BIOMEDICAL IMAGE PROCESSING IN PATHOLOGY: A REVIEW

H Nazeran, F Rice, W Moran*, and J Skinner**
School of Engineering, *Department of Mathematics, and **Department of Pathology
Flinders University of South Australia and Centre for Sensor Signal and Information Processing

ABSTRACT

Pathologists make a diagnostic decision by viewing a specimen and measuring various diagnostically important attributes of an isolated object such as size, shape, darkness, colour and texture. This is a complex process. In recent years, computer-aided image processing and analysis systems have played a significant role in quantitative pathology. This paper summarises basic image processing and analysis techniques and reviews related work in pathology and cytology based on computational image processing since 1987. Firstly, we present a general introduction to image enhancement, segmentation, morphometry and visualisation for those medical colleagues who may not have the necessary background in this area. (The mathematical treatment is kept to minimum and appropriate references are cited to satisfy the more mathematically oriented readers. Selected examples are provided to demonstrate the effects of various basic image processing algorithms on a MRI scan. It should be emphasised that the reviewed techniques are generally used as preprocessing steps in analysing microscopic images and powerful algorithms are more evolved and problem-specific.) Secondly, we review image cytometric and histometric methods, standards, calibration and applications. Finally, we touch upon three dimensional confocal image processing and analysis, applications of artificial neural networks, and optical disk database management for recording and retrieving a large number of digitised high resolution images. The development of integrated optical microscope and computer systems is also briefly described.

INTRODUCTION

Image processing and analysis in pathology is a multidisciplinary area of research and development. It is a facet of the technical disciplines of cytometry and histometry. Cytometry is the process of effecting independent measurements of morphological, biochemical, and physiological attributes of individual cells and cellular components (i.e. structure, content, and function) [1]. Histometry is the process of extracting these measurements from tissue components. The extracted features from large and representative samples enable us to characterise specimens in ways not solely by visual analyses, but also from the measured and computed properties of sample constituents. Figure 1 illustrates the major sciences and technologies involved in image processing and analysis applicable to quantitative pathology.

The left side of the figure shows core technologies which are applied in image cytometry and histometry, while the right side defines supporting technologies which contribute to the increasing level of new research and development opportunities in pathology. Many of the key and supporting technologies identified in Figure 1 involve optical and visual processes. Electronic imaging systems are essential for the detection and precise quantification of these processes. It is also significant that many of these technologies rely on the availability of computerised instrumentation and engineering techniques for the acquisition, storage, retrieval, communication, processing, analysis, and display of data.

Optical scientists and engineers are involved in fundamental aspects of optics, and computer scientists and mathematicians contribute computing environments and multidimensional processing methods. Biomedical engineers are interested in the prospects of using an array of powerful new tools and techniques to address complex biological or clinical questions. This paper summarises basic image processing and analysis techniques and reviews related work in pathology and cytology since 1987 that benefit from biomedical image processing.

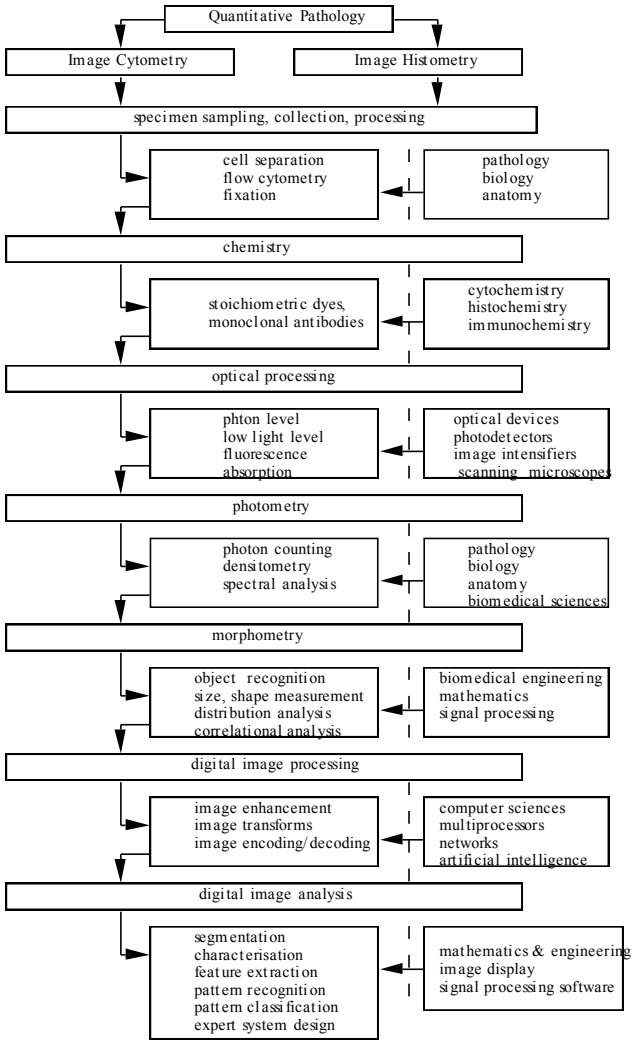

Figure 1. Multidisciplinary areas involved in image processing and analysis in pathology.

1.IMAGE ENHANCEMENT

Image enhancement refers to emphasising or sharpening of image features, such as edges, boundaries, or contrast to make a graphic display more useful for visualisation and analysis. The enhancement process does not increase the inherent information in the data, but it can provide a better match between image data and the human visual system or software analysis algorithm. Image enhancement includes gray-level and contrast manipulation, noise reduction, edge crispening and sharpening, filtering, interpolation and magnification, and psuedocolouring [2-7].

1.1 Enhancement in the Spatial Domain

1.1.1 Histogram Equalisation

In histogram equalisation, the goal is to obtain a uniform histogram for the output image. The procedure is a mapping (transformation) function of the form:

$$S_k = T(k) = \sum_{j=1}^{k} P_r(r_j) \qquad k = 1, 2, 3, ..., L$$

i.e. every pixel in the original image having value r_k is transformed in intensity to a value S_k. The objective of this transformation is to produce a histogram $p_S(s_j)$ which is more spread out than the original histogram. In theory, this histogram should be flat (i.e. uniform). In medical diagnostic imaging, a uniform histogram is not necessarily ideal and various different non-equalised (nonuniform) techniques are also used. Figures 2 a&b show a MRI scan and its histogram equalised image and their associated histograms. The histogram shows the number of pixels in the image that has each of the possible 256 gray level values (0 = dark and 255 = white.)

1.1.2 Local Enhancement

This procedure is to define an n ∗ m rectangular neighbourhood and move the centre of this area from pixel to pixel. At each location, the histogram of the n ∗ m points in the neighbourhood are computed and a histogram equalisation transformation function is obtained. The function is finally used to map the intensity of the pixel centred in the neighbourhood. The centre of the n ∗ m region is then moved to an adjacent pixel location and the procedure is repeated. Since only one new row or column of the neighbourhood changes during a pixel to pixel translation of the region, it is possible to update the histogram obtained in the previous
location with the new data introduced at each motion step.

Instead of using histogram, pixel intensity can be employed in local enhancement. The intensity mean and variance (standard deviation) are frequently used because of their relevance to the appearance of an image. The mean is a measure of average brightness and the variance is a measure of contrast [2-7]. A typical local transformation based on these concepts maps the intensity of an input image f(x,y) into a new image g(x,y) by performing the following transformation at each pixel location [2-7]:

$$g(x,y) = A(x,y) * [f(x,y) - m(x,y)] + m(x,y)$$

where

$$A(x,y) = c\frac{M}{\sigma(x,y)} \qquad 0<c<1$$

In this formulation m(x,y) and σ(x,y) are intensity mean and standard deviation computed in a neighbourhood of (x,y), M is the global mean of f(x,y), and c is constant in the range indicated above. It is important to note that A, m, and σ are variable quantities which depend on a predefined neighbourhood (x,y). Application of the local gain factor A(x,y) to the difference between f(x,y) and the local mean amplifies local variations. Since A(x,y) is

inversely proportional to the standard deviation of the intensity, areas with low contrast receive larger gain. The mean is added back in the first equation to restore the average intensity level of the image in the local region and restrict the variations of A(x,y) between two limits (A_{min}, A_{max}), in order to balance out larger excursions of intensity in isolated regions.(Please see references 2-7 for examples)

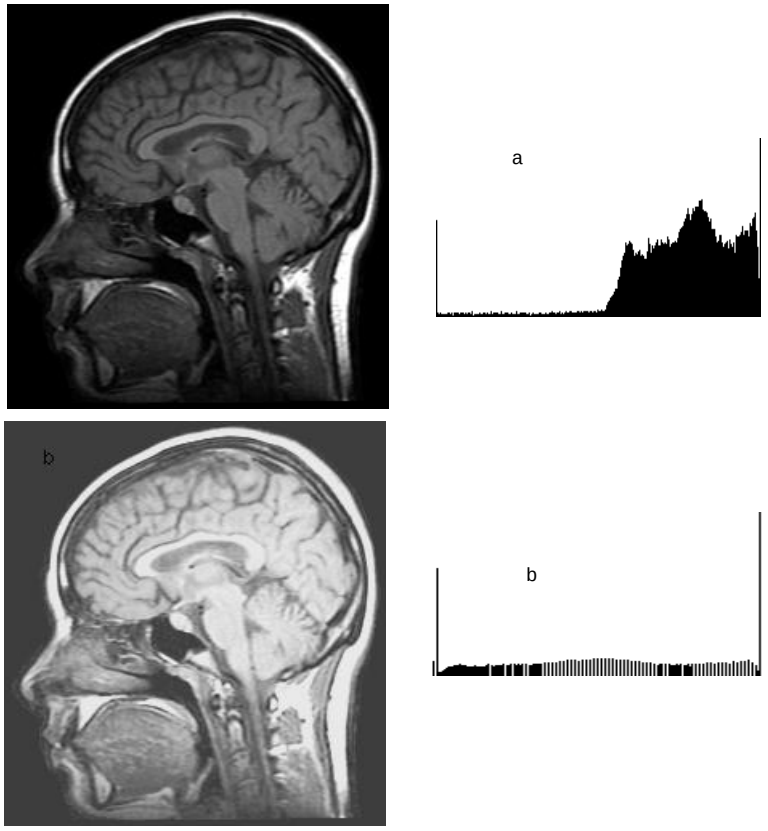

Figure 2. Histogram equalisation, a) MRI scan and its histogram, b) histogram equalised MRI scan and its histogram..

1.1.3 Smoothing

Smoothing operations are used for reducing noise and other spurious effects that may be present in an image as a result of sampling quantisation, transmission, or disturbances in the environment during image acquisition. Neighbourhood averaging is a spatial domain technique for image smoothing. Given an image f(x,y), the procedure is to generate a smoothed image g(x,y) whose intensity at every point (x,y) is obtained by averaging the intensity values of the pixels of f(x,y) contained in a predetermined neighbourhood of (x,y).

Here, S is the set of coordinates of points in the neighbourhood of f(x,y), including (x,y) itself, and N is the total number of points in the neighbourhood. Smoothing operations are usually implemented by using a m * m (i.e. 3 * 3) spatial masks, where the value of each pixel in the image is replaced with the weighted average of its 3 * 3 neighbourhood. Filtering (ie smoothing) operations are performed on a grayscale image or a psuedocoloured image originated from a grayscale image. Figures 3 a&b show the effect of smoothing the MRI scan corrupted with random spot noise with a smoothing filter to reduce noise. The 3 * 3 mask used in this operation is:

1	1	1
1	4	1
1	1	1

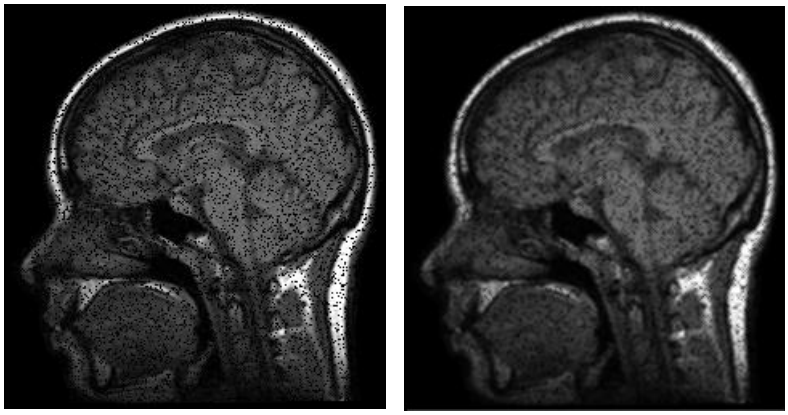

Figure 3. Effect of smoothing or low pass filtering, a) original image, b) smoothed image.

1.1.4 Median Filters

The principal function of median filtering is to replace the intensity of each pixel by the median of the intensities in a predefined neighbourhood of that pixel instead of the average. The median M of a set of values is such that half of the values in the set are less than M and half of the values are greater than M. In order to perform median filtering in a neighbourhood of a pixel we sort the values of the pixel and its neighbours, determine the median, and assign this value to the pixel. The median filtering operation makes pixels with very different intensities more like their neighbouring pixels. This method is particularly effective when noise pattern consists of strong spikelike components and where the characteristic to be preserved is edge sharpness.
Figures 4 a&b show the effect of filtering the MRI scan with a median filter. Please note the superior performance of this filtering operation in removing spikelike (salt and pepper) noise.

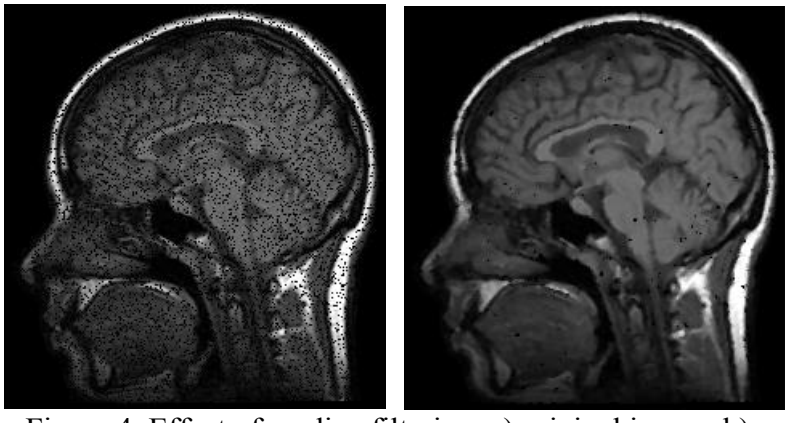

Figure 4. Effect of median filtering, a) original image, b) median filtered image.

1.2 Enhancement in the Frequency Domain

The frequency domain enhancement method is based on the convolution theorem. Convolution in the spatial domain corresponds to multiplication in the frequency domain. Therefore, image enhancement problems can be expressed as:

$$G(u,v) = H(u,v)\, F(u,v)$$

where G(u,v), H(u,v), and F(u,v) are two dimensional Fourier transforms of g(x,y) [the enhanced or filtered image], h(x,y) [impulse response of the enhancing operation or filter - a position invariant mask], and f(x,y) [the original image], respectively.
In a typical image enhancement application, the original image f(x,y) is given and the objective, after computation of F(u,v), is to design H(u,v) so that the enhanced image, g(x,y) = inverse Fourier transform of [H(u,v) F(u,v)], highlights the desired features of the original image. Figure 5 a&b show the effects of sharpening the MRI scan with a sharpening filter to increase contrast and accentuate detail. (The image is sharpened by finding the 2-D Fourier transform of a 3 x 3 sharpening mask, i.e.

-1	-1	-1
-1	-9	-1
-1	-1	-1

i.e. H(u,v) and multiplying it by the 2-D Fourier transform of the MRI scan i.e. F(u,v) and finally finding the inverse 2-D Fourier transform of the product H(u,v) F(u,v).

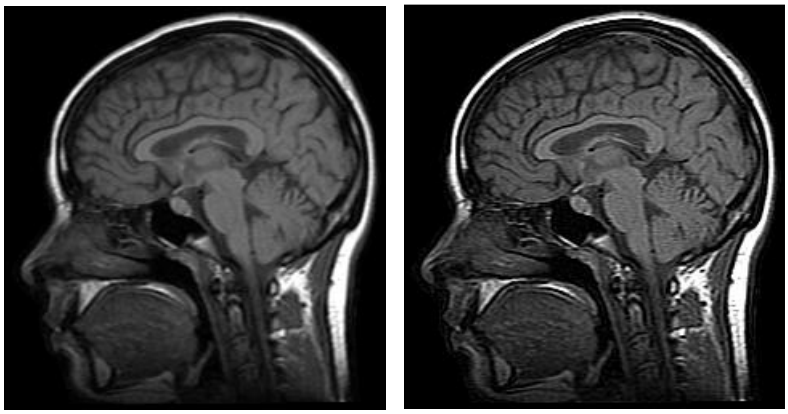

Figure 5. Image sharpening or high pass filtering, a) original image, b) sharpened image.

2.IMAGE PROCESSING AND ANALYSIS

In this section we briefly mention specimen preparation and review the main areas of image processing and analysis which are of particular interest in pathology. These areas are: image segmentation, morphometry, and image analysis.

2.1 Specimen Preparation

The basic requirements of a monolayer preparation are summarised by Shwarz et al. [8] as follows:

- regular and isolated cell deposits in an optimal cellular density
- location of cells in the same optical plane

- sufficient contrast between nucleus and cytoplasm as well as between cytoplasm and background
- appearance of cells according to their cytological classification with good reproducibility
- reproducibility of cellular staining
- clear background
- clear boundaries around the area of deposits

An overview of the different techniques for the preparation of specimens is given by Rosenthal and Manjikian [9].

2.2 Image Segmentation

Segmentation is the process that subdivides an image into its constituent parts or objects. Segmentation algorithms generally are based on one of the two basic properties of gray-level values: discontinuity and similarity [4]. In the first category, partitioning an image is based on abrupt changes in gray-level. The principal areas of interest within this category are the detection of isolated points and the detection of lines and edges in an image. The principal approaches in the second category are based on thresholding, region growing, and region splitting and merging [2-7].

2.2.1 Edge Detection

The edge detection methods are principally based upon the first and second partial derivatives at any point in the image and they can be performed by using the gradient operators or the Laplacian operators. In gradient operators the gradient vector magnitude and direction are given by :

$$G[f(x,y)] = \begin{bmatrix} G_x \\ G_y \end{bmatrix} = \begin{bmatrix} \frac{\delta f}{dx} \\ \frac{\delta f}{dy} \end{bmatrix}$$

$$\text{magnitude } [G] = G[f(x,y)] = [G_x^2 + G_y^2]^{\frac{1}{2}}$$

$$\text{phase } [G] = \alpha(x,y) = \tan^{-1} [\frac{G_y}{G_x}]$$

where G_X is the horizontal gradient and G_Y is the vertical gradient. There are a variety of methods for performing gradient computations and there are a large number of spatial masks designed for this purpose [5].

The Laplacian operator is a second derivative operator and is given by:

$$L[f(x,y)] = \frac{\delta_f^2}{\delta_x^2} + \frac{\delta_f^2}{\delta_y^2}$$

The Laplacian responds to transitions in intensity and is seldom used by itself for edge detection. Since it calculates the second derivative it is too sensitive to noise. Figure 6 a&b show the effect of edge detection in the MRI scan. This operation produces a binary image (6 b) with a white background and black outlines representing edges in the original image. In this operation the following masks have been used to find the horizontal and vertical gradients:

-1	0	1		1	1	1
-1	0	1	and	0	0	0
-1	0	1		-1	-1	-1

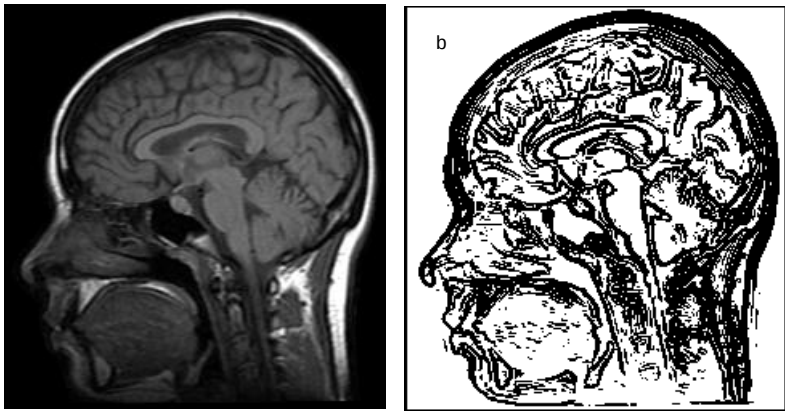

Figure 6. Edge detection, a) original image, b) edge detected image.

2.2.2 Thresholding

A simple approach for segmenting an image is to divide the gray scale into bands and to use thresholds to determine regions or to obtain boundary points. Threshold selection is an important step in this method. Some commonly used approaches are as follows [2-7]:

* The histogram of the image is examined for locating peaks and valleys. If it is multimodal then the valleys can be used for selecting thresholds.

* A threshold (T) is selected so that a predetermined fraction of the total number of samples are below T.

* Adaptive thresholding is performed by examining local neighbourhood histograms.

* Selective thresholding is performed by examining histograms only of those points that satisfy a chosen criterion.

* If a probabilistic model of different segmentation classes is known, the threshold is determined to minimise the probability of error or some other quantity.

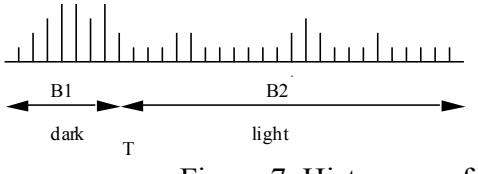

Figure 7. Histogram of an image.

The histogram in Figure 7 is characteristic of images consisting of objects superimposed on a dark background. To outline the boundary between objects and the background, the histogram is divided into two bands separated by a threshold T. The goal is to select T so that band B_1 contains, as closely as possible, the levels associated with the background while band B_2 contains the levels of the objects. As the image is scanned, a change in grey level from one band to the other denotes the presence of a boundary. In order to select boundaries in both the horizontal and the vertical directions, two passes through f(x,y) are required. Once B_1 and B_2 are selected, the procedure is as follows [3]:

Pass 1:

For each row in f(x,y) (i.e. x = 0, 1, ..., N-1), create a corresponding row in an intermediate image $g_1(x,y)$ using the following relation for y = 1, 2, ..., N-1:

$$g1(x,y) = \begin{cases} L_e & \text{if the levels of } f(x,y) \text{ and } f(x, y-1) \text{ are in different bands of gray scale} \\ L_b & \text{otherwise} \end{cases}$$

where L_e and L_b are specified edge and background levels, respectively.

Pass 2:

For each column in f(x,y) (i.e., y = 0, 1, ..., N-1) create a corresponding column in an intermediate image $g_2(x,y)$ using the following relation for x = 1, 2, ..., N-1:

$$g2(x,y) = \begin{cases} L_e & \text{if the levels of } f(x,y) \text{ and } f(x-1, y) \text{ are in different bands of gray scale} \\ L_b & \text{otherwise} \end{cases}$$

The desired image consisting of the points on the boundary of objects, different (as defined by T) from the background is obtained by using the following relation for x, y = 0, 1, ..., N-1:

$$g(x,y) = \begin{cases} L_e & \text{if either } g1(x,y) \text{ or } g2(x,y) \text{ is equal to } L_e \\ L_b & \text{otherwise} \end{cases}$$

In cytology, once objects of potential interest have been located, the analysis is focussed on the area around each cell deemed suitable for inspection. The aim is to identify the nuclear and cytoplasmic regions in the scene and then extract the necessary features that characterise each region.

To identify the constituent parts in each region the segmentation procedure performs two tasks: one to find the nucleus, the other to find the cytoplasm [10]. The resolution of the image and the quality of photometric properties such as gray-level value, colour or texture play an important role since they can affect the overall accuracy of the feature extraction procedure and classification results.

Image processing techniques such as smoothing, gradient operators, and iterative histogram modification are normally used to clarify the boundaries between background and cytoplasm and between cytoplasm and nucleus. Thresholding methods are based on optical density, local gradients, and histograms of different spectra. Density and gradient thresholding are the simplest and fastest segmentation techniques.

Liedke et al. have compiled an excellent reference source which reviews different methods of automated segmentation of cell images [11]. The techniques discussed include edge detection by thresholding, "blob" detection using the split-merge algorithm, global thresholding using gray-level histograms, hierarchical thresholding using colour information, global thresholding using two dimensional histograms and segmentation by "blob" labelling.

2.3 Morphometry

Morphometry is the quantitative description of a shape. In general, the term is more freely used with regard to the quantitative description of a structure. Morphometry could imply any type of quantitative analysis. In practice, the term is used to denote a particular area of quantitative pathology. It is restricted to the use of simple equipment for interactive quantitative image analysis of geometric features. Researchers use the full pattern recognition and diagnostic capabilities of morphometry in their analysis and measurement procedures.

Typical examples of quantitative analysis are mitotic counts and differential counts of leukocytes in a blood smear [12]. Being one of the oldest quantitative methods, the daily worldwide use of these analyses clearly indicates the versatility of such techniques in interactive morphometry. Other useful readings on morphometry are compiled in references [13,14].

2.4 Digital Image Analysis

In principle, digital image analysis should enable us to analyse architectural features of tissues and cells at high speed. Developments in this area are increasing at a rapid rate. Fully automated TV-image analysis has been used to establish cell cycle parameters based on autoradiographs of histological sections [15]. In oncological pathology and diagnostic cancer cytology, nuclear chromatin patterns represent the most important discriminating features between cells of a malignant neoplastic line from their benign counterparts. Therefore, methods for quantifying the chromatin distribution in cell nuclei are of greatest interest. Attempts to recognise dyskaryotic cells based on features such as nuclear heterogeneity, granularity, clumping and margination of chromatin have reached a high level [14,16].

3.BIOMEDICAL APPLICATIONS

The following part of this paper has three sections. The first section (3.1) reviews image cytometric and histometric methods as well as standards and calibration. Biomedical applications of image cytometry and histometry are included in this section. The second section (3.2) describes 3-dimensional confocal image processing and analysis. The third section (3.3) deals with computerised system development. This last section includes a very brief review of development of computer software and hardware, special purpose optical disk database management, and artificial neural networks.

3.1 Image Cytometry and Histometry: Methods, Standards and Calibration

Smeulders and Kate [17] have studied the accuracy of optical density measurement of cells and have analysed the factors which influence the accuracy and precision of this method.

They applied their research method to the measurement of DNA (deoxyribonucleic acid) content of Feulgen-stained cells using video microscopy. They have discussed many factors influencing the accuracy of density measurements such as staining, glare, diffraction, image sampling distribution, sampling density, noise and computational accuracy. They have reviewed these factors and have pointed out potential remedies.

The measurement of DNA in cells plays a key role in the detection and diagnosis of cancer [18]. Cancerous cells normally contain unusual and variable quantities of DNA. Since the fundamental discovery that normal cells could be distinguished from cancerous cells on the basis of their DNA content, measurement of nuclear DNA has proven to be a useful technique in detection and diagnosis of neoplasia. Cellular DNA content also can be of value in the classification of tumour types and in predicting biological behaviour.

Continuous motion imaging provides a method for the rapid quantitative analysis of slice mounted cell preparations [18]. The continuous motion imaging system is a new instrument which can in principle measure morphological, densitometric, and texture features of cells and other microscopic objects. Tucker et al. have described the continuous motion imaging system principles and techniques used to achieve densitometric measurements in the performance of the system in their paper. It is to be expected that continuous motion imaging systems will find increasing applications in the field of quantitative pathology, particularly in those tasks requiring rare event detection of small subpopulations of interesting cells hidden in much larger populations of uninteresting cells.

Histopathological and clinical differential diagnosis of melanoma and benign pigmented moles at early stages can be difficult. Abmayr et al. have used electron microscopy (EM) and light microscopy (LM) with high resolution TV-scanning and multivariate analysis methods to compare benign pigmented mole cells and malignant melanoma cells [19]. Comparative studies on EM and LM are useful to look for the reliability of light microscopic features for the recognition of malignant melanoma cells since the LM technique is more practical in routine work. Both EM and LM techniques can scan identical cells of benign pigmented moles and malignant melanoma. In electron microscopy, nuclei of melanoma cells appear usually more heterochromatic, whereas in light microscopy they appear more euchromatic. Using multivariate analysis methods Ambayr et al. found distinguishing features in EM images and transferred them to LM images. The correlation of EM and LM features were emphasised and helped to demonstrate that classification in LM is sufficiently good using texture features only, even though chromatin structure in the LM image appears out of focus.

A previous investigation of pigmented cells in electron microscopy showed that a specimen classification using only karyometric features was fairly reliable [20]. In their investigation Stotz et al. found that the features of chromatin texture were more discriminating than karyometric features when training sets of randomly selected individual cells were classified. Multivariate analysis improved the results of differentiation between individual malignant melanoma and benign pigmented moles. The nuclear area was not important for the classification of pigmented cells compared to chromatin features. The combination of euchromatin and heterochromatin features improved the results in both EM and LM.

Malignant change in human tissues is accompanied by deviations in the DNA content of the nuclei. A number of studies have been performed to elucidate the relationship between the clinical behaviour of these malignant lesions and the amount of DNA in the nucleus [21]. In their study Oud et al. showed that DNA histogram or DNA index analysis using image

cytometry in nuclei from paraffin-embedded tissue appeared to be a valuable method to characterise malignant processes. Image cytometry offers the advantage that in addition to DNA content, other DNA derived features such as nuclear size, shape and chromatin texture (DNA distribution in the nucleus) can be obtained. The DNA index can be computed and is comparable with that obtained from flow cytometry. In their paper, the authors also mention the clinical application of DNA index in dysgeminomas of the ovary and small-cell lung cancer.

Based on computer techniques, Melder and Koss at the Albert Einstein College of Medicine in New York have developed an image analysis system for diagnosis of bladder cancer [22]. The system converts optical images of cells into arbitrary numerical values based on light absorption and transmission. The digitised image is processed by a computer system that is capable of feature extraction and analysis. A broad variety of measurements of cells and their components can also be obtained resulting in a highly detailed study of individual cells or families of cells.

The identity of the cells can be controlled by visual examination, the cells can be rescanned to test the accuracy of the system, and the digitised images can be stored for future analytical study when machine generated data require a comparison with clinical findings.

Bronchocarcinoma has been one of the big killer cancers in the past years. Research has shown squamous cell carcinoma type to be the most readily detectable through the use of sputum cytology. Cytological screening of sputum specimens can be identified before they are evident through radiological screening [23]. This technique involves the microscopic screening of Papanicolaou prepared sputum specimens from the affected bronchus. Swank et al. have used high resolution image analysis techniques to detect and classify bronchial epithelial atypias from sputum. Different studies by Bartels and his colleagues has demonstrated the feasibility of this method in identifying premalignant atypias of the uterine cervix [24,25,26].

3.2 3-D Confocal Image Processing and Analysis

Confocal microscopy provides the ability to capture 3-D images of microscopic structures. But as the complexity of the tissues increases, direct viewing in 3-D becomes more difficult and confused due to the large number of structures involved [27]. One good solution to effectively study complex tissues is the numerical description of tissue organisation (e.g. position, size and orientation of each structure). An attempt at data reduction by means of manual tracing in 3-D has been made in the study of sea urchin embryo [27]. However, apart from being labour intensive, this 3-D digitisation technique suffers from the inaccuracies of manual 3-D tracing related to the depth of perception of the operator. To overcome the disadvantages of manual tracing, Samarabandn and his group have developed a number of image analysis algorithms. They also have designed a system to visualise and extract morphometric parameters from the data generated by confocal microscopy.

A confocal imaging system encodes structural information of the object into a digitised array. During the image generation procedure noise and artefacts are introduced. To reduce noise and artifacts image enhancement is needed to emphasise the biological structures of interest in the image. Three dimensional median filtering could be used to reduce spurious noise and difference of Gaussian (DOG) filtering could be utilised to enhance edges.

The presence of boundary concavities and hollow regions is a major source of problems in the segmentation of confocal images. To reduce these irregularities the authors used an approach based on multilevel neural networks to further enhance the images [27]. The network used consisted of three levels of n∗m computational elements where each layer receives activities from the level below it. They also applied region segmentation and boundary refinement in their research. The segmented image was used to extract morphometric parameters of microscopic structures such as surface area, volume, centre of gravity, eccentricity and skeleton. The complete procedure is illustrated in Figure 8.

Morgan et al. have reconstructed glial cells in the mouse central nervous system in three dimensions using confocal microscopy [28]. In their work, they labelled specific cells of intact tissue by immunostaining, optically sectioned the tissue, computationally reconstructed a three dimensional image data set from digitised confocal optical sections, and finally used surface rendering techniques to define boundaries of cells and to display individual cells.

Using confocal fluorescent microscopy Montag et al. have made a 3-D reconstruction of chromatin structures with a ray-tracing algorithm [29]. They recorded up to 32 optical sections in 500 nm steps in the z-axis. To achieve enhanced contrast and an outline of the unclear boundary image, filtering techniques were employed.

With the development of computer techniques and confocal microscopy, multi-dimensional representation has become possible. Kriete and Wagner have used computer-assisted confocal microscopy to study spatio-temporal changes of synaptic morphology in cultured fish retinas.

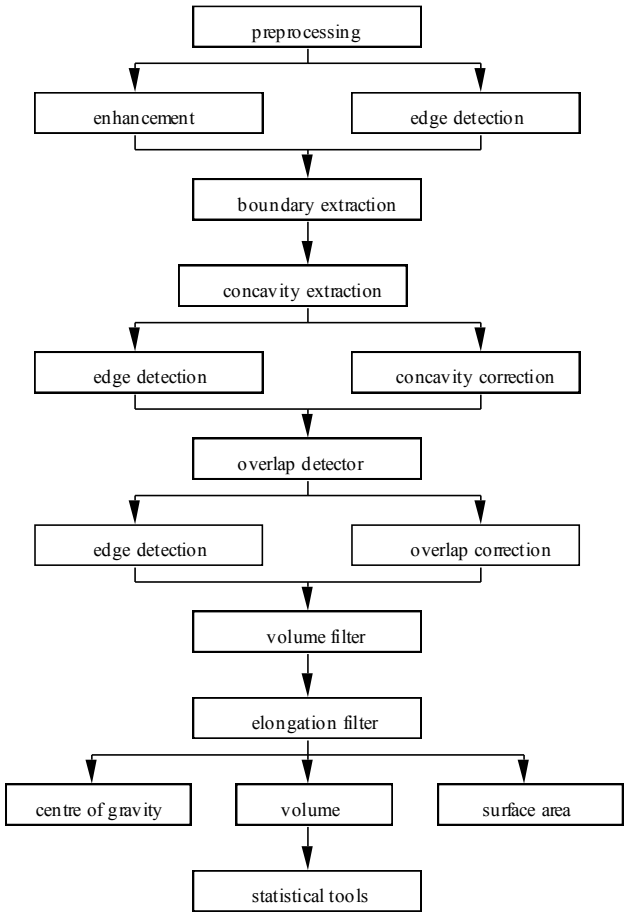

Figure 8. Segmentation procedure.

In their work [30], they used a specific volume rendering technique. Data volume acquired sequentially in time were reconstructed three dimensionally. The animated display of reconstructed views required large memory capacity to store and handle the enormous amount of data. In this study, their interest was only in visualisation of very subtle morphogenetic changes, so only the intensity information was stored by the volumetric elements. The time dependent intensity variation was added to the basic volumetric elements.

3.3 Computer System Development in Pathology

3.3.1 Workstations

Bacus et al. have developed a new fully integrated optical microscope and computer workstation system for the pathology laboratory [31]. Their system allows for acquisition and storage of digitised microscopic images, measurement of a standard set of cell features calibrated for accurate densitometry, and a comprehensive statistical analyses and display procedures. Their statistics programs emphasise the ability to discriminate between cells and cell population. Their system is used by practising pathologists in diagnostic and prognostic visual microscopic evaluations, i.e. to examine specimens from patients with cancer, infectious diseases, blood diseases, and genetic or constitutional abnormalities.

3.3.2 PC systems

A personal computer-based microphotometry system has been designed by Dytch et al. [32]. The system provides objective measurements of important diagnostic properties of histologic sections. The diagnostic features include detailed microscale measurements of individual nuclei such as total integrated nuclear optical density (DNA content), nuclear area, shapes, and texture. The microscale features may be measured in an individual nuclei. Additional diagnostic information, such as measurements of mitotic density, nuclear crowding, and relative nuclear orientation may be examined in the context provided by other nuclei and the structure of the histologic section as a whole.

The system hardware is of low cost, widespread availability and easy serviceability. The major hardware components are a microscope, a video camera and control unit, a video digitiser, a frame buffer, a display monitor, and a personal computer.

3.3.3 Optical Disks

In recent years, a new advancement in histopathology and analytical cytology has been possible due to the ability to record and retrieve a large number of digitised high resolution images of stained cells. This has resulted in the development of image cytometry database systems. Such systems employ an optical memory disk recorder for storage of an image database, a microcomputer for storage of a relational database containing descriptive features and graphics overlap parameters. Such systems can be used for varied purposes such as recording images of fixed and stained cells or tissue on a microscope slide, observing the behaviour of live cells growing in tissue culture, research and clinical applications. Clinical applications include storage of individual cases for presentation and consultation,

development of teaching techniques in cytology and pathology, time-lapse cinematography of live cells, and storage of an image database which is used to test various segmentation techniques.

3.3.4 Expert Systems

Knowledge engineering methods can be used to design machine vision systems for the histopathology laboratory which use computer resources efficiently and in an adaptive fashion. These methods offer the potential of giving the machine vision system a very good level of diagnostic judgement and they provide histopathologists with expert guidance in the handling, analysis, and interpretation of multivariate data.

An intelligent knowledge-based system in quantitative histopathology has been developed by Dytch et al. [28]. The system allows for real-time image data driven dynamic reconfiguration of the computer architecture, which is based on prior knowledge of the histopathologic sections to be processed. The system functions include an expert controlled scene segmentation, processing task scheduling, diagnostic expert system module, and validation procedures.

Scene segmentation in complex imagery is very difficult. Human observers preprocess imagery using prior knowledge. In examining histopathologic images, pathologists apply a large number of heuristic rules. These rules lend themselves ideally as a knowledge base to guide the scene segmentation process by a rule-based production system. This diagnostic expert system module allows the pathologists to enter the diagnostic categories and the clues, establish quantitative grading for the clues, and assign certainty factors. Several applications are described in Bartels et al. [33].

3.3.5 Neural Networks

Dytch and his colleagues have also developed an expert diagnostic system for the analysis of stratified epithelial tissues using objective histometric techniques [34]. A hierarchical design method with embedded expert systems directed by higher level meta-systems has been employed to evaluate specific diagnostic features. According to the authors, one approach is to directly use raw data derived from the digitised images of tissue sections as inputs to a neural network, bypassing the scene segmentation and feature extraction steps of the diagnostic process. The other approach is to use a neural network as a preprocessor to extract diagnostic clues which are difficult to define and relate to mathematical features, i.e. chromatin clumping and other pixel level textural features. The authors used neural networks as pattern recognisors, employed feature vectors extracted from histologic image data as input to the network, and derived diagnostic categorisation of image data as outputs from the network.

CONCLUSIONS

Quantitative pathology and cytology will benefit a great deal from the new advances in image processing and analysis technologies. The new research and development opportunities in pathology would rely heavily on the interdisciplinary interactions of a myriad of core and supporting technologies. Electronic imaging systems, computerised instrumentation, engineering techniques, advanced computing environments and sophisticated mathematical algorithms would play a fundamental role in the development of powerful new tools and techniques to address complex cytological and pathological morphometric questions.

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