Development of color micro optical-CT: Evaluation using phantom and biological samples

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ABSTRACT

Micro-optical computed tomography (MOCT) is a method for performing image reconstruction using microscopic images to obtain tomographic images of small samples. Compared with conventional observation methods, it offers the possibility to obtain tomograpic images without distortion, and create three-dimensional images. However, MOCT system which developed previously outputs monochrome images, while useful color information could not be obtained from the analysis of the sample. Therefore, we focused on the features that simplify the wavelength measurement of visible light, and developed a color MOCT system that can obtain color tomographic images. In this study, we acquired tomographic images of phantom and biological samples, and evaluated its usefulness. In this system, a digital single-lens reflex camera was used as a detector that was connected to a stereoscopic microscope, and projection images were obtained by rotating the sample. The sample was fixed in the test tube by carrageenan. The projection images were obtained from various projection angles followed by decomposing the R, G and B components. Subsequently, we performed image reconstruction for each component using filtered back projection. Finally, color tomographic image was obtained by combining the three-color component images. In the experiments, we scanned a color phantom and biological samples and evaluated the color and shape reproducibility. As a result, it was found that the color and shape of the tomographic images were similar to those of the samples. These results indicate that the proposed system may be useful to obtain the three-dimensional color structure of biological samples.

Keywords: CT, Optical, Image reconstruction, Color, microscope

1. INTRODUCTION

Optical computed tomography (CT) is a system to obtain a tomographic image using visible ray and near infrared ray unlike X-ray CT. Therefore, it has a feature that there is no radiation exposure. Optical CT is expected to be applied in various fields. As application examples, there is biometric optical CT[1], optical CT for analysis of gel dosimeter[2-4], and micro optical CT. We focus on the micro optical CT (MOCT). MOCT is a method for performing image reconstruction using microscopic images to obtain tomographic images of small samples. Compared with conventional observation method, it offers the possibility to obtain tomographic images without distortion, and create three-dimensional images. Therefore it is expected to be a new tomographic imaging technique[5-7]. However, MOCT system which developed previously outputs monochrome images, while useful color information could not be obtained from the analysis of the sample. Therefore, we focused on the features that simplify the wavelength measurement of visible light, and developed a color MOCT system that can obtain color tomographic images. In this study, we acquired tomographic images of phantom and biological samples using developed system, and evaluated its usefulness.

2. METHODS

Experimental system

In order to colorize MOCT, we developed simple experimental system. Appearance and configuration of a color micro optical-CT system is shown in Fig.1. In this system, digital single lens reflex camera (Canon EOS 60D) used as a detector was connected to a stereoscopic microscope (Carton, DSZT-44FT). Magnification of the microscope can range from x10 to x44, and total pixel resolution of camera connected to the microscope can range from 0.7 μm to 3.1 μm by

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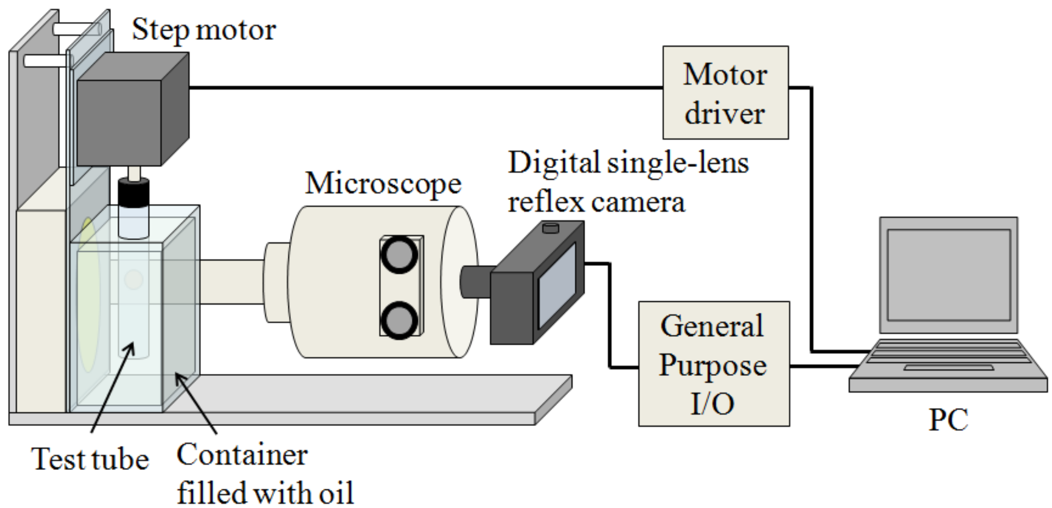
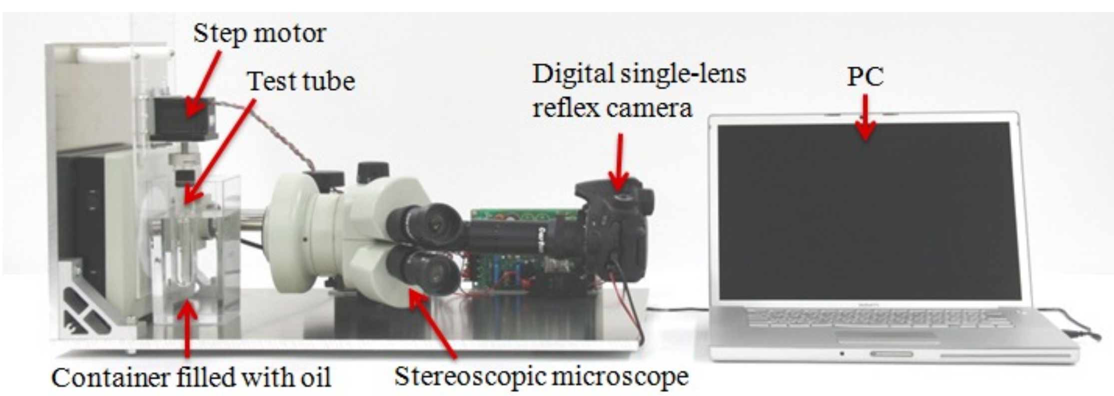
changing magnification. The projection images were obtained from various projection angles rotated by step motor (Sanyo denki, 103F7851). The sample was fixed in the test tube by carrageenan. Here, in order to reduce refraction of light, test tube was rotated in a container filled with oil. Therefore, the microscope was used to be laid sideways.

Color image reconstruction

Figure 2 shows the flow chart of image reconstruction for obtaining a tomographic image with the color information. First, a color sinogram is created using the projection image obtained by the experimental equipment. And then, a color sinogram is decomposed into RGB color components; image reconstruction is performed using each sinogram. As for the reconstruction algorithm, we introduced filtered back-projection for two-dimensional imaging, and FDK method [8] for three-dimensional imaging. Finally, color tomographic image is obtained by combining the three reconstructed images. Here, these processing were automated by in-house program that was developed by Microsoft Visual C ++ 2010.



(a) Configuration of the experimental system



(b) Appearance of the experimental system

Fig.1 Schematic of color MOCT

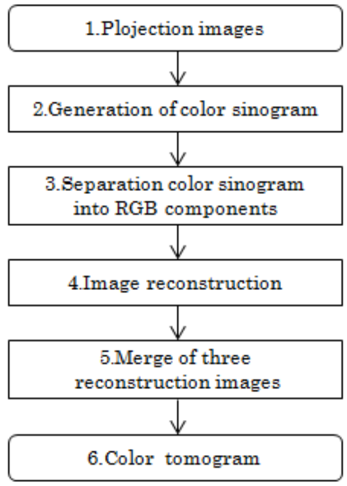


Fig.2 Flow of color image reconstruction

3. EXPERIMENTS

Evaluation methods

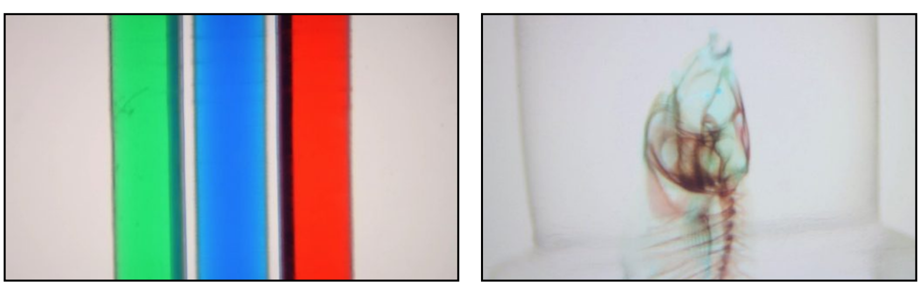
In the experiments, we evaluated the reproducibility of the color using a phantom, and the actual efficacy using biological sample.

(1) Color reproducibility

We first evaluated whether developed experimental system could obtain tomographic images including color information. For evaluation, we prepared the color phantom (Fig.3 (a)). It was acrylic rod in which three thin holes was created (diameter: 2 mm). These holes were filled with water colored by food coloring (red, green, and blue). After obtaining the tomographic image of the phantom, the color reproducibility was evaluated by comparing the hue of the projection image. Imaging condition was projection pitch of 1 degree, and number of projection of 360.

(2) Biological sample

The second point was whether it was able to obtain tomographic images of sample that has complex internal structure. For evaluation, we prepared the transparent specimen of killifish for biological sample (Fig.3 (b)). It was dyed bone to red and cartilage to blue. Imaging condition was projection pitch of 1 degree, and number of projection of 360.



(a) (b)

Fig.3 Projection images

(a) Color phantom: It was acrylic rod which thin holes were created (diameter: 2 mm).
Holes were filled with water colored by food coloring (red, green, and blue).

(b) Biological sample: It was transparent specimen of killifish which was dyed bone to red and cartilage to blue.

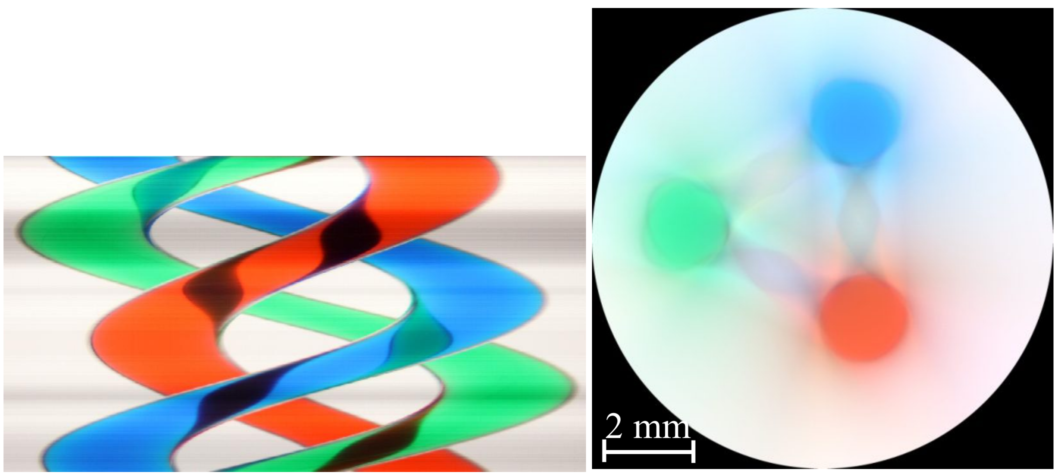
Results and discussion

(1) Color reproducibility

Figure 4 (a) shows sinogram of color phantom obtained by proposed system. And Fig.4 (b) shows 2D color tomographic image of color phantom. The shape and position of the holes could be identified. However, artifacts occurred along the tangent of the test tube containing the dye portion away from the test tube center. The refraction of light is considered as the main cause of artifacts. As a result of color evaluation, the errors of hue value between projection image and tomographic image were 1.02 (red-channel), 3.94 (green-channel), and 3.03 (blue-channel) as shown in Table.1. Therefore we found that the color of the tomographic image was close to that of the color phantom. From these results, we confirmed that developed experimental system was able to obtain a color tomographic image.

(2) Biological sample

Figure 5(a) shows 3D color tomographic image of biological sample. From the results, it was found that we could obtain a tomographic image of sample even if sample had complex internal structure. By displaying a volume image as shown in Fig. 5(b), the shape of the killifish's head was reproduced, and understanding of the structure of cartilage and bone became possible. These results indicate that our color micro optical-CT may be useful for the color analysis of small samples.

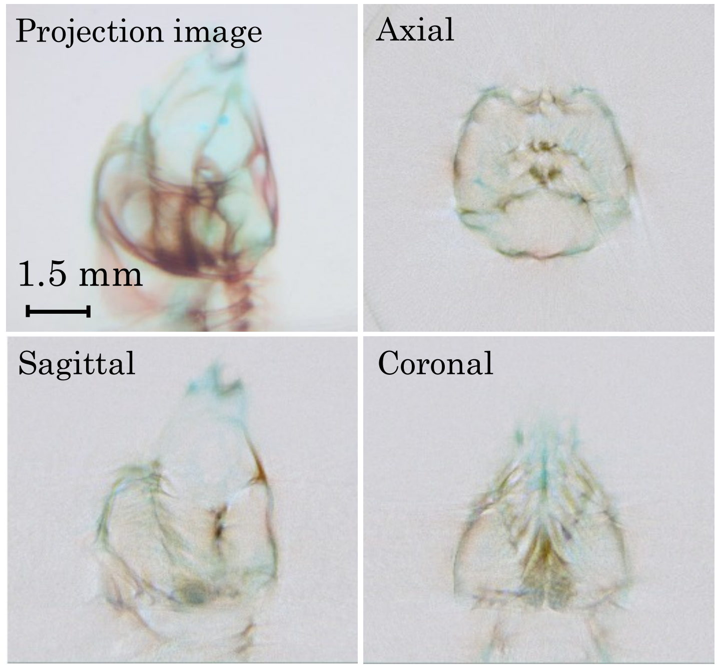


(a) (b)

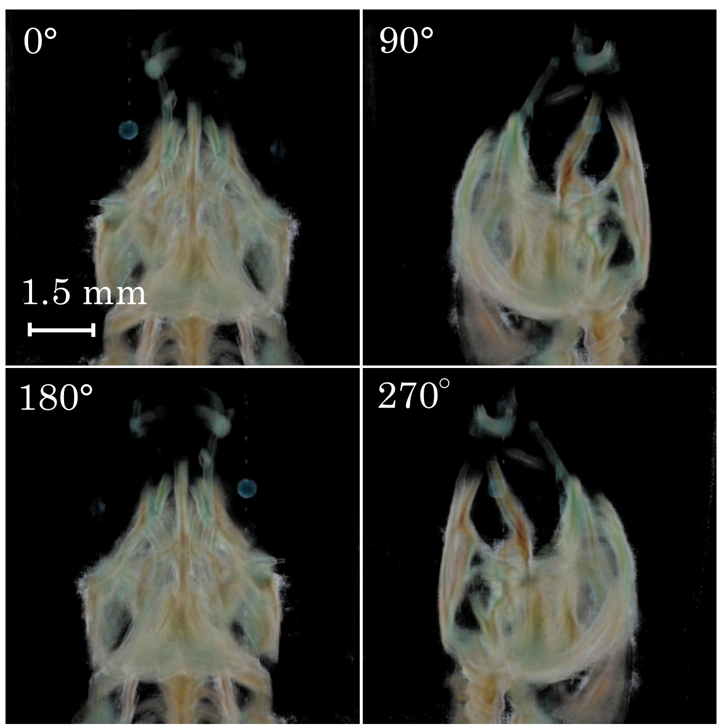
Fig.4 Result of color phantom

(a) Sinogram: In the image, horizontal direction indicates the detector positions and vertical direction indicates the rotation angle.

(b) 2D tomographic image



(a)



(b)

Fig.5 Results of biological sample

(a) MPR images: The upper right corner of the image shows the axial cross-section of biological sample, the lower left shows sagittal cross section, and the lower right shows coronal cross section.

(b) VR images: These captured images are volume rendered images of biological sample rotated every 90 degree.

Table 1 Color evaluation result

Color channel	Red	Green	Blue
Hue of the projection image [degree]	12.34	151.71	209.05
Hue of the tomographic image [degree]	11.32	155.65	206.02
Error [degree]	1.02	3.94	3.03

4. CONCLUSIONS

In this study, we aimed to develop imaging technique of a color MOCT and evaluate usefulness. In the experiments, we evaluated the effectiveness of this method by use of color phantom and biological samples. As a result of evaluation of the hue using color phantom, we found that the hue values of the tomographic image were close to those of the color phantom. Furthermore, in the evaluation using biological samples, understanding of the structure of cartilage and bone became possible by displaying a volume rendered image. These results indicate that our color MOCT may be useful for the color analysis of small samples.

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