PROGRESS REPORT
Division of Biomedical Technology

Hiroshi Takemura, Ph.D.

Members

Faculty members

Professor and Chairman

Hiroshi Takemura

Professor

Kohei Soga
Masanori Hayase
Atsuo Yasumori

Assistant Professor

Toshihiro Takamatsu
Doan Thi Kim Dung

Division of Biomedical Technology

Professor and Chairman: Hiroshi Takemura, Ph.D.

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".

1. Diagnosis of deep lesion by Near-Infrared Hyper-Spectrum Imaging

The Near-Infrared (NIR) spectrum, ranging in wavelength from 800 nm to 2500 nm, has properties that make it especially useful for bioimaging. NIR light is less scattered by biological tissues than ultraviolet or visible light, and radiation absorption by water is much lower in the NIR spectrum than in mid-infrared spectrum. This tends to make tissues transparent to NIR wavelengths. Thus, high transparency in the NIR spectrum makes possible non-destructive, non-invasive spectroscopic investigation of plants and human subjects. This allows safe, direct investigation of biomolecules in vivo. Hyper-Spectrum Imaging (HSI) is a potent imaging modality that provides spectroscopic information with high spatial resolution (precision of measurement) and has been applied to various research fields, including detecting epithelial tumors such as gastric cancer, without the use of fluorescent probes.

In our group, NIR-HSI system was built in National Cancer Center and investigate about

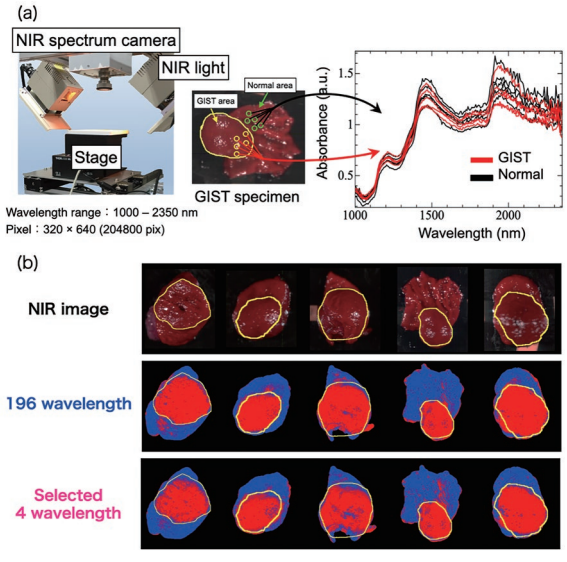

Figure 1. NIR-HSI setup and deep lesion prediction by machine learning method

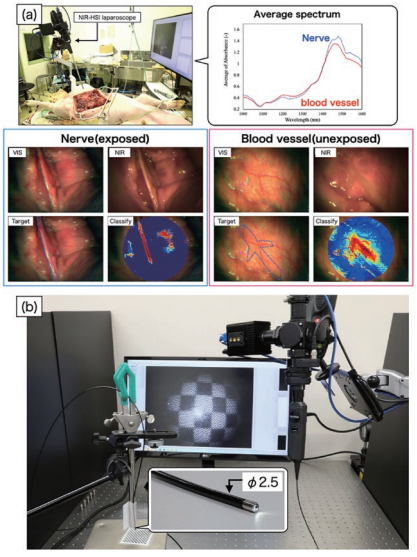

Figure 2. NIR-HSI system for laparoscope (a) and fiber scope(b)

diagnosis of gastrointestinal stromal tumor (GIST) where exist in underlying of mucosa (Figure 1(a)). And sectioning of normal and GIST was performed by machine learning method using the spectrum. This diagnostic system could identify GIST covered by normal mucosa, with high accuracy (approximately 80%). However, it is difficult to achieve real-time HSI because hyperspectral data has 196 wavelength bands and the data processing takes a long time. Then, we adopt selection method of high contribution wavelength for classification by machine learning. As the result, there are four wavelengths with large contributions for GIST identification in the 1000–1400 nm region. Visualizations of the identification using four wavelengths was no significant difference in 196 wavelength identification (Figure 1(b)). Therefore, it can be said that the result indicates its potential to achieve data acquisition and image processing in real time.

Currently, we have now developed NIR-HSI devices which can be used under endoscope. In our research, it is confirmed that laparoscope type (Figure 2(a)) and endoscope type (Figure 2(b)) have successfully performed NIR-HSI scans and classify unexposed target such as blood vessel. These endoscopic NIR-HSI devices can be expected as next generation technique of diagnosis and navigation surgery.

2. Development of robotic colonoscope

Currently, colorectal cancer is the second leading cause of death worldwide. Therefore, colon cancer screening tests such as colonoscopy and fecal occult blood test (FOBT) are performed to reduce the mortality and incidence of colorectal cancer. Although FOBT is a simple test, positive patients eventually require colonoscopy to confirm the presence of lesions. Thus, colonoscopy is gold standard to detect and diagnose colon cancer. However, performing a colonoscopy requires a high level of skill in colon insertion, making it challenging for beginner colonoscopists to achieve total colonoscopy. Furthermore, cases involving longer colons and adhesions require even more advanced techniques. Therefore, a new colonoscope that allows safe and easy colon insertion is desired.

In this study, a prototype of robotic colonoscope with double-balloon and double-bend tube was developed to simplify insertion(Figure 3). The device consists of an outer and an inner tube with a balloon and a bending function. Therefore, the mechanism is

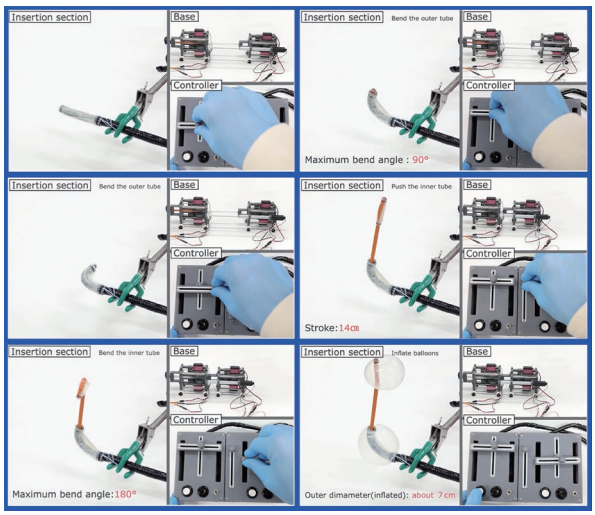

Figure.3. Motion of developed robotic colonoscope

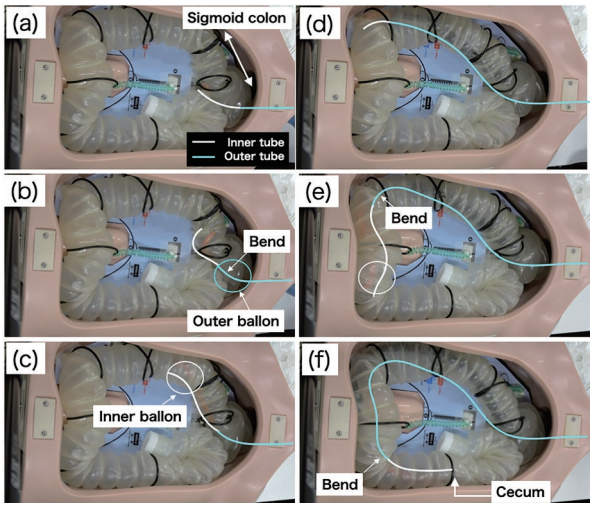

Figure 4. insertion test of colon model by developed device

characterized by the tip of the inner tube being pushed out to the off-axis by tip direction of outer tube. The insertion method is expected to avoid excessive stretching of the intestinal tract. They are independently and electrically manipulated to bend and push/pull using servo motor via a controller, so it has potential to insert total colon alone. In insertion test, the device could be reached to cecum of colon model (Case 1) by non-medical operator alone(Figure 4). In addition, the behavior was free from over-extended colon model, thus, it suggests that the mechanism of prototype can contribute to comfortable colonoscopy.

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.

(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 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

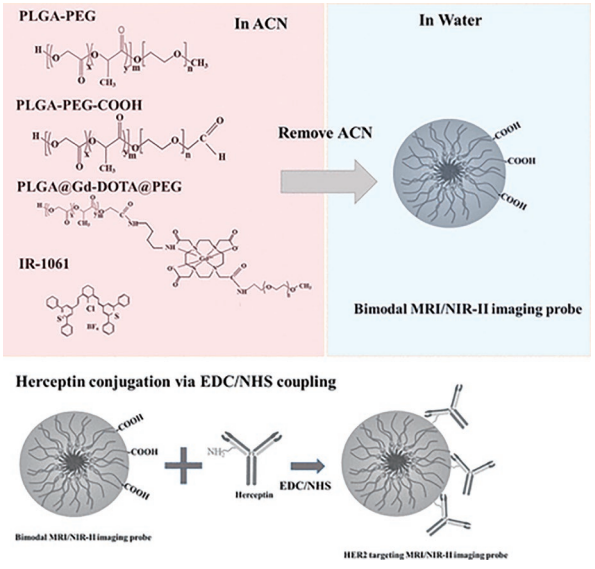

Figure 5. Schematic illustration of bimodal imaging probe development and Herceptin conjugation

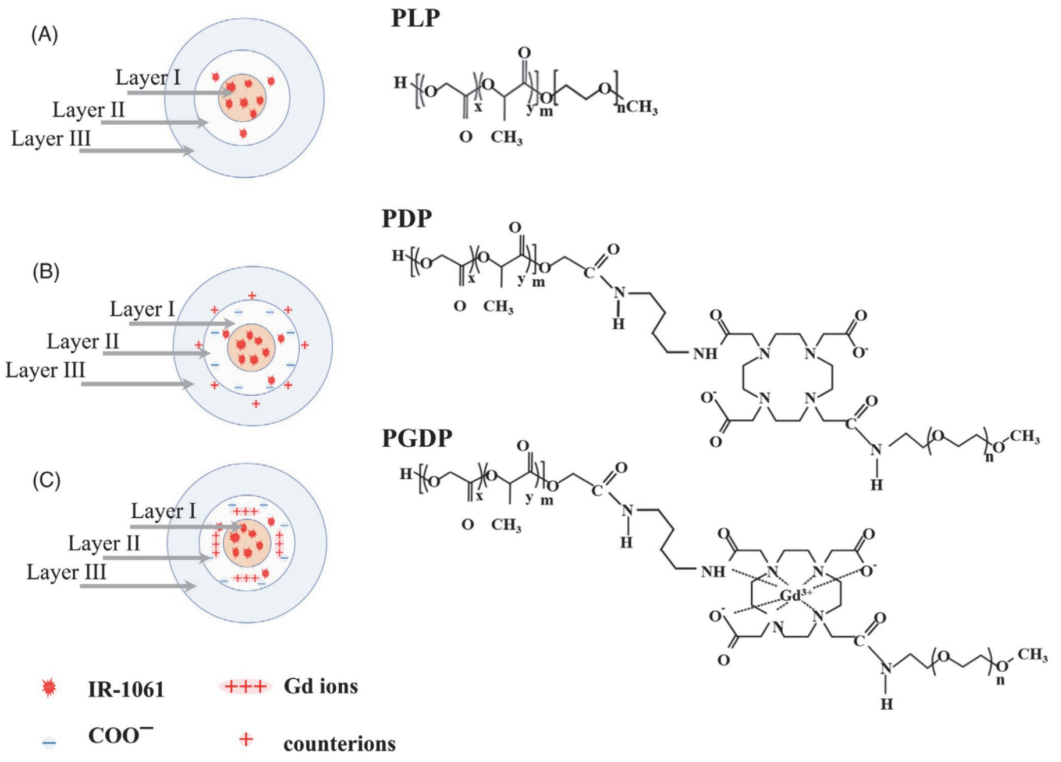

Figure 6. Illustration of IR-1061 molecules distributed inside the micelles throughout the inner core (layer I), the border of PLGA/PEG (layer II), and the outer shell of PEG. When DOTA was introduced at the border of PLGA/PEG, a double electrical layer was formed between the COOH groups and counter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

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, channel clogging around micro-posts in the DLD devices is problematic. We found that cell free DNAs are a major cause of the clogging, and demonstrated that mitigation of the clogging by addition of polyamine in blood specimens and enlargement of post array dimension. Optimization of polyamine concentration was performed using cancer patient bloods, and 500 μM showed the best results. Using the anti-clogging technique, enrichment of CTC from cancer patient bloods

has been performed since March 2022 and no serious clogging happened over 50 cases. But, even though the CTCs are enriched by the DLD microfluidic device, contamination with leukocytes is inevitable and elimination of leukocytes by a affinity capture on a microchannel wall was attempted as shown in Figure 7. After the leukocytes elimination, using TOK's SIEVEWELL™, all collected cells were observed and suspicious cells were selected for RNA analysis. The invention of polyamine addition for inhibition of the clogging was patented in 2023.

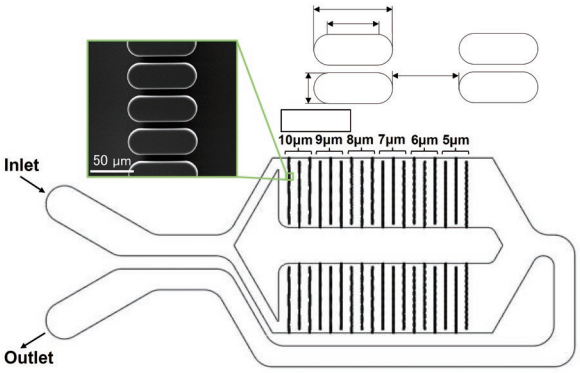

Figure 7. Schematic of the microchannel with slits. Because the size of the cells is not uniform, slit gaps were varied from 5 to 10μm. Si substrate was machined with deep-RIE.

Publications

1. Umezawa, M.; Ueya, Y.; Ichihashi, K.; Dung, D. T. K.; Soga, K., Controlling Molecular Dye Encapsulation in the Hydrophobic Core of Core–Shell Nanoparticles for In Vivo Imaging. *Biomedical Materials & Devices* **2023**.
2. Morikawa, D.; Nambu, K.; Morita, K.; Yoshida, H.; Soga, K., Effect of direct and alternating current (DC and AC) fields on creep behavior of 8 mol% Y2O3 stabilized cubic ZrO2 polycrystal. *J. Euro. Ceram. Soc*. **2023,** *43* (8), 3498-3506.
3. Hiroto, T.; Morino, Y.; Yoshikawa, M.; Soga, K.; Hayami, W.; Ogitsu, T.; Kimura, K., Does photoinduced structural change by β-rhombohedral boron occur at room temperature? *Solid State Sci*. **2023,** *142*, 107246.
4. Doan, T. K. D.; Umezawa, M.; Okubo, K.; Kamimura, M.; Soga, K., Enhancing near-infrared fluorescence intensity and stability of PLGA-b-PEG micelles by introducing Gd-DOTA at the core boundary. *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **2023,** *n/a* (n/a).
5. Doan, T. K. D.; Umezawa, M.; Ikeda, K.; Ohnuki, K.; Akatsuka, M.; Okubo, K.; Kamimura, M.; Yamaguchi, M.; Fujii, H.; Soga, K., 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. *ACS Applied Bio Materials* **2023,** *6* (7), 2644-2650.
6. Tomohiro Mitsui, Akino Mori, Toshihiro Takamatsu, Tomohiro Kadota, Konosuke Sato, Ryodai Fukushima, Kyohei Okubo, Masakazu Umezawa, Hiroshi Takemura, Hideo Yokota, Takeshi Kuwata, Takahiro Kinoshita, Hiroaki Ikematsu, Tomonori Yano, Shin Maeda, and Kohei Soga "Evaluating the identification of the extent of gastric cancer by over-1000 nm near-infrared hyperspectral imaging using surgical specimens," Journal of Biomedical Optics 28(8), 086001, 2023.
7. Toshihiro Takamatsu, Yuto Endo, Ryodai Fukushima, Tatsuki Yasue, Kensuke Shinmura, Hiroaki Ikematsu & Hiroshi Takemura, Robotic endoscope with double-balloon and double-bend tube for colonoscopy. Scientific Reports 13, 10494, 2023.
8. R. Fukushima, T. Takamatsu, A. Mori, K. Sato, K. Okubo, M. Umezawa, N. Takeshita, H. Hasegawa, H. Yokota, K. Soga, and H. Takemura "Hyperspectral imaging and detection mapping of in vivo biological tissues applying near-infrared laparoscope", Proc. SPIE Medical Imaging, 2023.
9. Ryodai Fukushima, Ren Iwanami, Akino Mori, Toshihiro Takamatsu, Hiroaki Ikematsu, Hideo

Yokota, Kouhei Soga, and Hiroshi Takemura, "Development of NIR-HSI Laparoscope for Distinguishing Deep Tumor Lesion without Labeling", 2022 Biophotonics Congress: Biomedical Optics, 2022.

10. Matsumae, T., Kariya, S., Kurashima, Y., Thu, L. H. H., Higurashi, E., Hayase, M., Takagi, H., Wafer-Scale Room-Temperature Bonding of Smooth Au/Ti-Based Getter Layer for Vacuum Packaging. Sensors, 22(21), 8144 (2022).
11. Kariya, S., Matsumae, T., Kurashima, Y., Takagi, H., Hayase, M., Higurashi, E., Bonding formation and gas absorption using Au/Pt/Ti layers for vacuum packaging. Microsystems & Nanoengineering, 8(1), 2 (2022).
12. Fukumoto, S., Matsumae, T., Kurashima, Y., Takagi, H., Hayase, M., Higurashi, E. , Hydrophilic direct bonding of GaN and Si substrates by wet treatments using H2SO4/H2O2 mixture and NH3/H2O2 mixture. Japanese journal of applied physics, 61(SF), SF1005(2022).