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" نظام يعتمد على الرؤية بالحاسب لقياس الأشكال البيولوجية "

A Machine Vision System for Measurement of Biological Shapes

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ملخص البحث:

يقدم البحث نظام تم بناؤه لقياس ومقارنة الأشكال البيولوجية واستخدم عمليا لقياس نسبة تحول الفضاريف الى عظام فى العمود الفقرى للفئران النجارب فى مراحل النمو المختلفه. ويتميز النظام المقترح بمرونته وقدرته على إجراء عمليات القياس تحت ظروف إضاءة متغيرة. يتكون النظام المستخدم فى عملية القياس من كاميرة فيديو مركبة على ميكروسكوب ضوئى لتكبير الفقرات العظمية للفئران قبل بدأ القياس و كارت لتحويل الصورة التناظرية المكبرة الى صورة رقمية كما يحتوى على ذاكرة موضعيه لتخزين الصورة الرقمية وكذلك مجموعة من العدسات ذات درجات التكبير المختلفة وحاسب شخصى . يمثل هذا النظام طريقة سهلة وأكثر دقة فى اجراء عمليات القياس التى تعتمد على استخلاص الخطوط الخارجية للفقرات بطريقة الشعاع الفطرى حيث يتم تعقب الإطارين الداخلى والخارجى للفقرة فى خطوة واحدة وذلك بعد تحويل الصورة الرمادية الى صورة ثنائية بطريقة ديناميكية تعتمد على محتوى الصورة ولا تتأثر بتغيرات شدة الاضاءة فى مجال القياس. بعد ذلك يمكن استخلاص سمات من الإطارين الخارجى والداخلى للفقرة ومنها مركز الثقل ، المحيطين الداخلى والخارجى ، مساحة القناه الشوكية ، مسلحة العظام ، أقصى وأدنى قطرين متعامدين يمران بمركز الثقل وأكبر وأصغر نصفى قطرين. وصممت البرامج اللازمة لاجراء عملية القياس بطريقة تسهل على الطبيب التعامل مع النظام . والبحث يوضح نتائج القياسات التى أجريت على ٢٤ فأر من فئران الألبينو البيضاء ذات الأعمار المختلفة والتى قسمت الى ستة مجموعات عمرية كل منها يحتوى على أربعة فئران من نفس السن وكانت الأعمار هى يوم ، عشرة أيام ، عشرون يوما ، ثلاثون يوما وستون يوما . ويتضح من الدراسة أن معدل النمو فى مساحة القناة الشوكية أكبر من معدل نمو العظام بالعمود الفقرى.

ABSTRACT

A machine vision system is developed for measurement and comparison of biological shapes such as the mouse vertebrae. The system is flexible and able to work under varying illumination conditions. The rate of growth and shape change of the vertebrae are evaluated quantitatively by using a new pattern recognition technique. The image segmentation process is made difficult since these images are plagued by poor contrast and dropouts. In this paper, a review of previous work is presented, along with how this problem can be viewed in the context of the computer vision area. The system consisting of a a video camera (Panasonic CCTV), digitizing unit with framestore, optics and a microcomputer measures the dimensions and compares the shapes of complex biological structures.

The image processing system helps automating the measurement problem of such complex shapes and objectifies the measurement results. Reproducibility is an interesting feature of the developed system. An assessment of the measurement accuracy and time duration was undertaken. Different steps in the implementation of this solution are discussed and results are presented.

Although our ultimate goal is automatic measurement of biological shape, attention will be restricted to a fast method for both parallel outlining of the vertebrae and feature extraction. Experimental results on mouse vertebrae are presented to successfully demonstrate the feasibility of the method for low quality images.

1. INTRODUCTION

Most of the findings on mouse vertebrae are based on subjective measurement results driven from the traditional manual measurement techniques. Current studies often require the tedious and time-consuming practice of having trained operators manually measure the vertebrae. This becomes increasingly labor intensive. Furthermore, automated shape measurement would improve the reliability of the quantitative analysis by eliminating the subjectivity of manual measurement. Objectifying the measurement therefore offers many advantages over the classical methods [1]. An extensive image analysis avoids neglecting important information and saves the tremendous effort during manual measurement.

Objective measurement of the cervical vertebrae requires quantitative analysis of the magnified image of the specimen. Quantitative analysis of the vertebral shape often uses shape features such as the enclosed area, perimeter, center of gravity, antroposterior and transverse diameters. This requires that the complete outer-and inner contours of the vertebra be detected from the digitized gray level image. These parameters are significant for analyzing the shape of the vertebrae and their medical significance will be stated in the next sections of the paper.

In section 2 of this paper we describe a computer vision system we have developed for automatic measurement of biological shape. In section 3 we discuss the material used in this investigation and the data acquisition. In section 4, a new method for detecting both inner and outer contours of the vertebra have been implemented. Section 5 is a discussion of the measurement results, its validity and medical significance. Section 6 is a brief summary of the paper.

2. DIGITAL IMAGE ANALYSIS SYSTEM FOR MEDICAL APPLICATIONS

A digital image analysis system (DIAS) developed by the first author for practical analysis of anatomical images will be pre-

sented in this paper. The development of such a system encompasses many aspects to be dealt with:

2.1. Constraints

Automating a measurement task underlies many constraints such as geometry of the set-up, illumination, imaging system architecture, and handling of biological objects. All these aspects interact with each other and have a great impact on the system performance and cost.

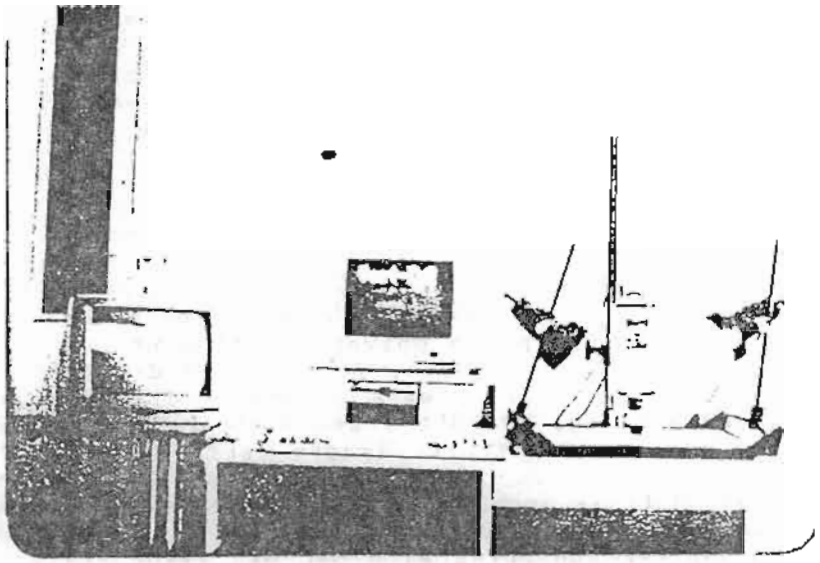

Fig. 1. The digital image analysis system DIAS

2.2. Illumination

The mouse vertebrae are very complex objects, which must be cleaned very well before the measurement process. The connective tissue imposes a heavy duty during the selection of the illumination technique. Our concern is to develop a system which is able to cope with its environment. We have thus deliberately chosen not to change the illumination conditions and to build a system able to work under non-uniform lighting conditions.

2.3. Architecture of the DIAS

The experimental set-up of our system as shown in Fig. 1. includes the following components:

- A microcomputer with 30 Mbyte hard disk for image archiving functions, one 5 1/4" disk drives and DMA interface.
- A CYCLOPE board for digitization and acquisition of video images [2]. An analog video signal is quantized in 8 bits at a rate of 10 MHz. A 512x512x8 bit buffer frame is available on board. This frame buffer can be accessed in two different modes: (1) either by direct access, or (2) by line access allowing one or several lines to be read or written by a single instruction. The CYCLOPE board possesses a 512x512x1 bit graphic plane which enables the text and the graphics to be overlaid in the video image with the required color.
- A CCTV Panasonic camera, model WV-1410/G.
- A group of standard achromatic objectives 4/0.4, 10/0.25 and 40/0.65.
- RGB -14 " monitor for real-time image presentation form either storage or from camera. Images can be displayed in gray value, black and white or in pseudocolors. Graphics can also be presented.
- Illumination and table

3. MATERIALS AND IMAGE ACQUISITION

The mice used in this study were bred in the animal house of the faculty of medicine in the University of Mansoura. Mice were killed at ages of 1 day, 10 days, 20 days, 30 days, 60 days and 90 days. The vertebrae C1-C7 were carefully dissected and then video digitized with the CYCLOPE frame Grabber. The vertebra was placed on the illuminated table. Images captured by a TV camera were fed to the DIAS. For image acquisition, a video Panasonic camera is used. A frame grabber quantizes the original image with 8 bits at 256 gray levels. The image analysis is performed with a 16 bit microcomputer supported with our own image analysis software.

4. IMAGE ANALYSIS

4.1. Ray-by-Ray dynamic thresholding in Area of Interest

There are many applications of image analysis where so much data has to be processed in a limited time that it is vital to attempt some speedup of an algorithm. Sometimes speedup is required for convenience; on other occasions it is needed so that the algorithm will operate in real time. Processing speed in our system is achieved primarily by a massive data reduction which depends critically on two main topics:

a- Processing the Area Of Interest (AOI) only
b- Ray by Ray dynamic thresholding in AOI

Since the images of the vertebra could not be guaranteed to be homogeneously stained it was necessary to implement a dynamic thresholding scheme. The existence of ambiguous regions (due to connective tissue) around the inner and outer borders of the vertebrae presents serious problems for contour searching algo-

rithms. Thresholding the AOI with Ray-by-Ray is an efficient technique. The profile of each radial contour-tracing-ray is analyzed to determine the suitable threshold limit for the gray value pixels along that ray. The threshold is given by:

$$T = (Max(g_i) - Min(g_i))/6 \qquad (1)$$

where g_i is the gray level of the ith pixel of the contour searching ray.

Application of this threshold value for contour tracing in each direction yielded satisfactory results, which could not in an obvious way be improved by a corresponding two dimensional alternative. An initial implementation of a global thresholding scheme was found inadequate.

4.2. Parallel radial contour searching (PRCS)

Both the inner - and outer contours of the vertebrae are extracted by a new technique known as the parallel radial- search (PRS). The vertebral image is quantized at 256 gray levels. The gray-level-image is adaptively thresholded for contour extraction. Local thresholds are determined from analysis of gray level profiles along the contour tracing rays. For radial searching a contour, the center of gravity of the vertebra is first determined by a heuristic technique. This technique is very fast and its accuracy lies within acceptable limits. The operator positions the vertebra under the microscope such that a marker cross on the RGB TV-screen lies within the spinal (neural) canal. The starting point determined by the marker is the initial point for the iterative process during the determination of the center of gravity. After that, an improved Bresenham-algorithm [3] is used for radial searching the contour. We put the most effort into speeding up the basic for-loop for computing the line scanning algorithm, since this is executed once for each pixel scanned. The call to the get_pixel procedure was replaced by in-line code for the get_pixel operation in order to remove the subroutine call overhead from the inner loop.

Radial lines passing through the center of gravity scan the gray level image until an inner contour point is found. The scanning continues with another adaptively determined threshold limit until an outer contour point is found. Radial lines are drawn every one degree resulting in a set of 180 scanning rays. Domain-knowledge based limits are imposed on the search to predict the pixel position in the case of missing edge points. After parallel extraction of both the inner-and outer contours, the contour coordinates are reorganized in two vectors in a clockwise direction.

The ray-by ray contour tracing approach presents limitations for the applicability of the system, but the benefits of significantly simple contour extraction and high speed makes it very

efficient in many applications. The nature of the vertebrae to be measured help avoiding the problems of this approach by changing boundary curvatures.

The contour of the vertebrae is defined by a closed curve and this curve is denoted by the coordinates of the vertices. Contour coordinates are recorded in a systematic clockwise fashion (positive order). The contour must be closed and this implies the first coordinate pair are identical to the last coordinate pair.

4.3. Feature extraction

The next stage of analysis concerns the extraction of the quantitative features from the contour lines. A Turbo-Pascal program library was written for radial search and feature extraction. The shape of the vertebra depends on its growth characteristics. Therefore, the following features are extracted from the traced boundaries:

1. coordinates of the center of gravity (X,Y)
2. inner perimeter
3. outer perimeter
4. spinal canal area
5. bone area (the total integrated optical density)
6. antroposterior and transverse diameters
7. minimal and maximal radii

Statistical features about the contour such as its perimeter, centroid and area are extracted [4]. These features are computed from the contour coordinates according to the following equations:

$$\text{Perimeter} = \Sigma_{J=2}^{N} \sqrt{[C(i)-C(i+2)]^2 + [C(i+1)-C(i+3)]^2} \qquad (2)$$

where
$I=J^2-3$, $J = 2, \ldots, N$ and
C is the vector containing the X and Y boundary coordinates of the contour with N points.

Centroid:

$$X = (\Sigma [(C(i)-C(1))]+[C(i+2)-C(1)]].H)/[3(\text{Area})+C(1)] \qquad (3)$$
$$Y = (\Sigma [(C(i+1)-C(2))]+[C(i+3)-C(2)]].H)/[3(\text{Area})+C(2)] \qquad (4)$$

where
$$H = (C(i+2)-C(1)).(C(i+1)-C(2))-(C(i)-(1)).(C(i+3)-C(2))$$

$$\text{Area} = .5\,|\Sigma_{J=2}^{N} H_J| \qquad (5)$$

5. SYSTEM SOFTWARE

The described system has a very large library of image analysis, presentation, and pattern recognition procedures which offers the user a wide spectrum of possibilities such as:

1. Easy man-machine communication through different capabilities: Turbo-Pascal programming and Menu driven programs.
2. Automatic measurement of complex biological shapes.
3. Interactive measurements
4. Manual measurement with cursor movement
5. Extraction of statistical features
6. Graphic capabilities such as plotting of histograms contours and binary images.
7. Integration of text in Arabic, English or German languages in the image content for presentation and archiving tasks.

6. EXPERIMENTAL RESULTS

We implemented a system for biological shape measurement which incorporates the ideas of the previous sections concerning image analysis. Specifically there are four basic steps:
(a) calibration of the microscopic image
(b) ray-by-ray dynamic thresholding
(c) parallel contour tracing
(d) feature extraction

In order to test the proposed approach with real images a total of 24 albino rats of different ages were used in this investigation . The rats were divided into six age groups. Each group was formed of 4 rats of the same age. The ages taken were 1, 10, 20, 30, 60 and 90 days respectively. The animals were injected intraperitoneally by a 2% neural solution of alizarin red S in a dose of 200 mg/kg body weight. The animals were killed next day and the cervical vertebrae were carefully dissected out. The dimension of the spinal canal during the postnatal development was determined by measuring the dorso-ventral and transverse diameters.

The transverse diameter of the first cervical vertebra was always greater than any of the other cervical vertebrae. The second cervical vertebra had the least transverse diameter which increased gradually from the axis to the seventh cervical vertebrae. The transverse diameter of all cervical vertebrae increased fairly rapidly during the first 30 days after birth, after that there was a slow increase in the transverse diameter (Fig. 2.).

The dorso-ventral diameter decreased from the first to the seventh cervical vertebrae. The decrease from the atlas to the axis vertebrae was marked then it became gradual from the second till the seventh vertebrae. The dorso-ventral diameter of the sixth and seventh vertebrae were nearly equal. There was gradual increase in the dorso-ventral diameter of all the cervical vertebrae till the thirty days of age, then there was slow increase. After sixty days the increase in the dorso-ventral was very slow (Fig. 3.).

During the first 20 days after birth the transverse diameter increased more than the dorso-ventral thus there was decrease in

the dorso-ventral/ transverse diameters ratio. After closure of the dorsal synchondrosis the dorso-ventral diameter increased more than the transverse so the ratio increased gradually from 20 to 60 days of age. During the following thirty days there was very slow increase in the ratio.

The changes of the dorsoventral to transverse diameters ratio reflects changes in the shape of the vertebrae [5]. As regard the atlas, at birth it was nearly circular and became ovoid in shape by 20 days of age. After that the lumen was changed into a pear-shaped form. The shape of the lumen of the axis ranged from almost circular to ovoid. In the following cervical vertebrae the ratio between the dorso-ventral and transverse diameters decreased in a cranio-caudal direction, so the oval shape of the lumen was more prominent in the posterior vertebrae (Fig. 4.).

Fig. 5. shows that the growth of the area of the vertebral foramen in C1 is very slow compared to that of the bone which changes very rapidly.

6. CONCLUSIONS

In this paper we have developed a system which shows the feasibility of both interactive and automated measurement of biological shapes. Our system is able to accurately measure the vertebra in a short time under varying lighting conditions.

A new technique based on radial parallel contour searching for measurement of complex biological shapes was presented. For the results shown, a radial search is used for contour extraction of a gray level image. A reliable way to measure the shape attributes of the vertebrae were developed and the results obtained were impressive. The experiments reported showed that our method holds promise. Several specimens have been measured. The most obvious area of future work is the application of our method to more complex images.

7. REFERENCES

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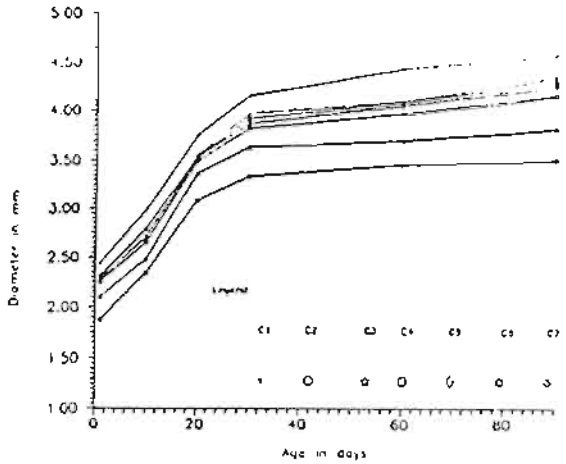

Fig. 2 The means of the maximum transverse diameters of the vertebral framina (c1-c7) at varying ages

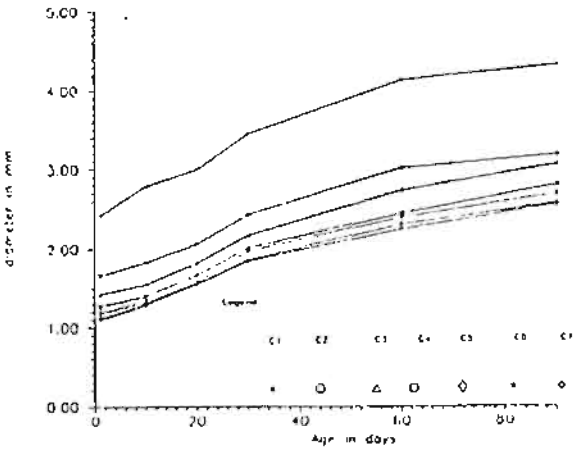

Fig. 3 The means of the midline dorso-ventral diameters of the vertebral foramina (c1-c7) at varying ages.

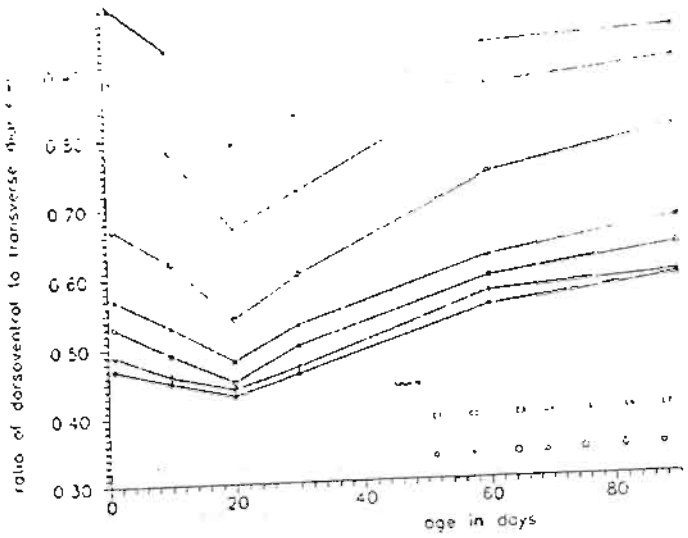

Fig. 4 The dorso-ventral/transverse diameter ratio of c1-c7 at varying ages

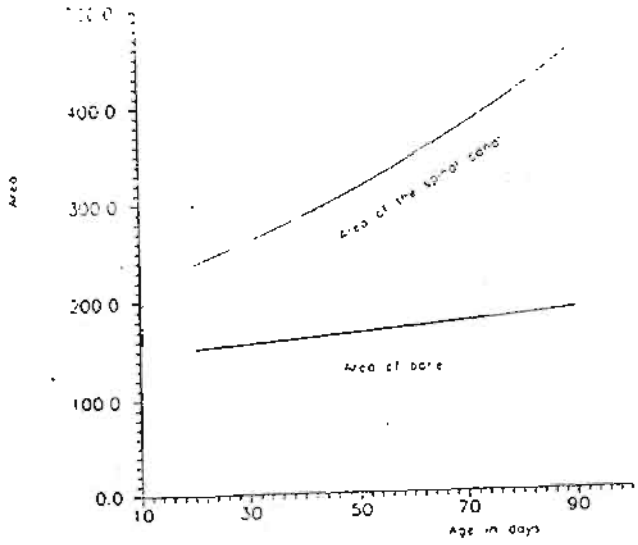

Fig. 5 The area of both bone and spinal canal for c1