Automatic Extraction of the Interest Organization from Full-Color Continuous Images for a Biological Sample

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Abstract: We presented the automatic extraction technique of a biological internal organization from full-color continuous images. It was implemented using the localized homogeneousness of color intensity, and also using the continuity between neighboring images. Moreover, we set the "four-level status value" of area condition as a value showing "area possibility. This played important role of preventing a miss-judgement of area definition. These our approach had a beneficial effect on tracking color and shape change of the interest area in continuous extraction. As a result, we succeeded in extraction of mouse's stomach from continuous 50 images.

1. Introduction

Recently, various methods have been proposed for constructing the computer-based 3D model of biological internal structure. However, the most methods reported to date suffer from poor spatial resolution of original cross-sectional images. Then, there are many researches to obtain the high-resolution cross-sectional image, which can be observed in detail. The Visible Human project, created by the U.S. National Library of Medicine, had delivered high-resolution images. They sliced the human body at 0.3 (μm /slice) intervals and succeeded in obtaining the full-color and high-resolution cross-sectional images. Moreover, our research team developed the new observation system for a biological sample: **3D-ISM** (***3-Dimentional Internal Structure Microscope***)[1][2]. Fig.1 shows the structure drawing of the 3D-ISM. This system provides us the full-color and higher resolution biological cross-sectional images, which sliced at micron order intervals. These images will be suited for constructing of high-quality models.

Yet to date, there is no 3D model reflecting the quality of these images. This is due to the fact that, for the majority of biological internal structure, the boundaries of each organization are not definite. It is difficult to segment each organization of the biological cross-sectional images. Moreover, the quantity of information has increased because of high resolution. According to these, conventional segmentation method is not effective.

So far, we have been examining the segmentation technique for a biological internal structure from full-color continuous cross-section image. The segmentation was implemented using the color information of the biological organization, and using the continuity between neighboring images. We reported of its elementary technique at ITC-CSCC 2001[3]. In that time, the segmentation had been achieved by setting binary value to each pixel. This binary value means the "inside" or the "outside" area of the interest organization. In this report, we set the "four-level status value" to each pixel, as a value showing "area possibility". It is expected that this four status will play a role to prevent a miss-judge of area definition, and a more accurate area judgment can be achieved.

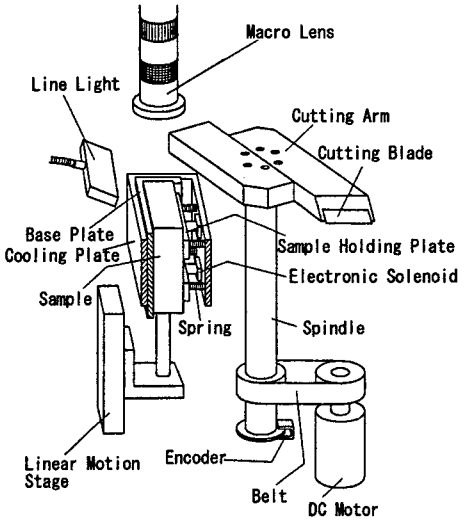

Fig. 1 Structure drawing of 3D-ISM

2. Extraction Method

Each pixel of this image is represented by a set of red, green and blue intensities in RGB color space. In the extraction that we proposed, to segment the organization, we must be define the boundary of the organization. Defining the boundary is accomplished by identifying the difference of the color intensities between neighboring pixels. However, it is difficult to identify its boundary, because there is hardly color change between their organizations. So as to become easy in identifying,we convert the sets of intensity in RGB color-space to another intensity value in HSVcolor-space. It shows the pixel color intensity by H (Hue), S (Saturation) and V (Value). Moreover, It was found through our research that neither H nor S change so much in the same organization.

In this section, we describe the extraction technique, using the intensity differences of H and S between the neighboring pixels, and also between neighboring slices.

2.1 Difference of Intensity in HSV Color-pace

In the HSV color space, a peculiar space is generated with each vector, hue, saturation and value. Fig.2 shows the HSV color-space.

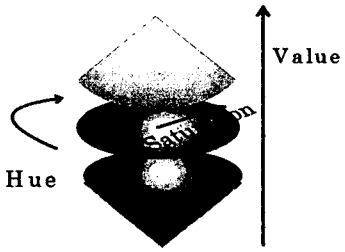

Fig. 2 HSV Color-Sapce

In this space, when deriving the difference of intensity between neighboring pixels, an effective value is not obtained by merely calculating the *Euclidean distance* between them. Therefore, we derive the difference by H (Hue) and S (Saturation) on *polar coordinates*. This difference δ between neighboring pixel is given by

$$y_1 = (s_{P_1}\cos(2\pi h_{P_1}) - s_{P_2}\cos(2\pi h_{P_2}))^2 \quad (1)$$

$$y_2 = (s_{P_1}\sin(2\pi h_{P_1}) - s_{P_2}\sin(2\pi h_{P_2}))^2 \quad (2)$$

$$\delta(P_1,P_2) = \sqrt{y_1 + y_2} \quad (3)$$

where (h_{P_1}, s_{P_1}) and (h_{P_2}, s_{P_2}) denote the intensity of H and S at pixel P_1 and P_2.

2.2 Region Growing by color intensity

Continuous extraction technique can be grouped into the following two steps.

In Flame N (N=0,1,2,3...),

1. Selecting quite the same area as the area selected in Flame N-1.
2. Tracking the moving area between Flame N and N-1.

Under 2 (*tracking the moving area*), we use the difference of intensity between neighboring pixels. This method is implemented by the following five steps.

A) Attention to pixel P_0 (the outside pixel of the boundary of the selected area).

B) Attention to the mask of size (7 × 7 pixel) where P_0 is center.

C) Computing the median intensity of inside and outside area, respectively.
This median intensity M is represented as,

$$M = \begin{cases} (M_{h_{in}}, M_{s_{in}}) & if \quad inside\ area \\ (M_{h_{out}}, M_{s_{out}}) & otherwise \end{cases} \quad (4)$$

D) Computing the difference of intensity between P_0 and M by the method described in section 2.1.
This difference d is defined as follows.

$$d = \begin{cases} d_{in} & (=\delta(P_0, M_{in})) \\ d_{out} & (=\delta(P_0, M_{out})) \end{cases} \quad (5)$$

E) Defining of the boundary by evaluating the homogeneousness of the pixels.
This is defined as

$$P_0 = \begin{cases} inside & if \quad d_{in} \le d_{out} \\ outside & otherwise \end{cases} \quad (6)$$

At all pixels in the vicinity of the boundary, A)~E) is implemented. This enables us to track a new boundary.

2.3 Refining the defined area

The images used in this research are continued at extremely thin intervals. Then, there is hardly change between neighboring two images. We use this feature so as to define the area of the interest organization with more accuracy.

Between the neighboring images, the pixel, which has a specificity of the difference, is reserved in the process of defining the area. To find this specific pixel, we show the intensity differences in Fig.3. This figure shows the distribution of the intensity differences between neighboring images. This difference φ is given by

$$\varphi(x,y,z) = \delta(P_i^N, P_i^{N-1}) \quad (7)$$

$$P_i^N = (x,y,N) \quad (8)$$

$$P_i^{N-1} = (x,y,N-1) \quad (9)$$

where P_i denotes the pixel of the inside area, and N denotes the flame number of cross-sectional image.

We see from Fig. 3 that the average of the intensity is nearly 0.015. Also, this distribution approximates the "*normal distribution*". The specific pixel can be found out as *outlier* of this distribution.

According to above-described process, continuous extraction can be implemented. We describe the flow of this process in the next following section.

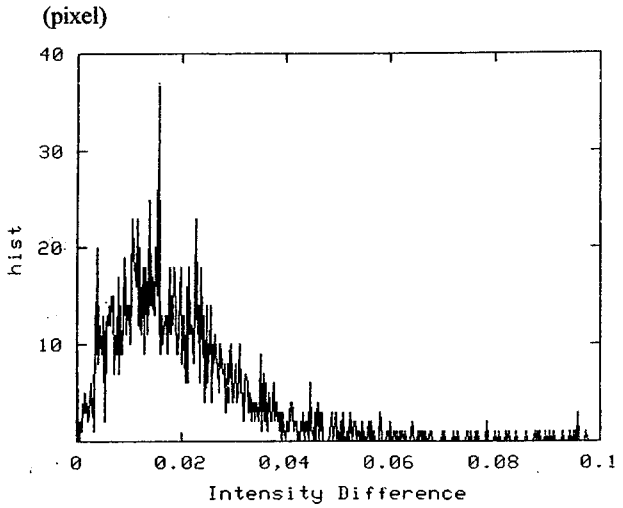

Fig. 3 Distribution of intensity difference

2.4 Defining algorithm of area condition

Fig.4 shows the flow of our proposed technique. Where A ~ D means the "status value". These are classified into the following four.

A : Completely inside area
(of the interest organization)

B : Reservation of defining area as "inside" .

C : Reservation of defining area as "outside"

D : Completely outside area

These value is set to each pixel. In Fig.4 , step (1) means the "Selecting quite the same area as the area selected in Flame N-1" described in section 2.2. At this time, we do not pay attention to D . Next, in step (2), we implement the process of "Region Growing" described in section 2.3. And next, in step (3), we implemented the process of "Refine the area" described in section 2.4.

At the end, we select the pixel which "status value" is A or B as the extracted area. (=inside area of the interest organization).

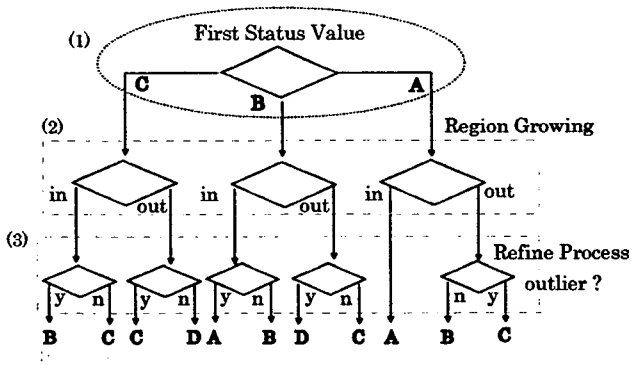

Fig. 4 Defining Algorithm

3. Experimental Result

Our experiment used the cross-sectional image of mouse from **3D-ISM**. The image were digitized at 320 × 240 (pixel μm /pix). From 3033 slices (30 μm /pix) of whole mouse, we used the 150 slices (stomach area). Fig.5 shows the original cross-sectional image. The closed region by block is the target point (=stomach area) in this experiment.

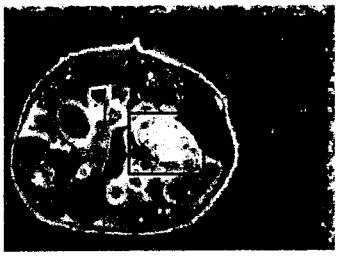

Fig. 5 Original image and Target

Fig.6 shows the extraction results partly. The upper column shows the original images. The lower column shows the result images. At first image, we selected the area by manually. The selected area does not need accuracy. After that, we implemented the segmentation technique that we proposed to define the accurate area of the interest organization.

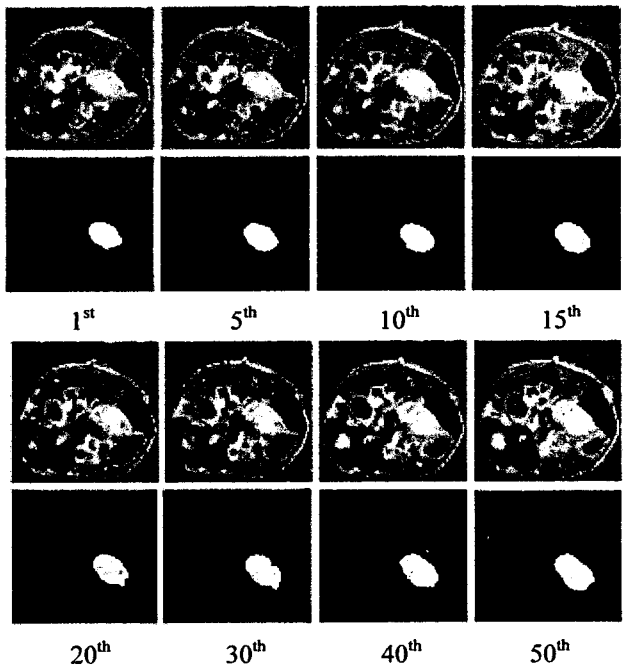

Fig. 6 Original image and Extraction Result

In Fig.6, from 1st slice to 50th slice, there is slightly area movement and shape change of the target area. We see from this figure that our proposed technique had been able to track their change. Moreover, we see that accuracy is automatically regained in the 40th slice though it was lost once in passing 20th.

Table.1 shows the correct answer rate. We selected the target area in five times by manually. And its majority was

set as a correct answer area. In this table, ***Answer*** shows the number of pixel inside/outside the target area by this majority. ***Result*** shows the number of the selected pixel inside/outside ***Answer***'s area by our proposed technique. In this case, Status value *A* and *B* were counted as inside the target area. On the other hand, *C* and *D* were counted as outside the target area. ***E*** shows the number of the error pixel.

Table. 1 Correct Answer Rate

Flame	*inside area*			*outside area*		
N.o.	*Result/Ans.*	*E*	*Rate %*	*Result/Ans.*	*E*	*Rate %*
1st	1451/1493	42	97.19	75263/75307	44	99.94
5th	1606/1672	66	96.05	75101/75128	27	99.96
10th	1663/1726	63	96.35	75015/75074	52	99.92
15th	1702/1738	36	97.93	74959/75062	103	99.86
20th	1847/1916	69	96.4	74815/74884	69	99.91
30th	1975/2093	118	94.36	74628/74707	79	99.89
40th	2097/2173	76	96.5	74545/74627	82	99.89
50th	2254/2329	75	96.78	74372/74471	99	99.87

Generally, the error range within 5 % is allowed in biological experiment. We also see from Table 1 that accuracy is regained in the 40th slice though the rate was below 95 % once in 30th. The size of the target changed from 1493 pixels to 2329 pixels. Our proposed technique also had been able to track this change.

This result clearly shows that our proposed method is useful for automatic extraction of full-color biological images.

5. Conclution

Computer based 3D model of biological internal structure is strongly hoped. It is necessary to extract internal organization from original continuous cross-sectional images. Then, we proposed the extraction technique from full-color biological images. This technique used the localized homogeneousness of color intensity, and also using the continuity between neighboring images. However, if the extraction had used only them, it was not robust extraction. We also proposed to set the "four-level status value" to each pixel as the value showing "area possibility". This played an important role in preventing proliferation of the miss-judgment during continuous extraction. The two middle status value *B* and *C*, as reservation of definition, prevented that the miss-judgments once negatively affected the extraction from a continuous images.

On the other hand, when the process of region growing, using the median value brought us an effective result. Before using the median value slice in our past experiments, the correct answer rate had been below 90 % in 50th. After using the median value, the correct answer rate kept over 95 %. As a result, we succeeded in more accurate extraction the area of biological organization from the full-color continuous images.

In future work, we will aim at the set the multistep "area status value". It is expected that we will be achieved to extract with more accuracy from more number of image.

Acknowledgements

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References

[1] Hideo YOKOTA, et al., "3Dimentional Digitizing for the Biological Sample Using a 3-Dimensional Internal Structure Microscope", Computational Biomechanics, pp. 69-79, May, 2000, RIKEN SYMPOSIUM.

[2] Hideo YOKOTA, et al., "Observation and measurement of freezed biological sample by 3-Dimentional Internal Structure Microscope (3D-ISM)", Cryobiol. And Cryotech., 44, pp. 1-9, 1998.

[3] Hideo YOKOTA, Satoko TAKEMOTO, et al., "Extract Distinction of a Certain Region of Full-Color Continuous image for a Biological Sample", ITC-CSCC 2001, Vol. 2, pp.1103-1106, 2001.

[4] Koichi SANO, et al, "Extraction of Soft Tissues by Region Growing and 3D Display", MEDICAL IMAGING TECHONOLOGY, Vol.13, No.3, pp.189-194, May, 1995.

[5] Clina Imelinska, et al, "Semi-automated color segmentation of anatomical tissue", Computerized Medecal Imaging and Graphics, Vol. 20, pp. 173-180, 2000.

[6] D.Androutsos, et al, "A Novel Vector-Based Approach to Color Image Retrieval Using a Vector Angular-Based Distance Measure", Computer Vision and Image Understanding, Vol. 75, Nos. 1/2, July/August, pp.46-58, 1999.

[7] Taizo ANAN, et al, "Stochastic Region Swelling-Shrinking Model for Region Extraction of Image", The journal of The Transactions of the Institute of Electronics, Vol. J80-D-2, No. 1, pp. 156-165, 1997.

[8] Nobutoshi Yamazaki, et al, "Segmentation of the Tissues from MR images Using Basic Anatomical Information", The journal of Transactions of the Society of Instrument and Control Engineers, Vol. 30, No. 11, pp. 1280-1286, 1994.