Attachable Micro-endoscopy System to Conventional Microscope for Live Mouse Organ Imaging using 4*f* Configuration

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Keywords: Micro-endoscopy, Confocal Endoscope, Confocal Endoscopy, GRIN Lens.

Abstract: The Micro-endoscopic technology combined with optical imaging system is essential for minimally invasive optical diagnosis and treatment in small animal disease models. Thus, the high resolution optical probe is required to achieve high resolution imaging. However, the optical imaging system requires highly precise and advanced technologies which are the main reasons for increasing system cost. Advancements in micro-optics and fiber optics technology have paved way in supporting compatibility among optical components. By providing compatibility between endoscopic system and existing conventional imaging equipment such as macro- or micro-scope, we could achieve in not only carrying out the high quality micro-endoscopic image procedure, but also reducing prices of the imaging system. The proposed system could be widely useful in the field of further biological study of animal disease model.

1 INTRODUCTION

For the basic biology and preclinical study, the experimental animal study is widely accepted for secure confirmation the biological hypothesis. However, the observation methods for in vivo monitoring inside of the experimental animal are very limited. Micro-endoscopic technology makes it possible to visualize the inner cells and organs in small animal with in-vivo and minimal invasively. As the technology of the optical fiber and micro-optics are developed, it has become essential equipment for optical imaging diagnosis and treatment in small animal disease models.

Recent advancement in micro-optics and fiber optics, the miniaturized optical endoscopy probe, such as GRIN lens assembly or fiber-bundles is utilized in the micro-endoscope. In addition to the technology, the high-resolution optical microscopy system is also required for micro-endoscopes to achieve high-quality imaging.

One of the widely used micro-endoscopy imaging system is the confocal endoscope which enables us high-contrast and high-resolution, real-time imaging by taking advantage of the confocal system. Comparing with other commercial devices from Karl Stortz, Mauna Kea Technologis and Olympus, the confocal endomicroscopy has higher resolution and enables minimum invasive. At the same time, the confocal fluorescence system allows optical sectioning of thick tissues. However, its highly precise micro-optical imaging system results in increasing system cost. Besides, conventional imaging microscopy from Leica, Zeiss and Olympus etc. has limited working space and this is major reason why the experimental mouse study could not extend their applications into in vivo or live status.

In this work, the attachable micro-endoscopy system is assembled based on commercialized confocal fluorescence microscope by attaching the additional optical components consist of 4*f* optical system, which relays the light path from the microscope to the endoscopy probe maintaining the optical properties of the microscope. That expands the capability of the microscope, not only in-vitro, but also in-vivo imaging as well. The micro-sized triplet GRIN(Graded-Index) lens probe is utilized to be inserted into the body of the small animal placed on motorized translation stage. The colon and pancreas cells of mice are visualized by using the implemented system for further biological study of animal disease model.

Bae Y., Kim J. and Kim J.
Attachable Micro-endoscopy System to Conventional Microscope for Live Mouse Organ Imaging using 4f Configuration.
DOI: 10.5220/0006101001370140
In *Proceedings of the 5th International Conference on Photonics, Optics and Laser Technology (PHOTOPTICS 2017)*, pages 137-140
ISBN: 978-989-758-223-3

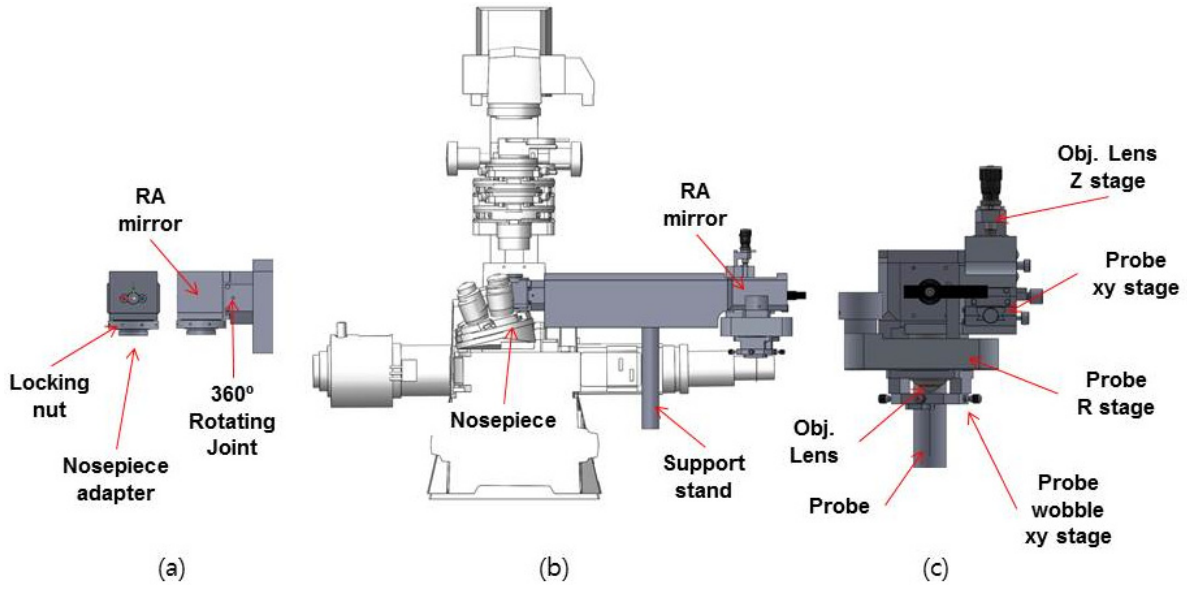

Figure 1: Illustration of the attachable micro-endoscopic system. (a) Junction part. (b) Relay optics part. (c) Endoscope holding part.

2 DESIGN OF ATTACHABLE MICRO-ENDOSCOPE SYSTEM

2.1 System Configuration

Fig. 1. shows the schematic illustration of the attachable micro-endoscopy system which consists of three parts, which are junction, relay optics and endoscope holding part.

Junction part depicted in Fig. 1. (a) connects the microscope to the endoscope for transmission of lights between the microscope and the endoscope probe. It is designed to be installed to the nosepiece of the microscope and rotated for compatibility of both upright and inverted microscope. The mounting adapter in the junction part is designed to accept the various threads of major microscope manufactures such as Leica, Zeiss, nikon and Olympus upright / inverted microscope etc.

The illumination light from the microscope is reflected by the mirror in the junction part and passes through the relay part. Then the relay part delivers the light to the endoscope probe as shown in Fig. 1.(b).

The delivered light is focused on the endoscope probe by the additional objective lens inside the endoscope holding part, shown in Fig. 1. (c). Emission light from the sample goes back through the attachable micro-endoscopy system then, makes the images of the sample by the microscope. In order to compensate the imaging wobbling during rotational sample scanning process, the wobble stage is built in the holding part. The distance between endoscope and objective lens can be controlled using Obj. Lens Z stage in Fig. 1. (c).

2.2 4*f* System for Light Delivery

In order to deliver the lights between the microscope and the probe of the endoscope, the 4*f* optical system is constructed in the relay optics part as shown in Fig. 2.

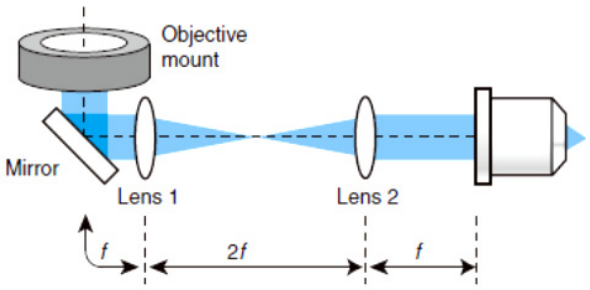

Figure 2: 4*f* optical relay system consists of two lens and mirror.

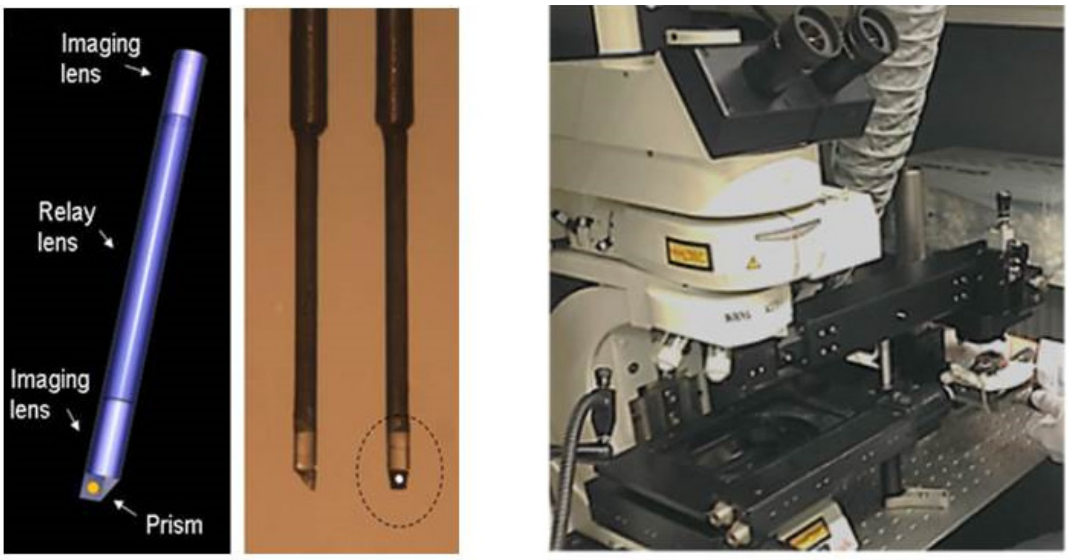

Figure 3: (a) Triplet sideview GRIN lens probe; The triplet GRIN probe consists of two imaging lens and relay lens with micro-prism . (b) Micro-Endoscopy experimental setup combined with conventional confocal upright microscope.

It extends the beam path of the microscope to the probe without loss of the lights power and changing in the properties of the microscope. The system is utilized two lenses with same focal length, f . Those are distance $2f$ each other and the objective mount and objective lens are placed on the each sides of the system apart from f . It is well-suited for beam scanning microscopes such as confocal microscopes in which the endoscopy is attached in our study. The focal plane of the image is adjusted by translating the axial position of the objective lens.

2.3 Endoscopy Probe and Complete System

Triplet GRIN lens is used as the endoscopy probe in this micro-endoscopy system, which is connected the endoscope holding part. It should be noted that the other types of endoscopy probe can be joined this system such as flexible fiber-bundles. Fig. 3(a) shows the triplet GRIN lens probe of side-view in which angled mirror prism is adhered on the tip of the imaging lens. The laser beam from the microscope goes through the probe then, forms a focal spot in front of the prism, which scans the sample. The probe is inserted in the body of the animal to scan the cells in the organ. The emission light from the sample is collected by the probe and goes back to the microscope.

There are several kinds of probe diameter from 0.35mm to 2.8mm. However, the probes of 0.35~1.0mm are mostly used for usual live mouse imaging, since minimum invasiveness is very critical in in vivo imaging. For the front-view probe, the probe has no prism on the tip and this probe is more appropriate for abdominal imaging for most organs. Thus, front-view probe is more appropriate for imaging most visceral organs such as liver, spleen, kidney and so on,. For the gastrointestinal and respiratory tracts such as colon, esophagus, trachea and so on, side-view probe is more appropriate. Thus, based on experimental purposes and directions, proper probes types, diameter and length should be chosen.

Fig. 3. (b) shows the attachable micro-endoscopy system built on the commercialized upright confocal microscope. The motorized translation stage is utilized to place the sample animal and scan the sample laterally. The micro probes are inserted into probes hole and fixed on endoscope holder. The complete micro-endoscopy combined with the confocal microscopy is applied for in-vivo fluorescence cellular imaging of internal organs in a mouse. As a result, the experimental space has enlarged and system costs are reduced.

3 EXPERIMENTAL RESULTS

The measured lateral and axial resolution of the attachable micro-endoscopy system are 1μm and 10μm, respectively within 300μm of field of view,

when diameter of 1mm probe is used. That shows sufficiently high-resolution of the system for application of cellular imaging of mouse. The optical penetration depth of a GRIN probes is limited to about 100μm in most organ tissue.

Fig. 4 shows the images of the cells in anesthetized mouse organs taken by our system. We visualized the mouse colon vasculature image after Acridine Orange IV injection. Fig. 4. (a), and (b) are the fluorescence image of mouse clone which clearly shows the single cells in the organ. By inserting front-view probe into live MIP+ mouse pancreas, the pancreatic islets GFP cells are imaged. Fig. 4. (c), and (d) are the in-vivo images of pancreatic islets GFP cell and, blood vessel of the mouse, respectively.

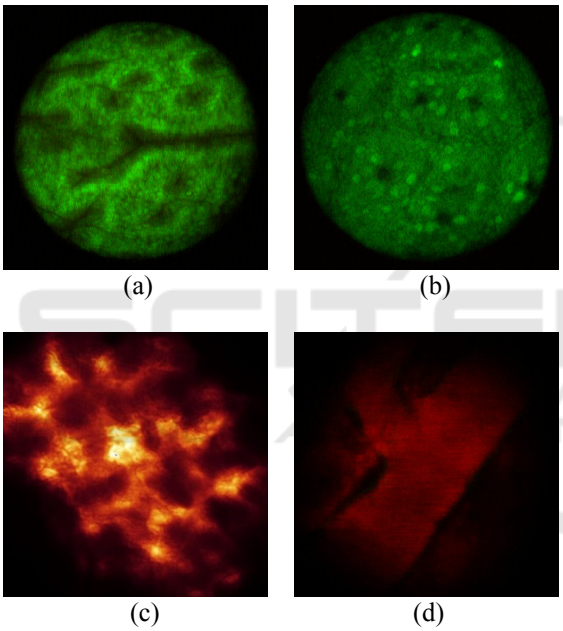

Figure 4: (a), (b) In-vivo images of mouse colon walls. (c), (d) In-vivo images of pancreatic islets GFP cell, and blood vessel of mouse, respectively.

4 CONCLUSIONS

In this paper, we present attachable micro-endoscopy system combined with conventional optical microscope. It features the compatibility with the most microscope manufactures' standards. The developed attachable micro-endoscope system is equipped to the conventional commercialized confocal microscope for in-vivo cellular imaging. The colon and pancreas cells of mice are visualized by using the implemented system for further biological study of animal disease model.

ACKNOWLEDGEMENTS

This work was supported by the Basic Science Research Program [2014R1A1A2057773, 2015K2A7A1035896] through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning and by a grant (2015-641, 2015-646, 2016-7212) from the Asan Institute for Life Science, Asan Medical Center, Seoul, Korea.

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