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A new light-field microscope system for high-resolution 3D bio-imaging

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With the recent development of systems biology, which views living organisms as "systems" consisting of small interacting elements such as molecules, cells, and even individuals, three-dimensional observation of biological phenomena defined on the millisecond scale is required. Therefore, a three-dimensional observation technique with both high spatial and temporal resolution to observe the behavior of each element in three-dimensional space is indispensable. Scanning microscopes such as confocal microscopes and light-sheet microscopes, which are generally used for three-dimensional observation, have a temporal resolution of about 30 fps at the fastest, resulting in a time difference between the start and end planes of scanning when performing spatial observation. Therefore, we focused on light-field microscopes that can acquire images in a single shot without scanning. However, light-field microscopes have the disadvantage of low spatial resolution. In addition, in order to clarify the correlation between neural firing patterns and behavior in methods such as optogenetics, where activity patterns are controlled by optical manipulation, feedback control is required to measure the activity of the neural network and perform optical manipulation of specific neurons according to the activity state of the network. In the case that such a control is required, the activity of the neural network must be measured. A three-dimensional observation technique with real-time performance is required when such control is needed. In light of the above, a light field microscope with high spatial and temporal resolution and real-time performance is desired. We have been developed a new light-field microscope for real-time observation of nematode cells. C. elegans has a transparent body, which allows observation of all cells from outside the body. C. elegans has been shown to have neural mechanisms similar to those of mammals, and the elucidation of these mechanisms has potential applications in the medical field. Conventional light-field microscopy has sufficient temporal resolution, but spatial resolution is insufficient for observation of C. elegans. There are two conventional light-field systems. One is called One F system, the other is called BA system. The One F system, which has an image sensor positioned at the focal plane of a microlens array (MLA) has a deep depth of field but a low xy resolution. The BA system can be constructed by placing the MLA and image sensor at the focal plane of the lens (MLA), and has the feature of high xy resolution but shallow depth of field. We have developed a new system, the One F-BA composite system that combines these two systems. When the sample is in the focal plane of the objective lens, the system behaves like the One F system. When the sample is out of the focal plane, the system behaves like the BA system, providing high xy resolution and a deep depth of field. With the proposed method, xy resolution of 2-5[μm] and depth of field of 70[μm] were achieved, enabling real-time observation of nematode cells.

1. Introduction

With the recent development of systems biology, which views living organisms as "systems" consisting of small interacting elements such as molecules, cells, and even individuals, three-dimensional observation of biological phenomena defined on the millisecond scale is required. Therefore, a three-dimensional observation technique with both high spatial and temporal resolution to observe the behavior of each element in three-dimensional space is indispensable. Scanning microscopes such as confocal microscopes and light-sheet microscopes, which are generally used for three-dimensional observation, have a temporal resolution of about 30 fps at the fastest, resulting in a time difference between the start and end planes of scanning when performing spatial observation. Therefore, we focused on light-field microscopes that can acquire images in a single shot without scanning. However, light-field microscopes have the disadvantage of low spatial resolution. In addition, in order to clarify the correlation between neural firing patterns and behavior in methods such as optogenetics, where activity patterns are controlled by optical manipulation, feedback control is required to measure the activity of the neural network and perform optical manipulation of specific neurons according to the activity state of the network. In the case that such a control is required, the activity

of the neural network must be measured. A three-dimensional observation technique with real-time performance is required when such control is needed. In light of the above, a light field microscope with high spatial and temporal resolution and real-time performance is desired.

2. Methodology

2.1 Setup of the light-field microscope

Figure 1 shows the experimental setup. By adjusting the arrangement of the camera, relay lens, and MLA, three different systems can be constructed: the One F system, the BA system, and the One F-BA composite system which is the proposed system in this study. The features and construction methods of these systems are described below. In the preparation stage, the MLA is placed at the focal plane of the imaging lens and the relay lens.

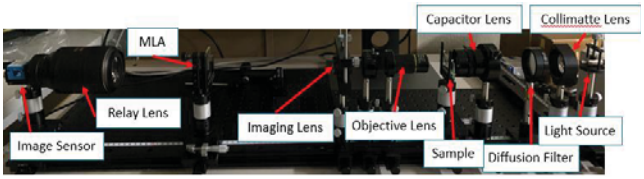

Fig. 1 Experimental Setup of the light-field microscope

2.2 One F system

A schematic of the One F system[1] is shown in Figure 2. The One F system can be constructed by moving the camera away from the MLA by the focal length of the MLA. In this system, the light emitted from the microlens reaches the image sensor without forming an image, resulting in a blurred image. However, since refocusing is created using one pixel at a time in the microlens image, the aperture is smaller and the depth of field is greater.

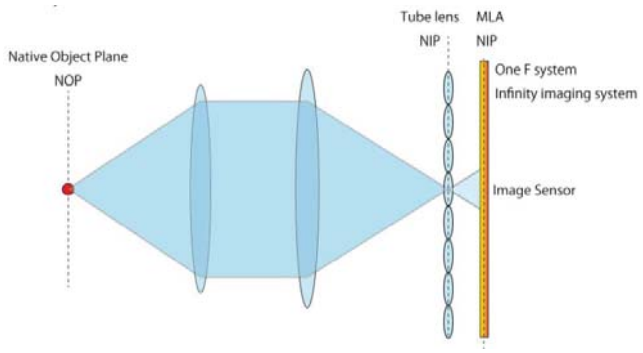

Fig. 2 Schematic diagram of the One F system

2.3 BA system

A schematic diagram of the BA system[2] is shown in Figure 3. The BA system can be constructed by placing the MLA and camera in positions where the lens formula is valid. Let a be the distance between the focal plane of the MLA and the imaging lens, and let b be the distance between the focal plane of the MLA and the relay lens. This system has high resolution because the light emitted from the ML reaches the image sensor through image formation. However, when the sample moves away from the focal plane of the objective lens, image formation occurs behind the image sensor, resulting in out-of-focus images that cannot be refocused. Therefore, the BA system has the disadvantage of a shallow depth of field.

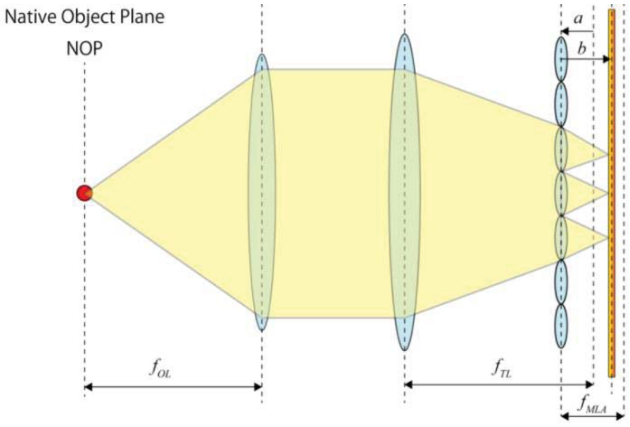

Fig. 3 Schematic diagram of the BA system

2.4 One F-BA composite system

One F-BA composite system is our proposed system. The schematic diagram is shown in Figure 4, and the system can be constructed by placing the MLA and camera at positions where a=0 and b=0.6-0.8f. The system behaves like a One F system when the sample is at the boundary plane of the objective lens and like a BA system when the sample is away from the boundary plane. Therefore, this system provides high resolution like the BA system and deep depth of field like the One F system.

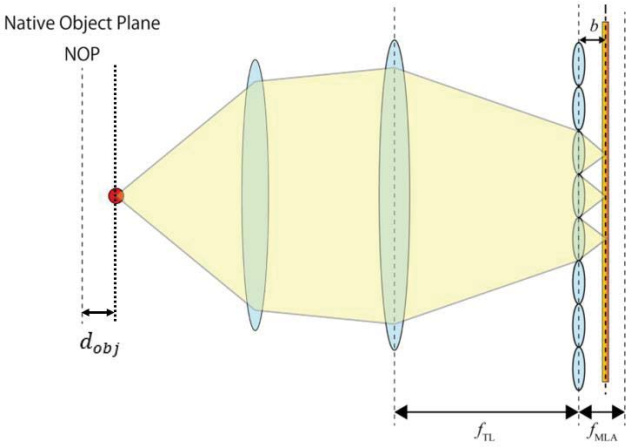

Fig. 4 Schematic diagram of the One F-BA composite system

3. Result and discussion

As shown in Figure 4, let d_{obj} be the distance between the focal plane of the objective lens and the test chart.Microlens images of the test chart were acquired by shifting the test chart by d_{obj} using the One F-BA composite system. The d_{obj} was acquired in 10[μm] increments from 0[μm] to the xy resolution of 5[μm]. The acquired

microlens images were refocused by post-processing, and the results were used to evaluate the resolution. As an example, the microlens image for a $d_{obj}$of 30 [μm] is shown in Figure 5 and the refocusing result is shown in Figure 6. The resolution is evaluated for each d_{obj}, and the xy-resolution is defined as the limit at which five bars can be distinguished. In the example shown in Figure 6, the resolution is 2[μm] because 5 bars can be identified up to the fourth 2[μm] from the top. The evaluation results of xy resolution are shown in Figure 7. From Figure 7, a depth of field of 20[μm] was obtained for xy resolution of 2[μm], a depth of field of 40[μm] for xy resolution of 3[μm], a depth of field of 60[μm] for xy resolution of 4[μm], and a depth of field of 70[μm] for xy resolution of 5[μm]

Fig. 5 Microlens image with d_{obj}=30

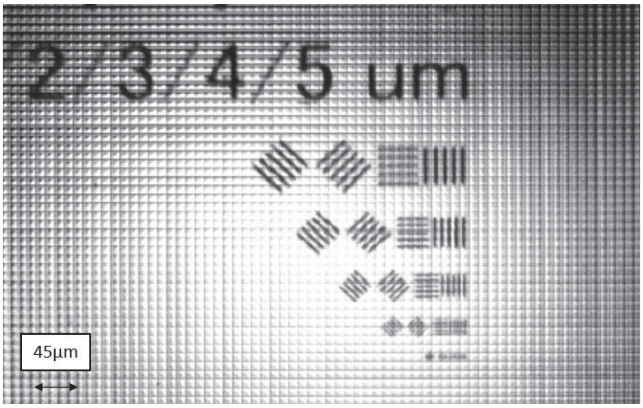

Fig. 6 Refocusing results for d_{obj}=30

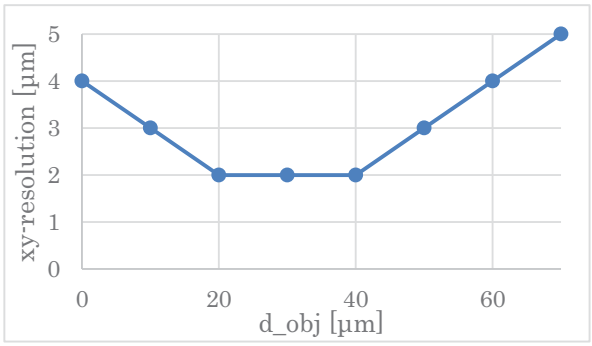

Fig. 7 Evaluation result of xy_resolution and d_{obj}

3. Conclusion

In this study, we proposed, developed, and implemented the One F-BA composite system. The xy resolution and depth of field were evaluated by refocusing from the obtained microlens images. A comparison of the resolution of each system is shown in Table 1. Table 1 shows that the One F-BA composite system provides a depth of field close to that of the One F system and xy resolution close to that of the BA system. Since nematode cell observation requires xy resolution of 3 [μm] and depth of field of about 50 [μm], we were able to develop a system suitable for 3D observation of C.elegans.

Table 1 Comparison of resolution of each system

	One F system	BA system	One F-BA composite system
xy resolution [μm]	5～10	2～3	2～5
depth of field [μm]	150	30	70

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