary curve and features of the image. There are several desirable advantages of active contour methods. They can achieve sub-pixel accuracy of the object boundaries, and prior knowledge about shape and intensity distribution can be incorporated for robust image segmentation. Also, the resultant object contours are quite regular which are convenient for further applications, such as shape analysis, classification and recognition (Li et al 2007).

The edge-based active contour models generally utilize the local image gradient information as the image-based force in order to guide the contour moving towards the object boundary. Though these models successfully segment images with strong gradient, they may suffer from some drawbacks when dealing with objects with weak boundary. Due to the gradient dependence, the models are normally prone to pass through the weak or blurred object boundaries. In addition, the edge-based active contours have small range capture, so the initial contour needs to be placed near the desired objects. Contrary to the edge-based models, the region-based active contour models use the region information such as color, intensity, and texture to guide the motion of the active curve (Shyu et al 2011).

Region-based active contour method has become quite popular for a range of applications such as detection of edges, motion tracking and stereo matching through the last decade. The characteristic of region-based active contours method is that the initial contour can be placed in any location of the image. It is because the region-based segmentation relies on the global energy minimization rather than local energy minimization. Therefore, less prior
knowledge about the shape of the objects present in the image is required than edge-based active contours (Baswaraj et al 2012).

The Chan-Vese method of active contour can be used to detect objects or features of the contours without gradient. This is not possible in classical snakes or active contours-based methods. This method is not based on an edge function to stop the evolving curve on the desired boundary. Also, the initial image need not be smoothened, even if it is very noisy and in this way, the locations of boundaries are detected and preserved. It can detect objects whose boundaries are not necessarily defined by gradient or with very smooth boundaries, for which the classical active contour models are not applicable. This method can automatically detect interior contours starting with only one initial curve. The position of the initial curve can be anywhere in the image, and it does not necessarily surround the objects to be detected (Chan and Vese 2001).

1.6.3 Segmentation Validation

The images segmented using active contours are validated with the manually segmented reference images. The manual segmentation of the images is regarded as a practical gold standard against which the segmentation algorithm can be compared (Crum et al 2006). The validation of segmentation is essential to understand the strengths, limitations and potential applications of a particular algorithm.

A strategy for evaluating single object segmentation is by using overlap measures and distance metrics. The accuracy of an individual experimental segmentation is usually given through the measure of a region’s overlap and its distance from the ground truth. Distance transforms are used in matching issues because they make distance-based similarity measures more practicable. The overlap measure is characterized by a similarity

An identification process is needed to be performed in the segmented image to determine if the remaining objects are bacilli thus enabling further classification of these images into positive and negative.

1.7 IDENTIFICATION OF BACILLI AND OUTLIERS

The segmented images may contain non-bacilli apart from the bacilli and probably overlapping bacilli may exist when the density of the bacilli is high. This reduces the presence of incorrectly labelled objects during the training procedure of the classification (Forero et al 2006). A definite procedure for identifying bacilli needs to be considered to diminish the error rate so that the outliers or the debris can be eliminated from the bacilli data set.

Objects are filtered according to their geometrical features as part of the identification process. The objects that are not of interest are rejected because they cannot be modelled or it is difficult to make an accurate ontology of the debris that may be present in a captured sputum smear images (Forero et al 2006, Khutlang et al 2010).

1.8 SEPARATION OF OVERLAPPING OBJECTS

The size and shape estimation done on the image without separating the touching and overlapping objects may lead to gross errors in identification and classification of images. However segmenting multiple objects in an image which overlap or occlude with other objects or regions of interest remains a challenging issue (Korath et al 2007, Wu and Shah 2011).
Separation of overlapping objects is carried out using the region- and shape-based analysis of the object. Among all proposed methods, thresholding, watershed, and deformable models or active contours remain the most popular approaches. In general, watershed segmentation is claimed to be more effective in terms of differentiating connected nuclei or cells. The watershed algorithm and its variants are enhanced pixel-oriented methods used in many applications. However, they are limited by noise and intensity fluctuations which generally result in over segmentation and need appropriate modification in post processing to enhance the accuracy to an acceptable level (Xue et al 2010, Park et al 2013).

Moreover, this watershed model is generally associated to a single label and a pixel cannot be granted membership to more than one label. Thus the existing methods of touching and occluded object separation such as watershed segmentation and morphological processing suffer from issues such as over segmentation and relatively large processing times (Korath et al 2007).

Simple intuitive Marker Controlled Watershed (MCW) segmentation method is fast and can be parallelized to produce a complete division of the image in separated regions. This avoids the need for any kind of contours joining to split the overlapping objects (Nee et al 2012).

Level set methods combined with prior shape information can provide accurate results but can be computationally demanding for large scale datasets, thus necessitating more time and subjective manual user intervention. The results from the level set approach show that under segmentation often occurs because it is difficult to divide a connected object without sufficient edge information. Overlapping makes the area of occluding portion large and there are no obvious gradient changes to map the contour of the exact boundary (Xue et al 2010).
Multi-phase level set provides a powerful framework for segmenting multiple connected objects. Nonetheless, these models are subject to the same labeling constraints so as to avoid assigning different labels to overlapping regions. One of the major drawbacks of the aforementioned continuous level set method is the ad-hoc style of parameter estimation (Wu and Shah 2011).

Another approach is to study the shape, in particular the concavities, of the object. This method tries to extract information about the way in which the object should be split (Wahlby et al 2002). Shape-based features are extracted for further analysis from the edited data set with outliers removed and overlapping bacilli separated.

1.9 FEATURE EXTRACTION

The method of feature analysis relies on an appropriate representation of shape or appearance and developing that representation for further classification. Based on the human interpretation of image information, appearance, shape, spectral, textural, and contextual features are some fundamental feature types that have been computerized in various image processing applications. Among these feature types, representing the appearance of medical images is the most discriminatory way that eases the classification procedure (Bagci et al 2012).

The bacilli in the sputum smear images characterize a rod like shape, presenting a straight, curved or bent shape (Forero et al 2006). Hence, this information is important for feature extraction and the classification scheme. Geometric shape descriptors and moment invariant features are derived to extract useful characteristics from the segmented images.
1.9.1 Geometric Features

Shape descriptors are a set of parameters that numerically represent each region or boundary in the segmented image. The representation of the bacilli should be preferably invariant against translations, rotations and scale changes in order to identify the tubercle bacilli (Forero et al 2003). This is because, they should not have any influence towards image magnification and direction and also location of the bacteria (Siena et al 2012).

The debris objects that often appear in zones near the decision regions increase the classification error. It can be observed that the rejected objects are well characterized by their invariant geometric features (Forero et al 2003).

Many shape description and similarity measurement techniques have been developed in the past and a number of new techniques have been proposed in recent years. The commonly used geometric shape descriptors include area, perimeter, eccentricity, compactness and axis ratio.

Contour- and region-based methods are the two different categories of shape-based feature extraction methods which are based on the use of shape boundary points as opposed to shape interior points. Under each class, different methods are further divided into structural and global approaches. This sub-class is based on whether the shape is represented as a whole or represented by segments or sections (primitives) (Mingqiang et al 2008).

1.9.2 Moment Invariant Features

Moment invariants have been widely applied to image pattern recognition in a variety of applications due to its invariant features on image translation, scaling and rotation. They are extensively applied to image pattern
recognition, registration and reconstruction of images (Huang and Leng 2010). Moment-based invariants are the most common region-based image invariants which are used as pattern features in many applications (Chen et al 2004).

Moment invariant features are derived to extract useful geometrical characteristics of different objects from the segmented images (Osman et al 2010, Ruberto and Morgera 2008). The seven Hu’s moment invariants are useful properties that are being unchanged under image scaling, translation and rotation. Orthogonal moments based on Zernike polynomials extract a set of features in which every feature represents unique information about an image (Chen et al 2004, Arvacheh and Tizhoosh 2005).

The invariant property exhibited by Hu’s and Zernike moments is found suitable to capture geometric properties of objects in medical images (Forero et al 2006, Fu et al 2006, Tahmasbi et al 2011).

1.9.3 Fourier Descriptors

Fourier Descriptors capture the global shape features by the first few low frequency terms, while the higher frequency terms capture the finer features of the shape. Fourteen Fourier coefficients are sufficient for describing the variable shape of TB bacilli. The advantages of the Fourier descriptors include noise sensitivity in the shape signature representation, easy normalization and information preserving capability (Veropoulos 2001, Zhang and Lu 2003).

1.10 FEATURE SELECTION

Feature selection methods select significant features which should be included in the reduced feature space based on a criterion function. Feature
selection leads to reduction in computational time since some of the features are discarded and the selected features retain their original physical interpretation. The retained features may be important for understanding the physical process that generates the patterns (Iqbal et al 2011).

Feature selection plays an important role in classification by removing insignificant features from the data set to provide better diagnosis, which is an important requirement in medical applications. When a large number of features are input to a classifier, some may be irrelevant while others will be redundant which will at best increase the complexity of the task, and at worst hinder the classification by increasing the inter-class variability (Reyes-Aldasoro and Bhalerao 2003).

If measures are not taken to reduce the number of features before classification, then it may reflect the noise or random error of the underlying data (Kassner and Thornhill 2010). This will most certainly result in over training as it gives too many degrees of freedom for the classifier. To get good generalization properties of the classifier, it is desirable to keep the number of features as low as possible. To perform the selection of features in an automatic fashion, a method to judge the quality of the resultant classifier is needed (Wahlby et al 2002).

The use of superfluous features often leads to inferior performance in pattern recognition. A general practical observation is that it is worth decreasing the dimensionality of the feature space while ensuring that the overall structure of the data points remains intact. A simple way to do this is by means of a transformation that linearly maps the initial feature space to a new one with fewer dimensions. The most popular technique, Principal Component Analysis (PCA) chooses the basis vectors of the transformed space as those directions of the original space to show large variance among the significant data (Wang et al 2007).
In the traditional Eigen space methods such as PCA, the feature space is transformed to a set of independent and orthogonal axes. This can be ranked by the extent of variation given by the associated Eigenvalues. However, while these Eigen space methods are optimal and effective, they still require the computation of all the features for the given data (Reyes-Aldasoro and Bhalerao 2003).

When linear transformations such as PCA are not powerful enough, feature extraction methods based on non-linear transformations can be attempted (Wang et al 2007). Linear techniques assume that the data lie on or near a linear sub-space of the high dimensional space. Non-linear techniques for dimensionality reduction do not rely on the linearity assumption as a result of which more complex embeddings of the data in the high dimensional space can be identified (Van der Maaten et al 2008).

By performing PCA in a kernel induced feature space, Kernel PCA (KPCA) extracts features that are non-linearly related to the input variables (Wang et al 2007). KPCA is the reformulation of traditional linear PCA in a high dimensional space that is constructed using a kernel function. In recent years, the reformulation of linear techniques using the kernel has led to the proposal of successful techniques such as regression and classification (Van der Maaten et al 2008). KPCA can take into account a wider class of higher order dependencies in the data and it has been reported to overcome the assumption of linear correlation among the data in PCA (Iqbal et al 2011).

1.11 CLASSIFICATION

Classifiers are widely used in discriminating pathological condition from the normal. The classification process involves grouping of data into pre-defined classes or finding the class to which a data belongs. This process plays an important role in medical image automation, which is a part of
decision making in medical image analysis. Machine learning based classification techniques provide support for many areas of health care, including prognosis, diagnosis and screening (Luukka 2011).

Artificial neural networks are claimed to be systems that enable the execution of a particular task without the need for a prior knowledge of it. They are capable of describing non-linearity but are considered as black boxes. On the other hand fuzzy logic is an effective rule-based modelling in soft computing that not only tolerates imprecise information but also makes a framework of approximate reasoning. The disadvantage of fuzzy logic is the lack of self learning capability (Giovanis 2010).

1.11.1 Integration of Classifier Learning Schemes

The combination of fuzzy logic reasoning and neural network learning can overcome the disadvantages of the above approaches. Integration of fuzzy reasoning with neural learning provides neural network the ability to express qualitative knowledge and network topological structure. This makes the initialization of network easier, avoid the local optimization of network training and ensure the stability of networks (Shi and He 2010, Yilmaz and Kaynar 2011). The merits of both neural and fuzzy systems can be integrated in a neuro fuzzy approach. Combined with the learning ability of artificial neural networks, the fuzzy inference system has proven to be a powerful mathematical construct which also enables the symbolic expression of machine learning results. The Adaptive Neuro Fuzzy Inference System (ANFIS) models are useful to handle various complicated issues with a capacity to learn by examples (Sun et al 2007, Yilmaz and Kaynar 2011). ANFIS combines both the learning capabilities of a neural network and reasoning capabilities of fuzzy logic to give enhanced prediction and classification capabilities (Giovanis 2010).
The Complex valued Adaptive Neuro Fuzzy Inference System (CANFIS) model is the result of the combination of adaptable fuzzy inputs with a neural network in order to get a rapid and more accurate classifier. With this combination, it is possible to use both advantages of fuzzy inference systems with the explanatory nature of rules (membership functions) and artificial neural network as a dynamic estimator (Tahmasebi and Hezarkhani 2012).

CANFIS offers a useful and consistent framework for describing representative adaptive network models and because of its systematic design methodology and reduced mean square error (Mizutani and Nishio 2002) it is used for classification applications (Mizutani and Nishio 2001, Malekzadeh and Akbarzadeh 2004).

The Extreme Learning Machine (ELM) is a neural network algorithm proposed recently as an efficient learning algorithm for Single hidden Layer Feed forward Neural network (SLFN). In ELM, the input weights and the hidden layer biases are chosen randomly, and the output weights (linking the hidden layer to the output layer) are analytically determined by using Moore Penrose (MP) generalized inverse. MP increases the learning speed by randomly generating weights and biases of hidden nodes rather than iteratively adjusting network parameters which are commonly adopted by gradient-based methods. It also avoids many difficulties faced by gradient-based learning methods such as stopping criteria, learning rate, learning epochs, and local minima. However, ELM usually needs higher number of hidden neurons due to the random determination of the input weights and hidden biases (Liu and Wang 2010).
To overcome the drawback, Differential Evolutionary Extreme Learning Machine (DE-ELM) has been proposed. It takes advantages of both ELM and Differential Evolution (DE) and removes redundancy among hidden nodes and achieves satisfactory performance with more compact and high speed neural networks.

The hybrid approach which takes advantages of both DE and ELM has a more compact network. The DE process is a global searching optimization method used to tune the input weights and hidden layer biases where the output weights are determined by the generalized inverse procedure. The ELM learning algorithm is faster and has good generalization ability. The ELM algorithm overcomes many issues in traditional gradient algorithms such as stopping criterion, learning rate, number of epochs and local minima. On account of these advantages and as it is a compact and high speed neural network, DE-ELM has been effectively used in the field of medical diagnosis (Huynh and Won 2008, Yusoff et al 2010, Monedero et al 2011).

1.11.2 Evaluation Measures of the Classifier

The classifier performance with two possible classes is evaluated based on the criteria obtained by applying the classifier to the test set, which include true positives, false positives, true negatives and false negatives (Kim et al 2007). These terms are used to describe the clinical efficiency of a classifier. Based on these criteria, the performance of the classifier is measured in terms of statistical quantities such as sensitivity, specificity and accuracy (Makinaci 2005). A more appropriate measure for the object level classification is the F-measure (Chang et al 2012).
1.12 OBJECTIVES OF THE THESIS

The objectives of the thesis are

- To perform and validate non-uniform illumination correction on different positive and negative sputum smear images
- To perform segmentation, to identify TB bacilli, outliers and overlapping bacilli from the sputum smear images and to validate segmentation with manually annotated images
- To separate overlapping bacilli in these images
- To extract useful shape descriptors from the identified TB objects
- To classify the images into TB positive and negative images

1.13 ORGANIZATION OF THE THESIS

The work reported in the thesis is organized in five chapters. Chapter 2 gives a comprehensive review of the literature on background of tuberculosis, diagnostic methods, microscopic image analysis and image processing techniques, non-uniform illumination correction methods, segmentation and validation, identification of TB objects into bacilli and outliers, separation of overlapping objects, shape-based geometric and moment features, hybrid machine learning techniques. Chapter 3 describes the methods for correction of non-uniform illumination, segmentation and validation methods, separation of overlapping objects, the feature extraction procedure and the methods of the hybrid machine learning techniques. Chapter 4 discusses on the results of the above mentioned methods. Chapter 5 deals with the significant conclusions and scope for future work.