AUTOMATIC EXTRACTION OF THE INTEREST ORGANIZATION OF A BIOLOGICAL SAMPLE FROM FULL-COLOR CONTINUOUS IMAGES

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Abstract. *We present a technique for automatic extraction of the internal organization of a biological sample from full-color continuous images by 3D-ISM. Conventional extraction, as typified by the Region Growing method, require various arbitrary conditions to be defined. On the other hand, this new technique extracts the information by applying the localized homogeneity of color intensity in HSV color space and the continuity between neighboring images. The technique can be applied without the need for arbitrary conditions or an anatomical knowledge base. Application examples are presented of extracting accurate information for the whole human eye from over 840 continuous images, and the mouse' stomach from about 140 continuous images.*

1. INTRODUCTION

A dynamic computer model of the human body is currently being constructed, and configuration information is indispensable to achieve this. Our research team is attempting to get this information by using full-color continuous cross-sectional images from 3D-ISM. It seems likely that detailed configuration information could be obtained from full-color cross-sectional images of the internal organization of a biological sample. Research using the dataset from the Visible Human Project (VHP) had so far been reported as color configuration information. This project, created by the U.S. National Library of Medicine, sliced the human body at intervals of 1 mm/slice and created an image from each cross section. The data set comprise full-color (RGB) images and matching images by X-ray CT. These images have one-to-one correspondence to each other and were created from 2000 slices [4].

The extraction method by Region Growing with color information has been proposed for this VHP data set [5]. However, this method needs an arbitrary threshold of RGB value, as well as at end condition, and geometric modeling with the help of an anatomical knowledge base has been applied to each extracted region to provide the structural outline. It is reasonable to speculate that this proposed method does not deliver an accurate result.

A method has also been proposed that involves only mapping the color data to the images by X-ray CT [6], but only configuration information of the surface could be acquired by this method. Furthermore, the internal organization that can be used is limited because of the poor spatial resolution of X-ray CT, and it is difficult to align positions between full-color and X-ray CT images.

Our proposal of the automatic extraction method from full-color biological images requires

no arbitrary threshold, no position adjustment, and no anatomical knowledge base, and exploits the advantage of images from 3D-ISM.

2. Extraction Method

Each pixel of the image from 3D-ISM is represented by a set of red, green and blue intensity in RGB color space. To segment each internal organization by our proposed extraction method, we must first define the boundary of the organization. This is accomplished by identifying the difference in the color intensity between neighboring pixels. However, it is often difficult to identify the boundary when there is hardly any color change between adjacent organizations, we convert the sets of color-intensity values in RGB color space to values in HSV color space. This type of color space shows the pixel color intensity by hue (H), saturation (S) and value (V). Fig.1 shows the color distribution of a slice in both types of color space, in which the dark areas represent the inside, and the pale areas represent the outside the organization. Although the three intensity values are not equivalent in HSV color space, we apply only the similarity of value within the same organization. It is clearly easier to segment an organization in HSV color space than in RGB color space, and it was found through this study that neither H nor S changed much in the same organization.

(a) RGB Color-space   (b) HSV Color-space
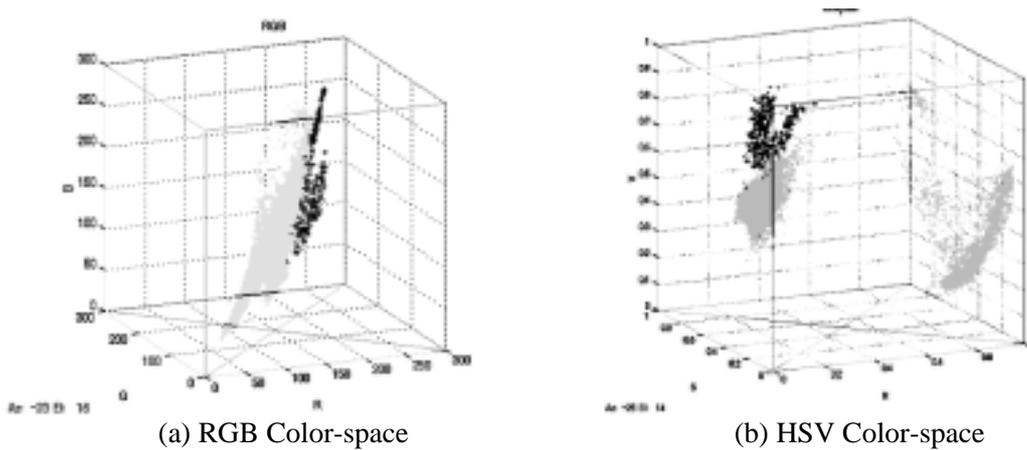
Fig.   1 Distribution of the arbitrary area

A peculiar area in HSV color space is generated with each vector for hue, saturation and value, as shown in Fig. 2. An effective value is not obtained by merely calculating the *Euclidean* distance between neighboring pixels in HSV color space when deriving the difference in intensity. This difference is derived from the hue (H) and saturation (S) in *polar coordinates*. The difference (δ) between neighboring pixels is given by

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

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

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

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.

Fig. 3 shows the intensity values of H and S in polar coordinates.
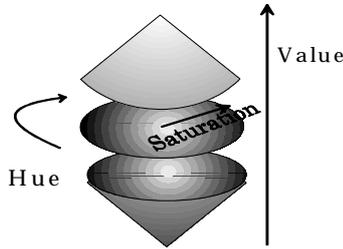
Fig. 2 HSV color space
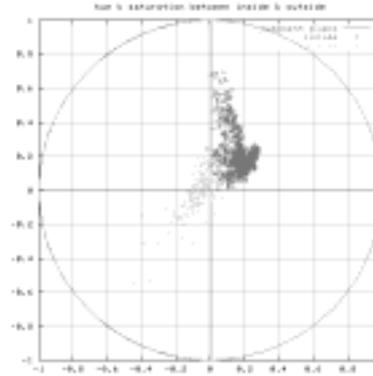
Fig. 3 Distribution of H and S intensity in polar coordinates

The dark points in Fig. 3 show the intensity value inside the arbitrary organization, while the pale points show part of the outside. This figure demonstrates that it is possible to segment between the inside and outside of an organization by computing the intensity difference of H and S in polar coordinates.

The Region Growing method is generally used for extracting from continuous images [7-8] requires an arbitrary condition to be set. On the other hand, our proposed method requires no arbitrary condition to achieve robust extraction. This progressive Region Growing method follows two steps:

For frame N (N=0,1,2,3...),
1. Selecting the same area as that selected in frame N-1.
2. Tracking the changing area between frames N and N-1.

When tracking the changing area in step 2, we use the difference in intensity between neighboring pixels that is achieved in the following five stages:
A) Attention to pixel P_0 (the outside pixel on the boundary of the selected area) in frame N.
B) Attention to the local area size ($7 \times 7 \times 4$ pixels) where P_0 represents the center point of the local area in flame N. Frames N-3, N-2 and N-1 refer to the upper neighboring images on frame N.
C) Computing the respective median intensity inside and outside the local area that were set in process stage B. This median intensity M is represented by

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

D) Computing the difference in intensity between P_0 and M by the method described by equations 1– 3.
This difference d is defined as follows:

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

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

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

Stages A–E are implemented at all pixels in the vicinity of the boundary and the process repeated until automatically stopping.   This is done for all slices so that a new boundary can be tracked.

3. Experimental Results

This extraction method was tested on the cross-sectional images of a human eye   (Fig. 4) and mouse (Fig. 5) from 3D-ISM. The images were digitized at 320 × 240 pixels/slice.   In the case of the human eye, we used 840 continuous slices ($30 \, \mu m$/pixel) for extracting the whole eye (**Experiment 1**), and 150 continuous slices for extracting the lens (**Experiment 3**).   In the case of the mouse, 3033 slices ($30 \, \mu m$/pixel) were used for the whole mouse, and 600 slices for the stomach (**Experiment 2**).   The boxed area in Fig. 4 shows the target point (lens), and in Fig. 5, the stomach.
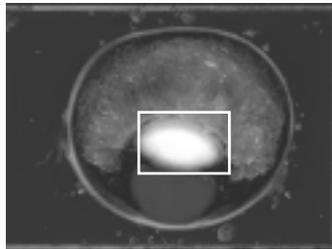
Fig.   4 Cross-sectional image of the human eye   **Fig.   5 Cross-sectional image of the mouse**

Before starting the extraction process, the area to be extracted is manually selected.   This area does not need to be selected with any accuracy.   The extraction method is then implemented and the area of interest accurately defined.   Figs. 6–8 show some of the extraction results. In Fig.6 and Fig. 7, the upper row showing the original images, and the lower row, the result images.

Experiment 1
When extracting the whole human eye, it was achieved by extracting the outside area of the whole eye. Outside area filled with embedding agent, and its color was only blue. In the case of one color, it seemed to be able to extract it by threshold. However, there is a color change under an actual environment, and it is difficult to extract by one threshold.
   On the other hand, because our extraction method does not need the threshold and can cope

with change between cross-sectional images, it seems to be possible to extract its area through all images. In Fig.6, the area of the whole human eye was successfully extracted from the all-continuous images. Fig.9 shows the constructed 3-D model of the whole eye by using this extraction result.

| 20th | 100th | 200th | 300th | 400th | 500th | 600th | 700th | 840th |
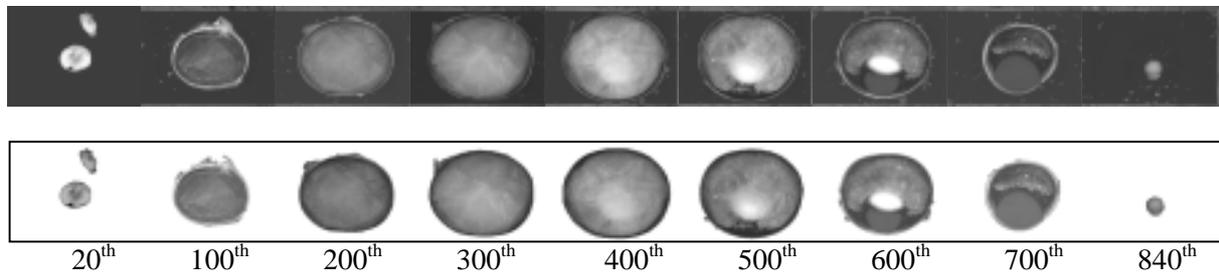
Fig. 6   Results of Experiment 1

Experiment 2
We extracted only the stomach area from mouse' cross sectional images. The results shown in Fig. 7 indicate a slight movement and shape change of the target area between the 1st slice and 150th slice. It can be seen that our proposed technique had been able to track this change up to the 100th slice, although it was not sufficiently accurate for the 140th slice and could hardly extract the stomach area after the 140th slice.

| 1st | 5th | 10th | 30th | 50th | 80th | 100th | 140th |
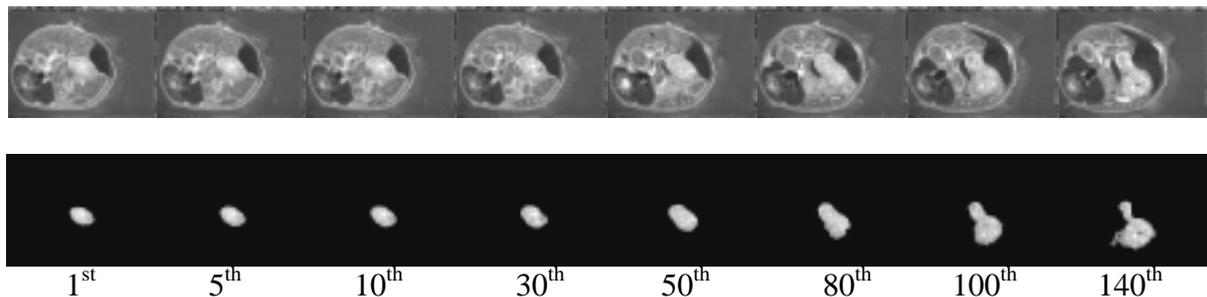
Fig. 7   Results of Experiment 2

Experiment 3
We extracted only the lens area from continuous 120 images of the human eye. The results shown in Fig. 8 indicate that it was possible to extract the rough area of lens through almost all images, although it is difficult to claim sufficient accuracy for the extraction procedure. Fig. 10 shows the 3-D model of the lens that was constructed from the results. It is apparent that separation from adjacent areas was not completely successful, and that improvement of the procedure is necessary.

| 1st | 20th | 40th | 60th | 80th | 100th | 120th |
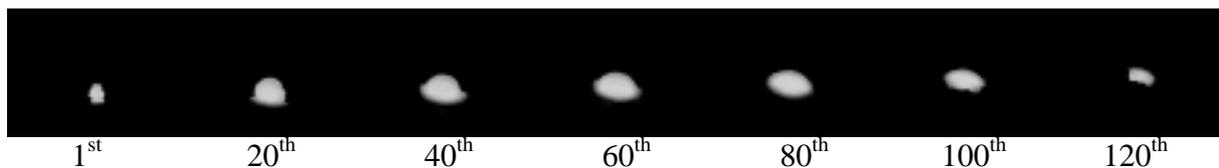
Fig. 8   Results of Experiment 3
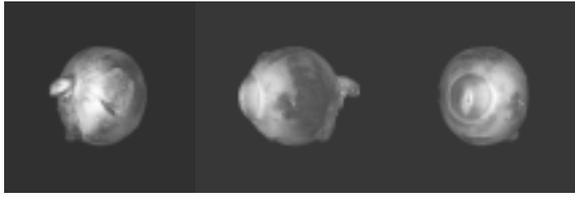
Fig. 9   3-D model of the whole huma n eye
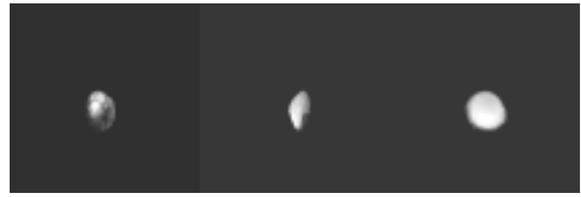
Fig. 10   3-D model of the lens

5.   Conclusions

There is a need for computer-based 3-D models of the internal organization of biological samples.   This requires extracting the configuration information from original images, and the proposed extraction method can be applied to full-color biological images created by 3D-ISM.

This method applies the localized homogeneity of color intensity in HSV color space, as well as the continuity between neighboring images.   Using the color intensity expressed by H and S in polar coordinates makes it easy to separate the selected area from other areas. We achieved the progressive Region Growing method to track the moving area in extraction. It is remarkable progressive point that these methods did not need the arbitrary condition. As a result, we succeeded in simple organization extraction from full-color continuous biological images.

As another approach, we are proposing method of "set the area possibility" to each pixel. This area possibility is also set by using the homogeneousness of color intensity in HSV color-space, and by using the continuity between neighboring images. This method was reported at JAMIT Annual Meeting in 2002 [9].

In future work, we will aim at using the ICA (Independent Component Analysis), when we segment the inside and outside area using H and S value.

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