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Segmentation and Analysis of Microscopic Osteosarcoma Bone Images

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Abstract

Characteristics of microscopic osteosarcoma bone cross section images carry essential clues for defining the important features in the bone cross section such as stroma, bone, malignant cell, myxoid matrix, artifact for different age groups and also for age related developments & diseases. The traditional approaches of bone microscopic image analysis rely primarily on manual processes with very limited number of bone samples which is very difficult to get reliable and consistent conclusions. A new method of hybrid technique of image segmentation which uses microscopic images for processing is proposed. This hybrid segmentation technique automates the bone image analysis process and is able to produce reliable results based on qualitative measurements of the features extracted from the microscopic bone images. The study of correlation of bone structural features and age related developments & diseases become feasible from large databases of bone images.

Key Words: – Ostosarcoma bone microscopic image, Image segmentation, Feature extraction, Qualitative image analysis.

1. Introduction

The characteristics of microscopic features in a bone cross section can be used to access the biological age of the bone and in histological studies of bones, such as in determination of age at death [1]. Reliable analysis of bone cross sections play a major role in understanding of bone growth and bone diseases such as cancer & osteoporosis. [2].

Traditionally used approaches for bone microscopic image analysis in research involve collecting a set of bone specimens and analyzing their cross sectional images. The microscopic images are acquired by using micro radiography, transmitted light scans, plain polarized light scans, circularly polarized light scans or laser technology. These different image acquisition techniques bring out different levels of mineralization in bone cross section in the form of grey level intensity variations. [3].

As the microstructures that are of interest are very small, high magnification is necessary for identification of bone features. Examination of bone microscopic images is usually a repetitive, time consuming and labor intensive process. The manual examination of microscopic images often produces subjective results and requires diligent concentration from a highly trained operator and also manual interpretation of microscopic bone images is error prone because of statistical, structural and temporal variations of objects in a raw bone images. Conventional bone feature extraction techniques are not sufficient to handle low resolution and noisy images. Hence there is a need for automated and reliable techniques to carry out image analysis. Automated qualitative image analysis involves acquiring digitized images of the bone cross sections followed by extraction of microstructures information.

Bone image analysis is used to identify and extract useful bone feature information from a bone microscopic image. The bone image feature analysis technique developed consists of steps such as pre-processing, object representation, feature extraction, classification and interpretation of an image.

Preprocessing of an image is performed for the improvement of the image data and also for identifying image features which are important for further processing. Pre-requisite for preprocessing of an image is knowledge about the image acquisition device, conditions under which the image was obtained and objects that are searched in the image. Representation of object includes quantifying abnormalities, visualization of structures.

This paper presents a hybrid technique of image segmentation such as region of interest technique combined with threshold technique and edge detection technique for effective and consistent extraction of bone features.

2. Methodology

The procedure and techniques carried out for bone image analysis is shown in flow chart.

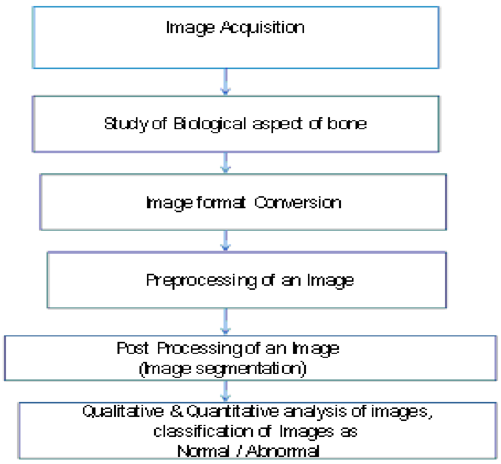

Fig.1 Work flow chart.

2.1 Image acquisition: Image acquisition is the first process involved. The microscopic bone cross section image acquired by using electronic microscope and is shown in Fig.2.

2.2 Biological aspect of bone: Important features in the bone cross section such as stroma, bone, malignant cell, myxoid matrix, artifact for different age groups and also for age related developments & diseases observed.

2.3 Image format conversion: The digital image obtained using electronic microscope is in RGB (Red, Green and Blue) format and converted to grey level image for further processing. The MATLAB tool is used for image format conversion from RGB to gray level.

2.4 Pre processing of an image:

The preprocessing operation is carried out to extract details that are obscured in an image or to highlight features of interest in an image.

The Osteosarcoma bone cancer cross section microscopic image obtained by using electronic microscope and is shown in Fig.2 & its histogram plot is shown in Fig.3a. The histogram plot is the plot of $h(r_k) = n_k$ versus rk or $p(r_k) = n_k/n$ versus r_k.where r_k is the k_{th} gray level and n_k is the number of pixels in the image having gray level r_k. The horizontal axis of histogram plot corresponds to grey level values, r_k. The vertical axis corresponds to values of $h(r_k) = n_k$ or $p(r_k) = n_k/n$ if the values are normalized [4]. With the histogram plot we can make out whether the image is dark or bright. The histogram equalization is performed to see the image clarity better and is shown in Fig.3b.

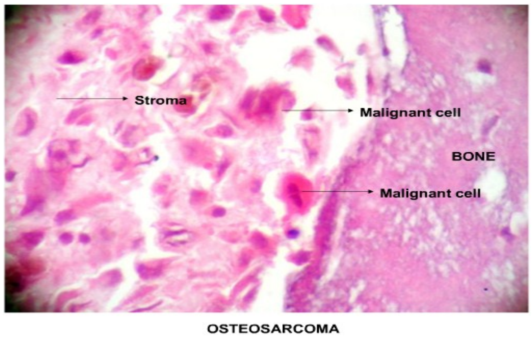

Fig. 2 Bone cross section image (Osteosarcoma)

(Source:Kidwai Institute of Oncology)

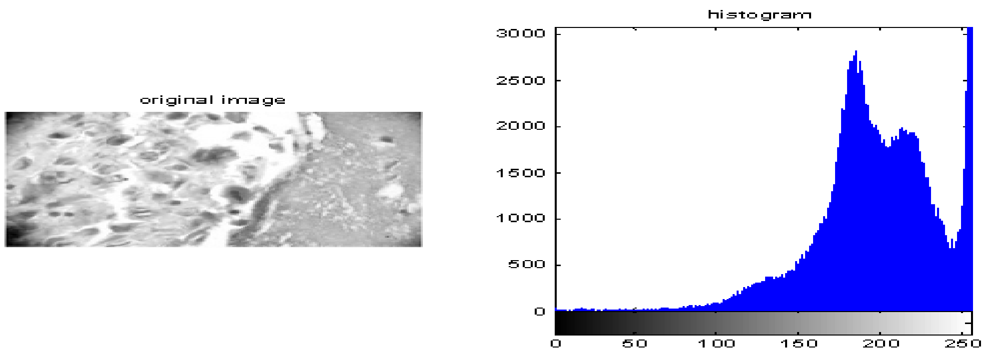

Fig. 3a Histogram of an image of an image. (X-axis- Gray value and y-axis-Number of occurrences of particular gray value)

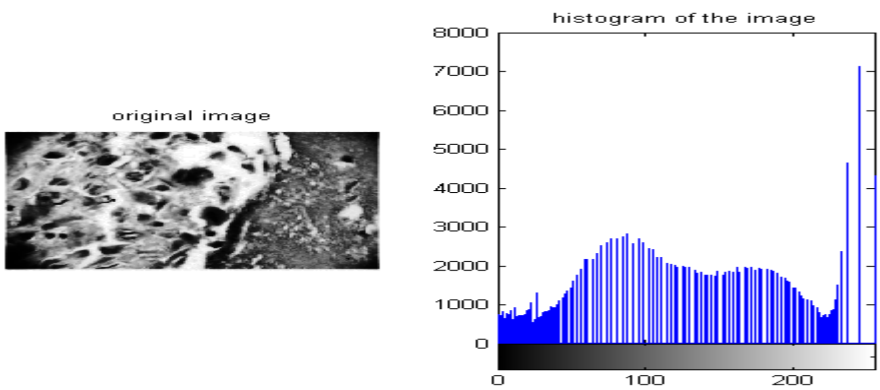

Fig. 3b Histogram equalization of an image. (X-axis- Gray value and y-axis-Number of occurrences of particular gray value)

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2.4.1 Zoom (magnifying) operation: Magnifying operation involves pixel duplication by creation of new pixel locations and assigning of grey levels to those new locations & is done by interpolation technique.

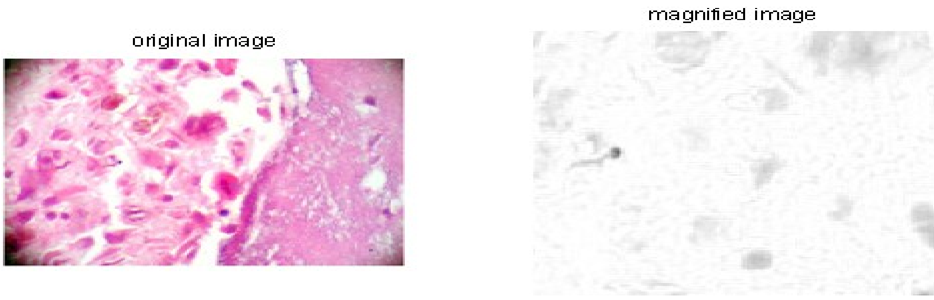


Fig. 4 Magnified parts of the selected image region of Fig.2.

2.4.2 Gray level slicing, Negative of an Image and Scaling of an Image:

Gray level slicing of an Image: Highlighting a specific range of gray levels in an image. There are two approaches of gray level slicing. One approach is to display a high value for all gray levels in the range of interest and second is to display low value for all other gray levels. This technique is applied for proper visualization in an image.

Negative of an Image: The negative of an image with gray levels in the range [0, L-1] is obtained by using the negative transformation and is given by the expression,

$$v = L - u \quad (1)$$

where L is the threshold value, u and v are the gray level values. This is done for proper visualization of bone features.

Scaling of an Image: The dynamic range of a typical unitary transformed image is so large that only a few pixels are visible. The dynamic range can be compressed via the logarithmic transformation,

$$v = c * \log(1+|u|) \quad (2)$$

Where c is the scaling constant. This transformation enhances the small magnitude pixels compared with those pixels with large magnitudes.

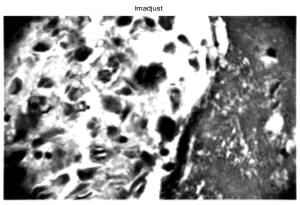
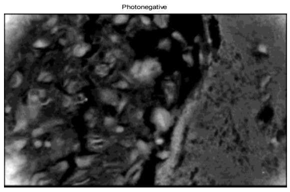


Image Gray scale expansion



Negative (photo negative) of an image

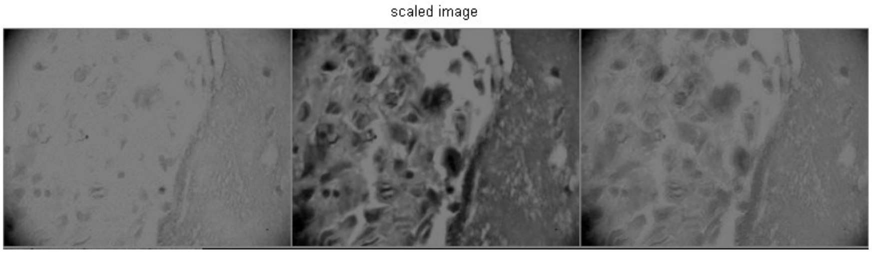


Fig. 5 Gray level slicing, negative and scaling of an image of Fig.2

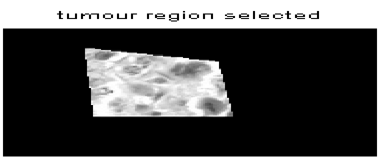
2.5 Image Segmentation Method

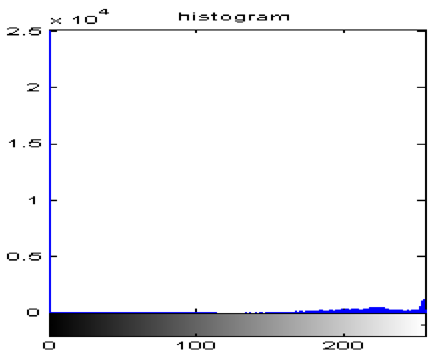
Image segmentation is the decomposition of a scene into its components for defining an object system. Segmentation of bone cross section images is to divide the bone images into anatomically significant regions.

Description, also called feature selection, deals with extracting attributes that result in some quantitative information used for differentiating one class of objects from another. Recognition is the process that assigns a label to an object based on its descriptor.

2.5.1 Region of Interest

A region of interest (ROI) data object can be used to control pixels within a source image processed by an operator to specify pixels processed by an operator will be recorded in a destination image [5]. The malignant tumors interested part separated from the original image for better visualization & understanding is shown in Fig.6(a) and its histogram plot is shown in Fig.6(b).





(a) (b)

Fig. 6 Region of interest selection and its histogram.

2.5.2 Edge Detection of an Image

Edge detection technique is used for detecting meaningful discontinuities in intensity values. The approach is to implement first and second order digital derivatives for the detection of edges in an image are used in this technique. The gradient of an image f(x, y) at location (x, y) is defined as the vector

$$\blacktriangledown f = \begin{bmatrix} Gx \\ Gy \end{bmatrix} = \begin{bmatrix} \Delta f / \Delta x \\ \Delta f / \Delta y \end{bmatrix} \quad (3)$$

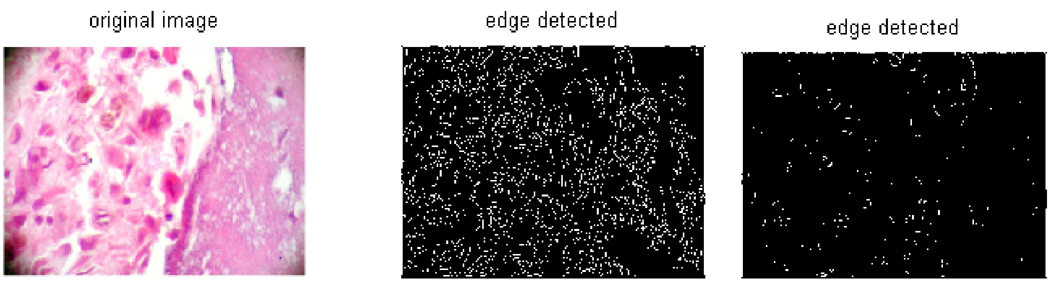
From vector analysis, the gradient vector points in the direction of maximum rate of change of f at coordinates (x, y). An important quantity in edge detection is the magnitude of this vector, denoted $\blacktriangledown f$, where

$$\blacktriangledown f = \text{mag}\ (\blacktriangledown f) = [G^2_x + G^2_y]^{1/2} \quad (4)$$

This quantity gives the maximum rate of increase of f(x, y) per unit distance in the direction of $\blacktriangledown f$. The direction of the gradient vector also is an important quantity. Let α(x, y) represent the direction angle of the vector $\blacktriangledown f$ at (x, y). Then, from vector analysis,

$$\alpha\ (x, y) = \tan^{-1}(G_y/G_x) \quad (5)$$

Angle is measured with respect to the x-axis. The direction of an edge at (x, y) is perpendicular to the direction of the gradient vector at that point [5].



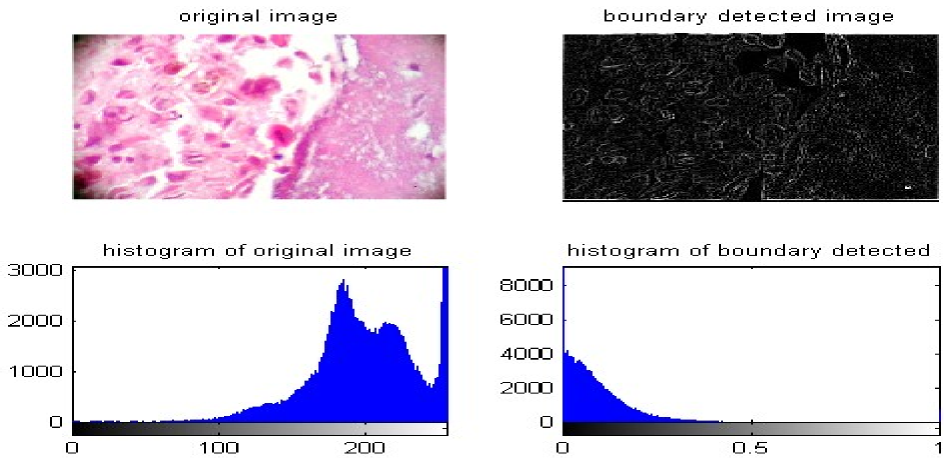
(a) **(b)** **(c)**

Fig. 7 Edge detection of an image of Fig.2

The edge detection of an original image with different gradient operators such as canny and sobel operators is shown in Fig.7. In edge detected image of Fig.7 (b) we can see the minute details of myxoid matrix and bone image features such as the edges of malignant tumor cells, & artifacts. In Fig.7(c) we can see the prominent edges of the image parts.

2.5.3 Boundary Detection of an Image

It is possible to segment an image into regions of common attribute by detecting the boundary of each region for which there is a significant change in attribute across the boundary. Boundary detection can be accomplished by means of edge detection. The histogram plot of original image is shown in Fig.8(a) and also the histogram plot of boundary detected image is shown in Fig.8(b).The boundary detected image is much darker. Wherever tumor cells, blood vessels and bony trabeculae are there & there we can make out the brighter parts of the image. The darker part of the image shows the myxoid matrix composition.



(a) (b)

Fig. 8 (a) Original image & its histogram plot (b) Boundary detected image & its histogram Plot.

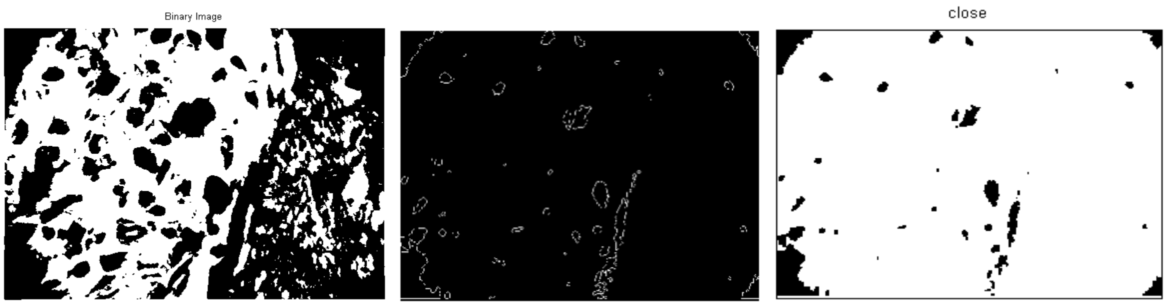


Fig.9 a.Binary Image of original Image Fig.9.b & c.Wanted grains marked and highlighted

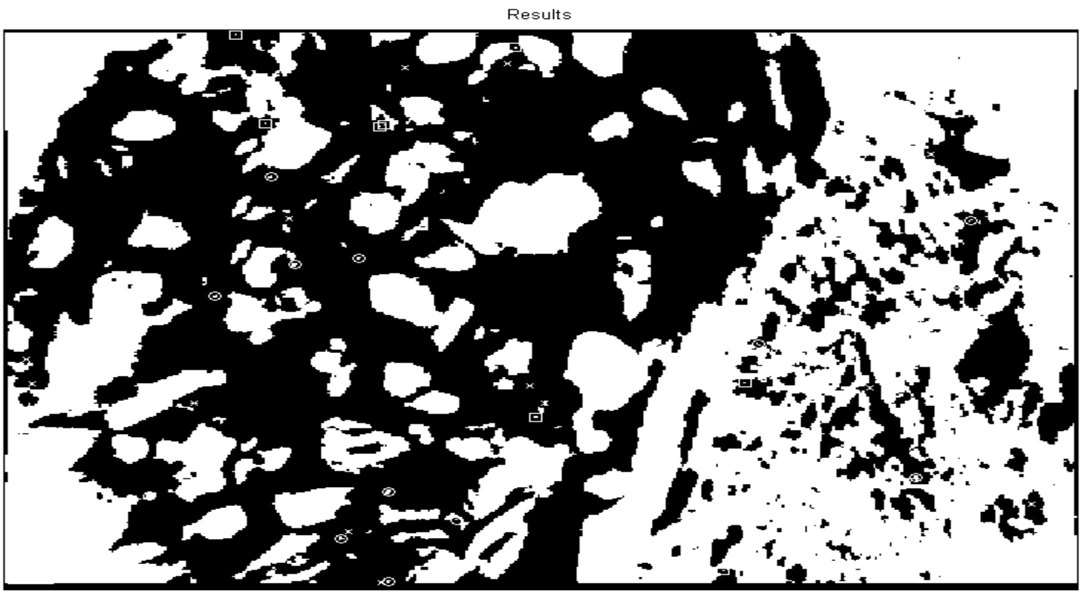


Fig. 10 Features extracted from an original image of Fig.2

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Binary image of Fig.9a shows the object parts with bright and dark gray level values for better visualization and understanding of each object spot.Fig.9 b&c shows the wanted grains marked and highlighted through morphological operation of remove and close. Morphological operation gives more structural and geometrical information. In Fig.10 marked all the parts based on their shape such as round, triangular, square because cancers cells are irregular in shape. These above image data will be more useful for classification of bone microscopic images as malignant or benign case of cancer by the physician.

2.5.4 Image Contour Tracing

Contour tracing (border following) is a technique that is applied to digital images in order to extract their boundary. The boundary of a given pattern P, is the set of border pixels of P. Since we are using a square tessellation, there are 2 kinds of boundary pixels: 4-border pixels and *8-border* pixels. A black pixel is considered a 4-border pixel if it shares an edge with at least one white pixel. On the other hand, a black pixel is considered an 8-border pixel if it shares an edge or a vertex with at least one white pixel.

It is not enough to identify the boundary pixels of a pattern in order to extract its contour. What it is needed an ordered sequence of the boundary pixels from which the general shape of the pattern is extracted.

2.5.4.1 Importance of Contour Tracing

Contour tracing is one of many preprocessing techniques performed on digital images in order to extract information about their general shape. Once the contour of a given pattern is extracted, it's different characteristics will be examined and used as features which will later on be used in pattern classification. Therefore, correct extraction of the contour will produce more accurate features which will increase the chances of correctly classifying a given pattern.

The contour pixels are generally a small subset of the total number of pixels representing a pattern. Therefore, the amount of computation is greatly reduced when we run feature extracting algorithms on the contour instead of on the whole pattern. Since the contour shares a lot of features with the original pattern, the feature extraction process becomes much more efficient when performed on the contour rather on the original pattern. In conclusion, contour tracing is often a major contributor to the efficiency of the feature extraction process -an essential process in the field of pattern recognition.

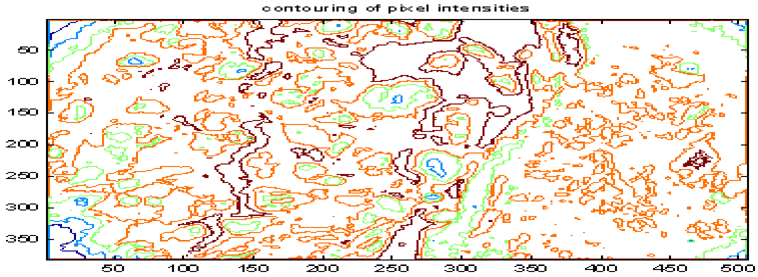

Fig.11 Contours of an image of Fig.2.

Figure 11 and figure 10 gives additional confirmation of cancers part and its growing information with qualitative information of image contour and marking of cell shapes such as round, square, triangular.

2.5.5 Detection and Measurement of Normal and Abnormal Parts

The interested objects of grains are detected and marked with different colors & is shown in Fig.12. Also calculated the area of affected and non affected object grains and tabulated in table as shown below Table 1. for the beginning case of osteosarcoma cancer case. The detailed and comparative study of fast growth of tumor cells can be identified for consecutive samples at regular intervals and also the effect of drug on cancer can be cross verified after taking sample of tumor by knowing its area of affected and non-affected grains.

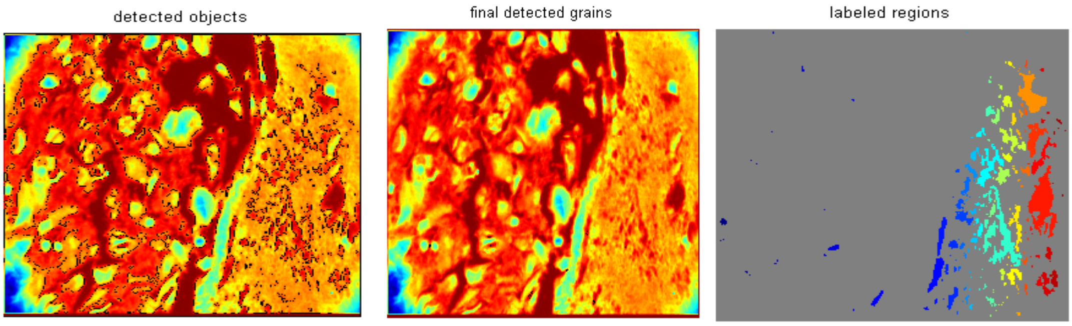

Fig.12 Final detected objects of interest of Fig.2

Total area of an image	Image area affected	Image area unaffected	Percentage affected
585585	209	585376	0.0356

Table 1. Area of affected and non-affected grains of osteosarcoma in terms of percentage

3. Results

The microscopic osteosarcoma bone image samples are processed for removing the unwanted signal noise for better vision and for making differentiation among different parts of the image. From the processed images it can be observed that the artifact region, tumor cells and myxoid matrix regions can be identified and seen clearly. The boundary detected image and edge detection of an image clearly shows each part boundary & if any breakages in boundary can be identified clearly. Through region of interest selection we can select interested region for better elaboration, labeling and identifying pitfalls in it for further research. The tumor cells and other cells identified & marked based on their shape. The area of affected and non affected grains are calculated and tabulated. The segmented images were analyzed for qualitative and quantitative image analysis for making classification of images as cancer affected or not in terms of percentage. This hybrid technique of image segmentation helps doctors and researchers for diagnosis plan, drug development and delivery plan.

4. Conclusions

Bone microscopic images are normally poor in contrast and noisy. Important features such as stroma, malignant cells, myxoid matrix and blood vessels are not well defined in the image. To process images of such quality significant challenges are to be faced. This paper work presents a new hybrid segmentation technique for qualitative and quantitative osteosarcoma bone image analysis. It focuses on various image processing techniques like histogram generation, image enhancement, magnification, filtering, edge detection, boundary detection, morphological operations, image contour and hybrid segmentation technique. With this system, it is now possible to process a large number of bone microscopic images more effectively for diagnosis, research and also for education purpose. By this new hybrid segmentation technique we can clearly identify and classify the normal cells with the abnormal cells along with the percentage of abnormality.

5. References

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