PROGRESS REPORT
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A project laboratory is set in NEXT Medical Device Innovation Center, National Cancer Center (NCC) Hospital East, which is equipped with common research facilities of TUS and NCC. Proper research staff members of the laboratory (cross appointment assistant professors) are also affiliated to both TUS and NCC. By the direct collaboration between academic researchers and medical doctors, we are developing innovative medical devices for realizing "hyper-assisted medical care" that assists medical care with "abilities beyond human skill".

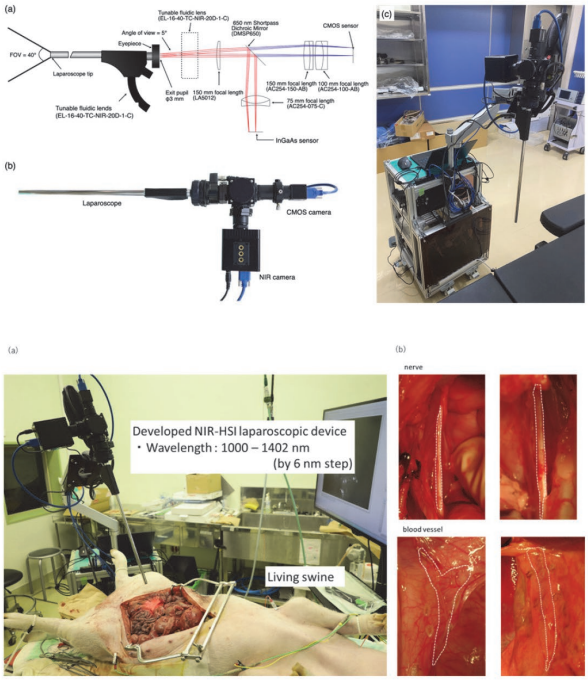

Figure 1. Diagram of an optical system of the laparoscope using a visible camera and picture of the laparoscope system using visible camera.

1. Laparoscopic Near-infrared Hyperspectral Imaging System

Cancer is a leading cause of death, with surgical resection being a critical treatment. Minimally invasive surgery (MIS) has grown, necessitating advanced support systems for safer operations. Near-infrared (NIR) light penetrates deeper into tissues and provides detailed molecular information. This study developed an NIR-Hyperspectral Imaging (HSI) laparoscope using a supercontinuum light source and Acousto-Optic Tunable Filter for rapid wavelength switching, aiming for high-speed NIR-HSI during surgery.

A laparoscopic NIR-HSI system using a custom rigid endoscope and an optical system centered on an acousto-optic tunable filter and SC light source are developed. We investigated its performance by measuring light intensity, spectral performance, and resolution. Ex vivo SWIR-HSI

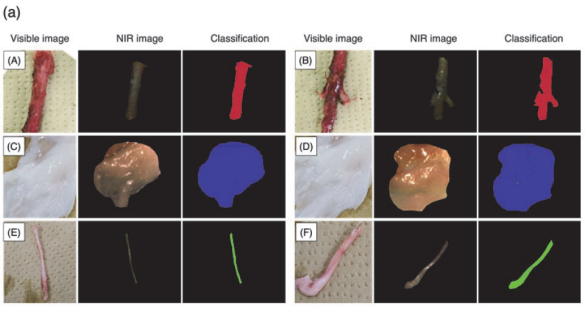

Figure 2. (a) Neural network classification of ex-vivo pig tissues. (A) and (B) are artery, (C) and (D) are fat, (E) and (F) are nerve. Left images are Visible images. Center images are NIR pseudo color images with assigned to 1036 nm for red, 1282 nm for green, and 1372 nm for blue. Right images are classified image by neural network. Each red, blue, and green pixel indicate classified artery, fat, and nerve, respectively.

imaging was conducted on pig arterial, mesentery, and nerve tissues, followed by in vivo imaging to identify nerves and blood vessels in deep tissue. Data processing involved pixel calibration and machine learning for tissue identification. A three-layer neural network was trained using leave-one-out cross-validation. The model's accuracy was assessed through specificity, sensitivity, and overall accuracy metrics.

The device showed varying light intensities across different wavelengths, with peak intensity around 18 mW near 1064 nm. Accurate spectral separation was confirmed for wavelengths beyond 1000 nm. Ex vivo imaging distinguished blood vessels, fat, and nerves with high accuracy. In vivo imaging on an anesthetized pig used machine learning to segment exposed nerves and non-exposed blood vessels. Visible and near-infrared pseudo-color images were created for analysis. Segmentation accuracy for exposed nerves was 88.4%, with a recall of 68.7% and specificity of 89.1%. For non-exposed blood vessels, accuracy was 83.2%, with a recall of 60.2% and specificity of 88.0%. This confirms the system's effectiveness for tissue identification during surgery.

We developed a laparoscopic NIR-HSI system with an acousto-optic tunable filter and SC light source. The device effectively distinguished blood vessels and nerves in both ex vivo and in vivo conditions

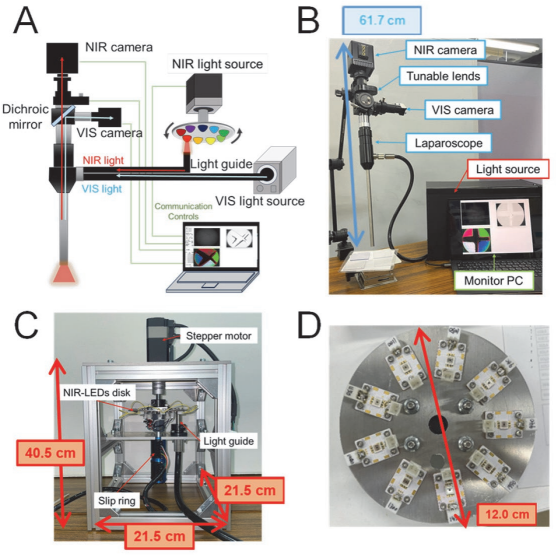

Figure.3. (A) Diagram of the system. (B) NIR-MSI rigid endo-scope system. (C) NIR light source. (D) LEDs mounted disk.

2. Near-Infrared Multispectral Imaging Rigid Endoscope System for High-Speed Target Identification Using LED Rotating Light Source

Near-Infrared Multispectral Imaging (NIR-MSI) has attracted attention in the surgical field owing to its ability to pen-etrate tissues and analyze molecular vibrations with high spatial resolution, thus enabling the identification of deep or similarly colored tissues. However, conventional devices have problems such as non-

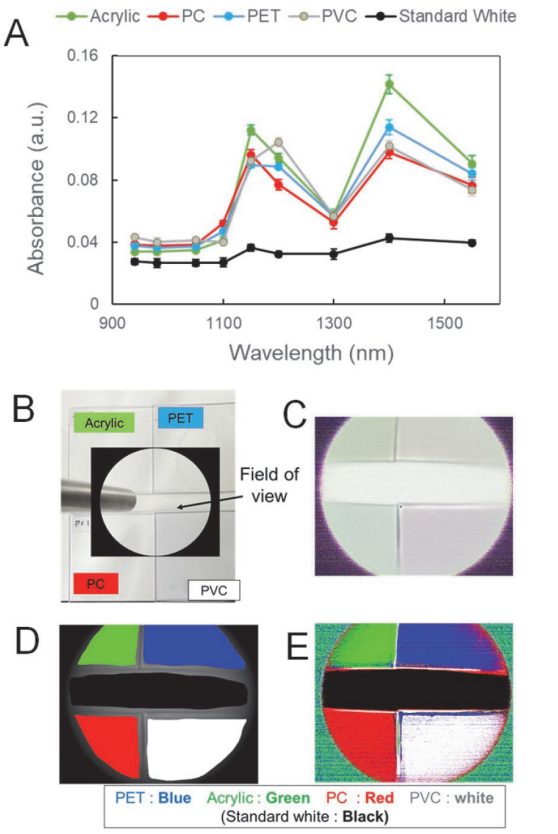

Figure 4. (A) Average spectrum of five various targets. (B) Im-age of 4 type of resins taken by visible camera. (C) Merged color image taken using the NIR camera (R:1450 nm, G: 1300 nm, B: 1200 nm). (D) Annotation image of resin classification. (E) Result of Neural network classification.

portability and long imaging times, making the surgical use of NIR-MSI challenging.

In this study, we developed a prototype of an NIR-MSI rigid endoscope system capable of high-speed imaging and tis-sue classification. This system is constructed using a custom-made rigid endoscope that can relay NIR images, an In-GaAs camera, and a lighting device containing nine high-power LEDs between 940–1535 nm. The developed device enables continuous processing of light irradiation, data acquisition, neural network analysis, and result display using customized software.

Tissue transparency and component classification were investigated to verify this system. Tissue transparency showed that text could be recognized through a 5 mm-thick layer of chicken breast. This system completed imaging and neural network analysis of four color-matched transparent resins, which are difficult to distinguish under visible light, within 2 s, with an average accuracy of 96 %.

The system has future potential to be applied to the identification of deep/similarly colored tissues with noninvasive and real-time endoscopic surgery.

3. Development of bimodal over-1000 nm (OTN) near infrared (NIR) fluorescence and magnetic resonance (MR) imaging probe for breast cancer detection

One of the research missions of the division is the development of bimodal over-1000 nm (OTN) near infrared (NIR) fluorescence and magnetic resonance (MR) imaging probe for breast cancer detection. In addition to the development to the last year, we've reported the following two studies.

(1) *Influence of Carboxyl Group Ratios on the Design of Breast Cancer Targeting Bimodal MR/NIR-II Imaging Probe from PLGA@Gd-DOTA@PEG Micelles Conjugating Herceptin*

We developed a small MRI/NIR-II probe to target HER2 (tetanucleotide) breast cancer cells. The probe is composed of PLGA-b-PEG micelles encapsulated NIR-II, and Gd-DOTA is conjugated at the border of PLGA/PEG. Herceptin was then conjugated to carboxyl residues of PLGA-b-PEG chains. We examined the influence of carboxyl group ratios on the probe property stability and Herceptin concentration and the binding affinity to HER2(+) cells corresponding to the −COOH ratios. The binding assays demonstrated that the optimal surface ratio of −COOH is 5%, which is less affected by fluorescence reduction and which exhibited the highest antigen-capturing activity.

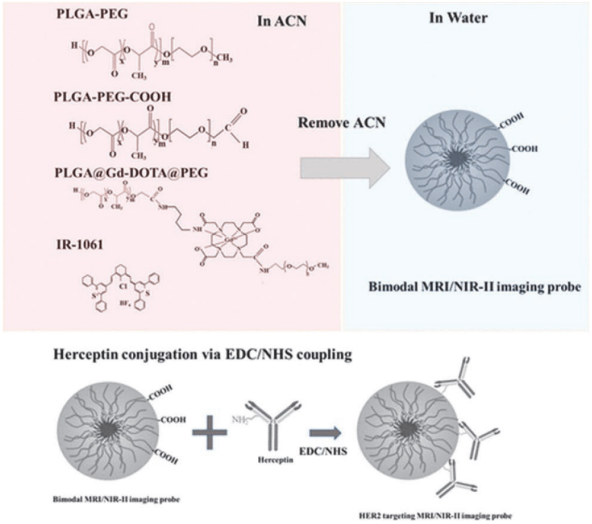

Figure 5. Schematic illustration of bimodal imaging probe development and Herceptin conjugation

(2) *Enhancing near-infrared fluorescence intensity and stability of PLGA-b-PEG micelles by introducing Gd-DOTA at the core boundary*

Micelles have been extensively used in biomedicine as potential carriers of hydrophobic fluorescent dyes. Their small diameters can potentially enable them to evade recognition by the reticuloendothelial system, resulting in prolonged circulation. Nevertheless, their lack of stability in physiological environments limits the imaging utility of micelles. In particular, when a dye sensitive to water, such as IR-1061, is encapsulated in the micelle core, the destabilized structure leads to interactions between water and

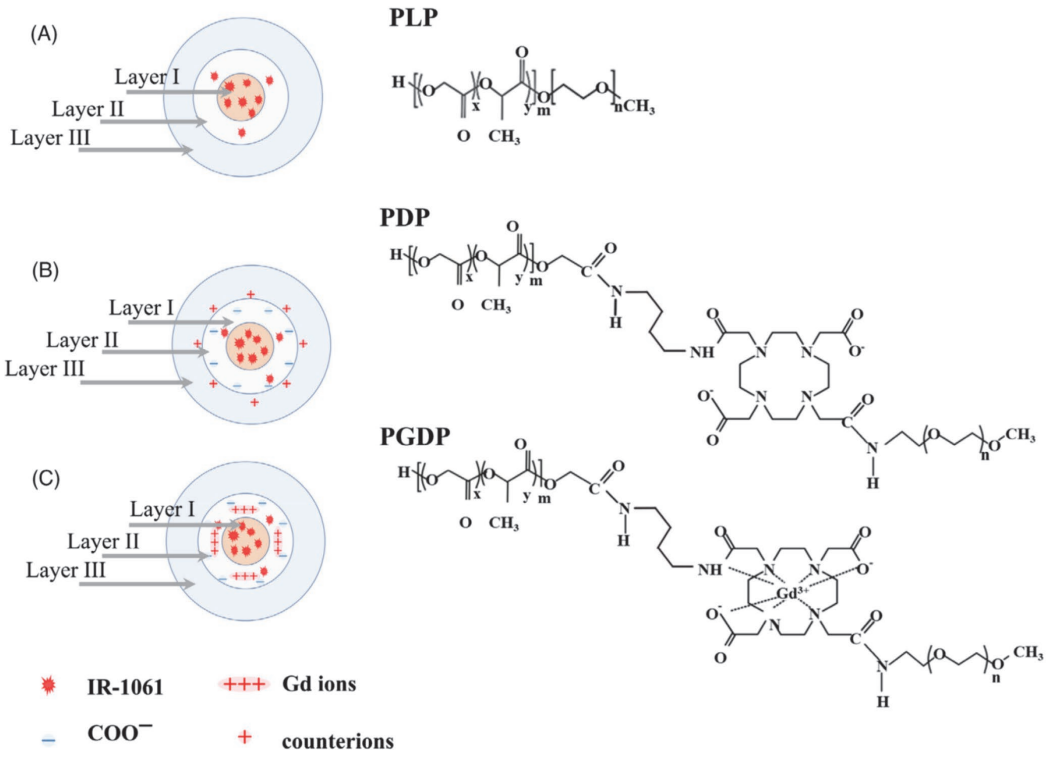

Figure 6. Illustration of IR-1061 molecules distributed inside the micelles throughout the inner core (layer I), the border of PLGA/PEG (layer II), and the outer shell of PEG. When DOTA was introduced at the border of PLGA/PEG, a double electrical layer was formed between the COOH groups and counter ions. In the case of Gd-DOTA, a double electrical layer was formed between COOH and Gd3+ ions at the center of adjacent DOTA molecules.

dye, degrading the fluorescence. In this study, we investigated a method to improve micelle stability utilizing the electrical effect of gadolinium (Gd3+) and tetraazacyclododecane tetraacetic acid (DOTA), introduced into the micelles. Three micellar structures, one containing a poly(lactic-co-glycolic acid)-block-poly(ethylene glycol) (PLGA-b-PEG) block copolymer, and two other structures, including PLGA-b-PEG with DOTA or Gd-DOTA introduced at the boundary of PLGA and PEG, were prepared with IR-1061 in the core. Structures that contained DOTA at the border of the PLGA core and PEG shell exhibited much higher fluorescence intensity than probes without DOTA. With Gd3+ ions at the DOTA center, fluorescence stability was enhanced remarkably in physiological environments. Most interesting is the finding that fluorescence is enhanced with increased Gd-DOTA concentrations. In conclusion, we found that overall fluorescence and stability are improved by introducing Gd-DOTA at the boundary of the PLGA core and PEG shell. Improving micelle stability is crucial for further biomedical applications of micellar probes such as bimodal fluorescence and magnetic resonance imaging.

4. Circulating Tumor Cell Capture by Microfluidic Devices

As a major factor of metastases, circulating tumor cells (CTC), which are leaking from primary tumor into peripheral blood, has attracted large attention. It is assumed that CTC reaches distant organs along the blood circulation and subsequent growth will cause the metastasis. If we could capture CTCs, useful knowledge can be obtained for novel treatments. Previous studies indicate that CTCs are generally larger and harder than leukocytes, and we have developed deterministic lateral displacement (DLD)

microfluidic devices for separating large cells from peripheral bloods. However, we have been struggling with the clogging of microchannels. Finally, we managed to solve it by condensing DNA in the blood, and we have successfully conducted CTC concentration experiments from the blood of cancer patients continuously over 40 times . Our next challenge is to improve the concentration efficiency. Selection based solely on size has not been able to prevent the contamination of larger white blood cells. Therefore, we were considering utilizing specific adsorption to attach white blood cells to the walls of the microchannel. However, it was found that simply flowing cells slowly through the microchannel does not result in specific adsorption. Since the cell surface is not smooth, it is assumed that antigens located in the recesses do not come into contact with the antibodies on the wall. We have examined a microfluidic device as shown in Figure 7, aiming to press cells against the wall. We observed that stronger the pressure against the wall, the more the specific adsorption was promoted, and we are working on further optimization.

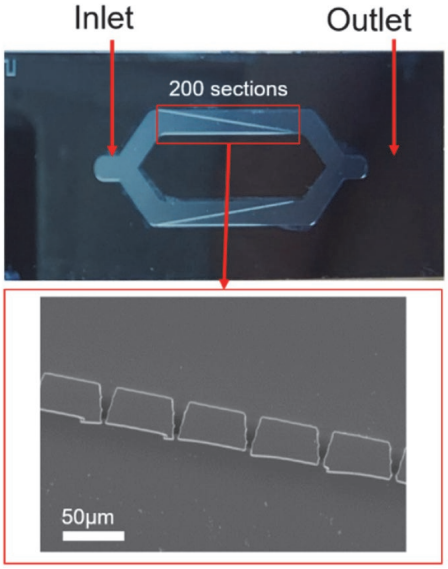

Figure 7. Prototype microfluidic device for elimination of white blood cells. Cells are sucked at slits in the figure, and are pressed onto the channel walls around the slits. Larger pressure showed rapid specific adsorption.

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Publications

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