2-4. International Joint Research Faculty Members

No.	Name	Project Title
2-4-1	**Takumi HIGAKI** FAST	Digital Plant Cell Biology
2-4-2	**Takahiro HOSONO** FAST	Environmental Diagnosis on Earth Surface Systems
2-4-3	**Kei ISHIDA** CWMD	Deep Learning for Hydrology
2-4-4	**Makiko KOBAYASHI** FAST	Advanced Biomedical Evaluation System
2-4-5	**Ruda LEE** IINa	Reprogramming Multi-Drug Resistance Breast Cancer for Women's health and quality of life
2-4-6	**Yuta NAKASHIMA** FAST	Novel Cancer Medical Technology Using Liquid Biopsy
2-4-7	**Shinichi OHIRA** FAST	Separation, Synthesis, and Detection by Means of Ionic Solutes Handling
2-4-8	**Atsushi SAINOKI** FAST	Development of Microbially-Aided Carbon Sequestration Technology
2-4-9	**Mitsuru SASAKI** IINa	Environmentally Promising Processes for Medical and Skincare Nanomaterials
2-4-10	**Keitaro TAKAHASHI** FAST	Study of First-Generation Objects in the Universe with Radio Telescopes

FAST: Faculty of Advanced Science and Technology

CWMD: Center for Water Cycle, Marine Environment and Disaster Management

IINa: Institute of Industrial Nanomaterials

No. 2-4-1	Digital Plant Cell Biology		
Name	Takumi HIGAKI		
Affiliation	Faculty of Advanced Science and Technology Email: thigaki@kumamoto-u.ac.jp	Title	Professor
Research Field	Environmental bioscience		
Cluster Members			
Name	Affiliation/Title		
Bo LIU	Professor / University of California at Davis U.S.A.		
Kae AKITA	Assistant Professor / Kitasato University		

[Details of Activities]

With recent advances in bioimaging equipment such as microscopes, the information processing of bioimages is attracting attention as a new research field in bioinformatics. In this 'Research Cluster' project, we aim to develop and validate microscopic image analysis techniques to quantitatively evaluate the dynamics of intracellular structures in plant cells. Specifically, we will develop biological image analysis frameworks that quantitatively assess the multidimensional biological features of the cytoskeleton, and utilize machine learning to make biological discoveries. This fiscal year, as indicated in the publications listed below, we have published eight papers in high-impact journals such as *Science* and *Current Biology*, and released five press releases from Kumamoto University. Here, we briefly report on three particularly outstanding achievements:

1. Nishimura et al. (2023) *Science*

Plants exhibit gravitropism, directing their roots towards nutrient-rich soil and their stems towards light and reproductive advantages. Gravitropism is observed in plant organs containing cells with amyloplasts that sediment in the direction of gravity. The "starch-statolith hypothesis," proposed over 100 years ago, suggests that the sedimentation of these granules enables gravity sensing. However, the physical process of amyloplast sedimentation and its conversion and transmission to other signals within the cell were not understood. In collaboration with Professor Morita of NIBB, we demonstrated, using image analysis techniques, that when amyloplasts sediment close to the plasma membrane, the LAZY1-LIKE (LZY) protein present in amyloplasts moves to the plasma membrane, allowing the cell to sense the position of the amyloplasts.

2. Kikukawa et al. (2023) *Plant Cell Physiol*

The interdigitated pavement cell shape is suggested to be mechanically rational at both the cellular and tissue levels, but the biological significance of the cell shape is not fully understood. This study investigates the significance of the jigsaw puzzle-like shape of pavement cells for cotyledon morphogenesis in *Arabidopsis*, using a transgenic line overexpressing *RIC1* that leads to simple elongation of these cells. Analyses revealed that *RIC1* overexpression causes abnormal cotyledon shapes with marginal protrusions, possibly due to changes in pavement cell shape and reduction in cell wall cellulose content. A mathematical model and computer simulations suggest

that irregular cotyledon shape and marginal protrusions are due to local growth variation and that an organ-level regulatory mechanism is required.

3. Hiromoto et al. (2024) *Sci Rep*

We developed an image analysis method that comprehensively evaluates the distribution of various intracellular structures. In collaboration with Professor Ueda of Tohoku University, we analyzed the process from the onset of cell elongation of the plant zygote to the first asymmetric cell division using this image analysis technique. The results revealed that the intracellular structure distribution was already biased at an early stage after fertilization, it elongates while maintaining this biased distribution, and there is a correlation between this intracellular structure distribution and the position of the asymmetric cell division site.

Additionally, the invited lecture was given at the International Microscopy Congress (IMC20) held at BEXCO in Busan, South Korea, under the title "Quantitative evaluation of cytoskeleton organization using fluorescence microscopic image analysis." A photo of the lecture is shown below.

In the next fiscal year, we will continue to contribute to solving cell biological problems through the development of image analysis methods. Although the name of this project is Digital Plant Biology, we plan to actively work on image analysis of animal cells as well as plant cells.

Publications

1. Hitora Y, El-Desoky AH, Sadahiro Y, Sejiyama A, Kinoshita A, Ise Y, Angkouw ED, Mangindaan REP, Higaki T, Tsukamoto S (2024) Neopetromin, a cyclic tripeptide with a C-N cross-link, from the marine sponge *Neopetrosia sp.*, that causes vacuole fragmentation in tobacco BY-2 cells. ***J Nat Prod***. in press. https://doi.org/10.1021/acs.jnatprod.4c00158
2. Hiromoto Y, Minamino N, Kikuchi S, Kimata Y, Matsumoto H, Nakagawa S, Ueda M, Higaki T (2023) Comprehensive and quantitative analysis of intracellular structure polarization at the apical-basal axis in elongating *Arabidopsis* zygotes. ***Sci Rep*** 13: 22879. https://doi.org/10.1038/s41598-023-50020-8 (Kumamoto U Press Release at https://www.kumamoto-u.ac.jp/whatsnew/sizen/20240110)
3. Ichita M, Higaki T. (2023) Intracellular trafficking regulation of plasma membrane H+-ATPase and environmental response in plants. ***Cytologia*** 88: 169-173. (Published: 27 Sep 2023) https://doi.org/10.1508/cytologia.88.169
4. Kikukawa K, Takigawa-Imamura H, Soga K, Kotake T, Higaki T (2023) Smooth elongation of pavement cells induced by *RIC1* overexpression leads to marginal protrusions of the cotyledon in *Arabidopsis thaliana*. ***Plant Cell Physiol*** 64: 1356–1371. (Published 18 Sep) https://doi.org/10.1093/pcp/pcad094 (Kumamoto U Press Release at https://www.kumamoto-u.ac.jp/whatsnew/sizen/20230914)
5. Hirano T, Ebine K, Ueda T, Higaki T, Watanabe-Nakayama T, Konno H, Takigawa-Imamura H, Sato MH (2023) The SYP123-VAMP727 SNARE complex delivers secondary cell wall components for root hair shank hardening in *Arabidopsis*. ***Plant Cell*** 35: 4347–4365.

(Published 15 Sep 2023) https://doi.org/10.1093/plcell/koad240 (Kumamoto U Press Release at https://www.kumamoto-u.ac.jp/whatsnew/sizen/202301006)

6. Yue Y, Hotta T, Higaki T, Verhey KJ, Ohi R (2023) Microtubule detyrosination by VASH1/SVBP is regulated by the conformational state of tubulin in the lattice. ***Curr Biol*** 33: 4111–4123. (Published 15 Sep 2023) https://doi.org/10.1016/j.cub.2023.07.062
7. Matsumoto T, Higaki T, Takatsuka H, Kutsuna N, Ogata Y, Hasezawa S, Umeda M, Inada N (2023) *Arabidopsis thaliana* subclass I ACTIN DEPOLYMERIZING FACTORs regulate nuclear organization and gene expression. ***Plant Cell Physiol*** 64: 1231–1242. (Published: 18 Aug 2023) https://doi.org/10.1093/pcp/pcad092
8. Nishimura T, Mori S, Shikata H, Nakamura M, Hashiguchi Y, Abe Y, Hagihara T, Yoshikawa HY, Toyota M, Higaki T, Morita MT (2023) Cell polarity linked to gravity sensing is generated by protein translocation from statoliths to the plasma membrane. ***Science*** 381: 1006–1010. (Published: 11 Aug 2023) https://doi.org/10.1126/science.adh9978 (Kumamoto U Press Release at https://www.kumamoto-u.ac.jp/whatsnew/sizen/20230809)
9. Wint H, Li J, Abe T, Yamada H, Higaki T, Nasu Y, Watanabe M, Takei K, Takeda T (2023) Pacsin 2-dependent N-cadherin internalization regulates the migration behaviour of malignant cancer cells. ***J Cell Sci*** 136: jcs260827. (Published: 3 May 2023) https://doi.org/10.1242/jcs.260827 (Kumamoto U Press Release at https://www.kumamoto-u.ac.jp/whatsnew/seimei/20230612)
10. Yoshida D, Akita K, Higaki T (2023) Machine learning and feature analysis of the cortical microtubule organization of Arabidopsis cotyledon pavement cells. Protoplasma 260, 3, 987-998. (Published: May 2023)

No.2-4-2	Environmental Diagnosis on Earth Surface Systems		
Name	Takahiro HOSONO		
Affiliation	Faculty of Advanced Science and Technology Email: hosono@kumamoto-u.ac.jp	Title	Professor
Research Field	Environment-friendly technology / Strengthening resilience / Data science and AI		

Cluster Members	
Name	Affiliation/Title
Jens HARTMANN	Institute for Geology, University of Hamburg, Germany / Professor, *IROAST Visiting Professor*
Gibran ROMERO MUJALLI	Institute for Geology, University of Hamburg, Germany / Postdoc
Marino Domenico BARBERIO	National Institute of Geophysics and Volcanology, Italy / Researcher
Yu ZHI-QIANG	Faculty of Advanced Science and Technology, Kumamoto University / Postdoc
Kimpei ICHIYANAGI	Faculty of Advanced Science and Technology, Kumamoto Univesity, Japan / Associate Professor

[Details of Activities]

1. Research outline and its perspective

Our research group investigates hydrological phenomenon and hydrochemical issues on near-surface environments including atmosphere, watershed, aquifers, and costal environments on regional scale, using various physicochemical parameters, isotopes, simulation tools, statistical and AI approaches, and large datasets. Effects of human impacts as well as natural disasters on earth systems including floods, earthquakes and volcanism are also major concerns of this research cluster. Our research with multidisciplinary approach provides important base for developing sustainable and well-being society.

Photo 1. Artesian well in Kumamoto

2. Research progress and results in the fiscal year

Our team mainly carried out the following five activities planned at the end of last year.

1. Developing regional groundwater flow simulation model: Against the backdrop of increasing demand of groundwater resource due to the expansion of semiconductor-related companies, we have incorporated the latest geological information and water use information and updated the

world's most detailed physically based regional groundwater flow simulation model, by applying GETFLOWS. The Kumamoto University, Kumamoto Prefecture, Kumamoto City, and local companies combined their expertise to achieve this goal.

2. Numerical and statistical study : In order to understand the characteristics and causes of groundwater level fluctuations in Kumamoto on regional scale, we collected as much long-term data as possible on water levels, Lake Ezu spring discharge volume, and various meteorological parameters, for the period before the occurrence of the 2016 Kumamoto earthquake. We performed trend and statistical analysis and clarified their spatiotemporal characteristics. Cluster member Dr. Zhi-Qiang Yu took the lead in carrying out the study, and the results are currently being submitted to an academic journal for peer review.
3. Assessment for groundwater contamination: In order to evaluate the long-term state of groundwater nitrate-nitrogen pollution in Kumamoto, we integrated trend analysis based on agriculture and forestry census archives data with long-term water quality and nitrogen-oxygen stable isotope analysis, and their spatiotemporal transition characteristics were revealed. I devoted myself in writing research paper as a first author, and the results were published in Ecological Indicators, Elsevier B.V. IROAST provided financial support (US$2600 ≒ JPY 321,354) for publication fees. I would like to express my gratitude for the great support. In addition, similar themes were implemented on Okinawa Island, and the results were published in two international journals through PhD study of the international student.
4. Characterization of river water quality on national scale: Aiming to visualize land-sea interactions and material circulation in Japan, we conducted a water sampling survey on rivers that had no reports in existing materials in order to understand the basic water quality characteristics of 109 first-class rivers nationwide (Photo 2). In addition, I discussed the concept of this research with Professor Jens Hartmann.
5. Water quality research in the Kirishima area: Dr. Gibran Romero Mujalli is taking the lead in writing a paper based on the data accumulated so far. We are currently at the stage of finalizing the manuscript in cooperation with Professor Jens Hartmann, who came to Japan at the end of this year (Photos 2 and 3).

Photo 2. River water sampling survey

Photo 3. Discussion with Prof. Jens Hartmann

3. Research plan for the next fiscal year

We are planning next semester year's research based on the research results we obtained this semester year. First, while trying to improve the reproducibility of the groundwater flow simulation model built this semester year, we will also try to build a nitrogen dynamics simulation model next semester year by using improved groundwater flow simulation model. Also, while achieving publication for the research topic discussing groundwater level fluctuations focused on the period before the Kumamoto Earthquake, we will further conduct numerical and trend analysis

with the aim of accurately evaluating the impact of the earthquake, targeting the period during 2016 and 2022, when the impact of the Kumamoto earthquake has been confirmed. The impact of groundwater pumping on regional groundwater levels due to economic development in the future will need to be determined based on the data at the period after the impact of the earthquake has returned to normal, therefore, it is important to accurately estimate the impact of the earthquake. In addition, we aim to publish research regarding water quality formation in the Kirishima area in international journals. Finally, we have a plan to accept doctoral student from Italy in 2025, and will enhance discussion on issues that will enable the acceleration of joint research through degree research.

4. List of journal papers

Maruyama, R., Yasumoto, K., Mizusawa, N., Iijima, M., Yasumoto-Hirose, M., Iguchi, A., Hermawan, O.R., **Hosono, T.**, Takada, R., Song, K.-H., Shinjo, R., Watabe, S., Yasumoto, J., 2024. Metagenomic analysis of the microbial communities and associated network of nitrogen metabolism genes in the Ryukyu limestone aquifer. Scientific Reports 14, 4356. https://doi.org/10.1038/s41598-024-54614-8

Hermawan, O.R., **Hosono, T.**, Yasumoto, J., Yasumoto, K., Song, K.-H., Maruyama, R., Iijima, M., Yasumoto-Hirose, M., Takada, R., Hijikawa, K., Shinjo, R., 2024. Mechanism of denitrification in subsurface-dammed Ryukyu limestone. Science of the Total Environment 912, 169457. https://doi.org/10.1016/j.scitotenv.2023.169457

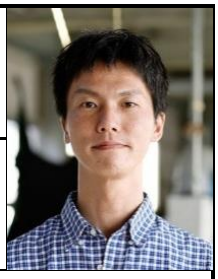
Hosono, T., Taniguchi, K., Rahman, A.T.M.S., Yamamoto, T., Takayama, K., **Yu, Z.-Q.**, Aihara, T., Ikehara, T., Amano, H., Tanimizu, M., Nakagawa, K., 2023. Stable N and O isotopic indicators coupled with social data analysis revealed long-term shift in the cause of groundwater nitrate pollution: insights into future water resource management. Ecological Indicators, 154, 110670. https://doi.org/10.1016/j.ecolind.2023.110670

Hermawan, O.R., **Hosono, T.**, Yasumoto, J., Yasumoto, K., Song, K.-H., Maruyama, R., Iijima, M., Yasumoto-Hirose, M., Takada, R., Hijikawa, K., Shinjo, R., 2023. Effective use of farmland soil samples for N and O isotopic source fingerprinting of groundwater nitrate contamination in the subsurface dammed limestone aquifer, Southern Okinawa Island, Japan. Journal of Hydrology, 619, 129364. https://doi.org/10.1016/j.jhydrol.2023.129364

Mizota, C., Hosono, T., Okumura, A., Yamanaka, T., 2023. Nitrogen cycling in western India as revealed by nitrogen isotopes and the historic production of saltpetre. Archaeometry, 65, 3, 635-652. https://doi.org/10.1111/arcm.12830

5. List of awards, grants, and patents

- JSPS Grant-in-Aid for Scientific Research A (22H00563), 2022-2025, Leader: Takahiro Hosono

No.2-4-3	Deep Learning for Hydrology		
Name	Kei ISHIDA		
Affiliation	CWMD Email: keiishida@kumamoto-u.ac.jp	Title	Associate Professor
Research Field	Environment-friendly technology / Strengthening resilience / Data science and AI		

Cluster Members	
Name	Affiliation/Title
Motoki AMAGASAKI	FAST・Professor
Masato KIYAMA	FAST・Assistant Professor
Ali ERCAN	Middle East Technical University, Türkiye・Associate Professor
Tongbi TU	Sun Yat-Sen University, China・Associate Professor

[Details of Activities]

1. Research outline and its perspective

Our research cluster explores the potential uses of deep learning in hydrology. Given the rapid advancements in this field, we employ the latest techniques from various research domains to address hydrological challenges. Our aim is to identify the most appropriate deep-learning methods for each problem and to develop new techniques specifically designed for hydrological applications.

2. Research progress and results in the fiscal year

In pursuit of advancing hydrology, we have initiated an investigation of the potential of a novel deep learning approach known as graph neural network (GNN). GNN is designed to take into account the connections among nodes, which may prove beneficial in aiding models to acquire and comprehend geographical spatial relationships within hydrological challenges. Subsequently, we have commenced the integration of GNNs into our hydrological problem work, with the objective of unveiling new insights and enhancing the accuracy of hydrological simulations. Specifically, we have applied GNN for flood forecasting this fiscal yar. There are various architectures of GNNs, and we selected the most appropriate one for flood forecasting. By leveraging the characteristics of GNNs, we have made it possible to include river connectivity and the difference in gradient of sub-basins in the flood forecast. This is a capability that has not been possible with the deep learning methods employed in flood forecasting until now. The outcomes of this research are being documented in an academic paper.

Currently, considerable attention and recognition are being directed towards ChatGPT, a highly advanced and large-scale language model that has been developed using cutting-edge deep learning techniques. This innovative model has the potential to generate a wide range of textual content, including programming code. This fiscal year, we have initiated extensive research and evaluation of ChatGPT's abilities as an indispensable support tool in addressing various

hydrological issues. We have initiated the development of a method leveraging ChatGPT to automatically execute tasks that were previously reserved for experts, thereby obviating the necessity for experts.

Additional studies involving deep learning were also conducted on various other subjects. One such study involved gathering soil test data from boring data collected across Japan. By utilizing deep learning, we were able to develop a method for accurately estimating hydraulic conductivity from different types of Soil Test Data. Furthermore, by using a variety of observation data and enhancing the architecture, we were able to improve the precision of flood forecasting through the use of deep learning. In addition,

Moreover, using the outcomes of profound learning conducted up until the previous year, we drafted and submitted two papers to international journals. This fiscal year, we invited Dr. Ali Ercan, an international collaborator, to Japan (Photo. 1). Additionally, I visited Middle East Technical University in Türkiye, where Dr. Ercan is affiliated, (Photo. 2) to promote and foster joint research endeavors.

Photo 1 Meeting at Kumamoto University

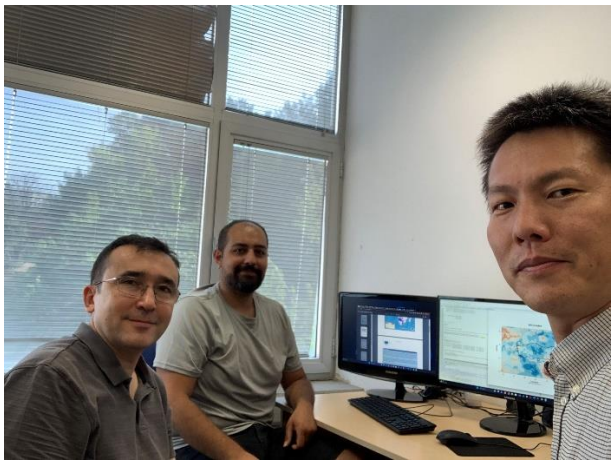


Photo 2 Meeting at Middle East Technical University, Turkey.

3. Research plan for the next fiscal year

During the forthcoming fiscal year, we will proceed with our ongoing research utilizing graph neural networks (GNN). We will complete and submit a manuscript on GNNs to an international journal, which we have been working on since last year. Additionally, we will explore the potential application of GNNs to various issues beyond flood forecasting, as we successfully employed GNNs for this purpose in the current year.

Research utilizing ChatGPT will persist. Over the next fiscal year, we will explore not only ChatGPT, but also other generative AI models such as Claude and Gemini. In the previous year, our research was based on publicly available online data. This year, we intend to collaborate with a civil engineering consulting company to obtain additional data.

As we have in previous years, we will continue to undertake a variety of studies this year, including estimating hydraulic conductivity and enhancing flood forecasting. Once sufficient findings have been obtained, we plan to publish our results in international journals.

No. 2-4-4	Advanced Biomedical Evaluation System		
Name	Makiko KOBAYASHI		
Affiliation	Faculty of Advanced Science and Technology Email: kobayashi@cs.kumamoto-u.ac.jp	Title	Professor
Research Field	Biotechnology & healthcare technology / Strengthening resilience / Advanced materials / Data science and AI		

Cluster Members

Name	Affiliation/Title
Toshitaka YAMAKAWA	Nara Institute of Science and Technology / Affilliate Professor
Masayuki TANABE	FAST, Kumamoto University / Assistant Professor
Rajendra Udyavara ACHARYA	University of Southern Queensland, Australia / Professor
Oliver FAUST	Faculty of Science and Engineering, Anglia Ruskin University, UK / Associate Professor
Ru san TAN	National Heart Centre / Doctor

[Details of Activities]

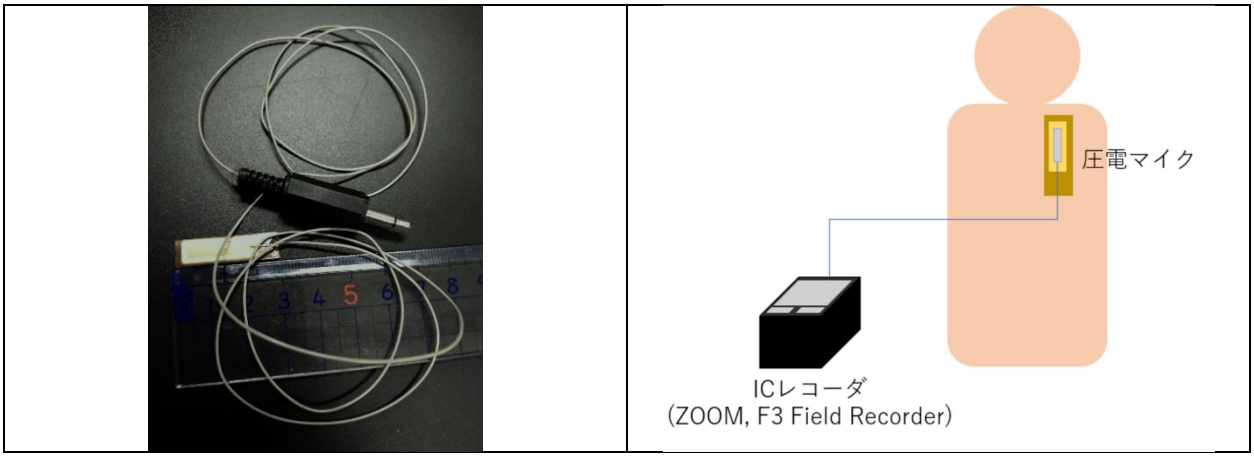
1. Research outline and its perspective

For the sustainable continuity of society, medical DX (Digital Transformation) is indispensable. This necessitates a system incorporating inexpensive wearable sensors that automatically assess acquired data and connect patients to hospitals when necessary. Our research cluster aims to establish a system where results obtained from high-performance flexible piezoelectric sensors and measurement system (Kumamoto University's proprietary) are automatically diagnosed by AI technology on the Australian team.

2. Research progress and results in the fiscal year

At Kumamoto University, research and development on flexible ultrasound and piezoelectric sensors utilizing porous piezoelectric films have been conducted. Recording heart sounds experiment was conducted using flexible piezoelectric devices. The device, as shown in the figure, consists of a porous PZT piezoelectric film (thickness: 80-100 μm) formed on a SUS substrate (thickness: 50 μm, length: 28 mm, width: 8 mm), with a length of 21 mm and a width of 6 mm. A top electrode with a thickness of 10 μm, with a length of 18 mm and a width of 4 mm was fabricated.

In this study, heart sound measurements were attempted on one adult (male) participant as shown in the figure. The developed stethoscope was connected to an IC recorder. The participant conducted recordings by placing the developed microphone on their chest while seated. As a result, successful recordings of heart sounds were achieved at the tricuspid area located at the left sternal border of the fourth intercostal space. For blood to be pumped out of the heart, the opening and closing of the atrioventricular and semilunar valves are necessary. Heart sounds are composed of the sound of the atrioventricular valves closing (first sound) and the sound of the semilunar valves closing (second sound). It is known that the main components of these sounds are around 100Hz. Frequency analysis of the recorded heart sounds revealed that they are primarily composed of sounds below 100Hz, with two peaks observed around 10Hz and 30Hz. This result is consistent with previous studies on heart sound analysis, thus it also proved the successful measurement of heart sounds.



3. Research plan for the next fiscal year

Kobayashi will visit a research institution in Australia to reaffirm the research structure and future tasks. Initially, experiments will utilize a simulated robot in Kumamoto University. Improvements to the wearable stethoscope will be made by Kumamoto University side, while automatic detection of abnormal heart sounds and lung noises measured using commercially available electronic stethoscopes will be carried out by Australia side. Discussions will be held to determine where the clinical study will take place among Kumamoto University, Singapore, and Australia.

4. List of journal papers (with IROAST as your affiliation) published between April 2023 and March 2024.

1. Thin, flexible, and biocompatible medical ultrasound array transducer using a sol–gel composite spray technique, Masayuki Tanabe, Kosuke Sato, Toru Uda and Makiko Kobayashi, Jpn. J. Appl. Phys. 62 SJ1034 (2023) https://doi.org/10.35848/1347-4065/acbc27
2. Innovative Fibromyalgia Detection Approach Based on Quantum-Inspired 3LBP Feature Extractor Using ECG Signal, Prabal Datta Barua, Makiko Kobayashi, Masayuki Tanabe, Mehmet Baygin, Jose Kunnel Paul, Thomas Iype, Sengul Dogan, Turker Tuncer, Ru San Tan, U. Rajendra Acharya, IEEE Access 11, 101359-101372 (2023)

https://doi.org/10.1109/ACCESS.2023.3315149

3. FF-BTP Model for Novel Sound-Based Community Emotion Detection, Arif Metehan Yildiz, Masayuki Tanabe, Makiko Kobayashi, Ilknur Tuncer, Prabal Datta Barua, Sengul Dogan, Turker Tuncer, Ru San Tan, U. Rajendra Acharya, IEEE Access 11, 108705-108715 (2023)
https://doi.org/10.1109/ACCESS.2023.3318751
4. GCLP: An automated asthma detection model based on global chaotic logistic pattern using cough sounds, Kilic, Mehmet; Barua, Prabal Datta; Keles, Tugce; Yildiz, Arif Metehan; Tuncer, Ilknur; Dogan, Sengul; Baygin, Mehmet; Tuncer, Turker; Kuluozturk, Mutlu; Tan, Ru-San; Acharya, U. Rajendra
Engineering Applications of Artificial Intelligence, 127, Part A, 107184, 2024
https://doi.org/10.1016/j.engappai.2023.107184
5. A Novel Attention-Based Model for Semantic Segmentation of Prostate Glands Using Histopathological Images, Mahesh Anil Inamdar; U. Raghavendra; Anjan Gudigar;Sarvesh Bhandary; Massimo Salvi; Ravinesh C. Deo; Prabal Datta Barua; Edward J. Ciaccio; Filippo Molinari; U. Rajendra Acharya, IEEE Access 11, 108982-108994 (2023)
https://doi.org/10.1109/ACCESS.2023.3321273
6. Brain tumor detection and screening using artificial intelligence techniques: Current trends and future perspectives, Raghavendra, U.; Gudigar, Anjan; Paul, Aritra; Goutham, T. S.; Inamdar, Mahesh Anil; Hegde, Ajay; Devi, Aruna; Ooi, Chui Ping; Deo, Ravinesh C.; Barua, Prabal Datta; Molinari, Filippo; Ciaccio, Edward J.; Acharya, U. Rajendra, Computers in Biology and Medicine 163, 107063 (2023)
https://doi.org/10.1016/j.compbiomed.2023.107063
7. Automated detection of scaphoid fractures using deep neural networks in radiographs, Amanpreet Singh, Ali Abbasian Ardakani, Hui Wen Loh, P. V. Anamika, U. Rajendra Acharya, Sidharth Kamath, Anil K. Bhat, Engineering Applications of Artificial Intelligence, 122, 106165, 2023
https://doi.org/10.1016/j.engappai.2023.106165
8. Automated detection and screening of depression using continuous wavelet transform with electroencephalogram signals, U. Raghavendra, Anjan Gudigar, Yashas Chakole, Praneet Kasula, D. P. Subha, Nahrizul Adib Kadri, Edward J. Ciaccio, U. Rajendra Acharya, Expert Systems 40 (4), e12803 (2023)
https://doi.org/10.1111/exsy.12803
9. A Data-Driven Hybrid Methodology Using Randomized Low-Rank DMD Approximation and Flat-Top FIR Filter for Voltage Fluctuations Monitoring in Grid-Connected Distributed Generation Systems, Mohan, Neethu; Kumar, S. Sachin; Soman, K. P.; Sujadevi, V. G.; Poornachandran, Prabaharan; Acharya, U. Rajendra, IEEE Access 11, 39228 - 39242, 2023
https://doi.org/10.1109/ACCESS.2023.3267125
10. SchizoNET: a robust and accurate Margenau–Hill time-frequency distribution based deep neural network model for schizophrenia detection using EEG signals, Smith K Khare, Varun Bajaj and U Rajendra Acharya, Physiological Measurement 44, 3, 035005 (2023)
https://doi.org/10.1088/1361-6579/acbc06
Published in the previous year, but included in this report due to being unlisted in the previous year.
11. Automated detection of airflow obstructive diseases: A systematic review of the last decade (2013-2022), Shuting Xu, Ravinesh C Deo, Jeffrey Soar, Prabal Datta Barua, Oliver Faust, Nusrat Homaira, Adam Jaffe, Arm Luthful Kabir, U Rajendra Acharya, Computer Methods and Programs in Biomedicine 241, 107746, 2023
https://doi.org/10.1016/j.cmpb.2023.107746
12. An explainable and interpretable model for attention deficit hyperactivity disorder in children using EEG signals, Smith K Khare, U Rajendra Acharya, Computers in Biology and Medicine 155, 106676 (2023)
https://doi.org/10.1016/j.compbiomed.2023.106676
Published in the previous year, but included in this report due to being unlisted in the previous year.

13. Automated warfarin dose prediction for Asian, American, and Caucasian populations using a deep neural network, Jahmunah, V.; Chen, Sylvia; Oh, Shu Lih; Acharya, U. Rajendra; Chowbay, Balram, Computers in Biology and Medicine, 153, 106548, 2023 https://doi.org/10.1016/j.compbiomed.2023.106548
**Published in the previous year, but included in this report due to being unlisted in the previous year.*
14. An Explainable Deep Learning Model to Prediction Dental Caries Using Panoramic Radiograph Images, Oztekin, Faruk; Katar, Oguzhan; Sadak, Ferhat; Yildirim, Muhammed; Cakar, Hakan; Aydogan, Murat; Ozpolat, Zeynep; Yildirim, Tuba Talo; Yildirim, Ozal; Faust, Oliver; Acharya, U. Rajendra, Diagnostics, 13 (2), 226, 2023 https://doi.org/10.3390/diagnostics13020226
**Published in the previous year, but included in this report due to being unlisted in the previous year.*
15. Uncertainty quantification in DenseNet model using myocardial infarction ECG signals, Jahmunah, V.; Ng, E. Y. K.; Tan, Ru-San; Oh, Shu Lih; Acharya, U. Rajendra, Computer Methods and Programs in Biomedicine, 229, 107308, 2023 https://doi.org/10.1016/j.cmpb.2022.107308
**Published in the previous year, but included in this report due to being unlisted in the previous year.*

5. List of awards, grants, and patents

N/A

No. 2-4-5	Reprogramming Multi-Drug Resistance Breast Cancer for Women's health and quality of life		
Name	Ruda LEE		
Affiliation	Institute of Industrial Nanomaterials (IINa) Email: aeju-lee@kumamoto-u.ac.jp	Title	Associate Professor
Research Field	Biotechnology & healthcare technology		
Cluster Members			
Name	Affiliation/Title		
Seung-Hae KWON	Korea Basic Science Institute, Korea Principal investigator		
Jungkyu KIM	University of Utah, USA Associate Professor		

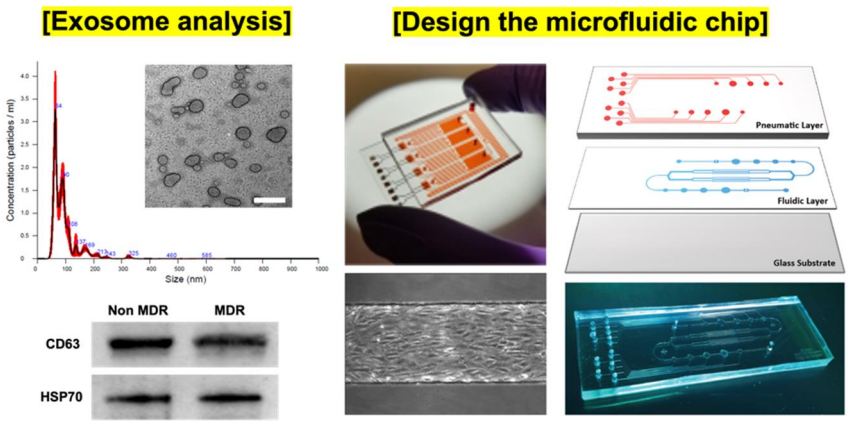
[Details of Activities]

1. Research outline and its perspective

Chemotherapy has been used for primary conventional treatment for cancer, but multidrug resistance (MDR) poses a significant challenge in chemotherapy. Several complex regulatory signaling mechanisms dynamically crosstalk to initiate, establish, and maintain tolerance/resistance to different chemotherapeutic agents in breast cancer.

2. Research progress and results in the fiscal year

In this research, we mainly worked for exosome tracking under a microfluidic channel and will evaluate the exosome-mediated reprogramming of the MDR to drug-sensitive conditions. The research clusters were designed for gravity-related microfluidic chips and formed a cancer environment. The cellular interaction between the drug-sensitive and MDR cells was tracked by super-resolution microscopy and indicated the movement of extracellular vesicles (EVs).



3. Research plan for the next fiscal year

Exosome extraction and evaluation methods were established for future investigations. Next year, exosome movement will be monitored using RFP- and GFP-transfected cell lines. Changes in cell line characteristics will be scrutinized through DNAseq and RNAseq analyses. Subsequently, treatment strategies will be explored using nanoplatforms based on insights gained from gene expression differences.

4. List of journal papers (with IROAST as your affiliation) published between April 2023 and March 2024.

Ruda LEE

1) Ruda Lee, Sho Tanigawa, Yong Il Park, Hoon Kim. Antimetabolite Prodrug Delivery for Non-small Cell Lung Cancer, *Korean Society for Biotechnology and Bioengineering Journal*, 38: 236-243, 2023

5. List of awards, grants, patents, and other achievements

Ruda LEE

1) FY2022 JSPS, Grant-in-Aid for Scientific Research (C)
2) Brain Pool Korea 2022
3) FY2023 Kumamoto University Research Achievement Awards
4) Ruda Lee, Yong Il Park. Exosome detection for early cancer diagnosis. *Springer Handbook of Cancer and Immunology*, Sep 2023 **Publication of Book*

Seung-Hae KWON

N/A

Jungkyu KIM

1) US patent, Jungkyu Kim, Minju Kim, Kanghoon Choi, Zachary Ryan Estlack. Dynamic cell culture platform for combinatorial and biomechanical stimulation. 2023

No. 2-4-6	Novel cancer medical technology using liquid biopsy		
Name	Yuta NAKASHIMA		
Affiliation	Faculty of Advanced Science and Technology Email: yuta-n@mech.kumamoto-u.ac.jp	Title	Associate Professor
Research Field	Biotechnology & healthcare technology		

Cluster Members	
Name	Affiliation/Title
Chengkou LEE	National University of Singapore/Professor
Wataru IWASAKI	National Institute of Advanced Industrial Science and Technology/Chief research officer
Yoichi SAITO	Faculty of Advanced Science and Technology, Kumamoto University/Assistant Professor
Mami AKAIKE	Graduate School of Science and Technology, Kumamoto University/Doctoral student

[Details of Activities]

1. Research outline and its perspective

This research objective is to develop the palm size medical devices for cancer detection, post-surgical management, medical treatment, drug discovery, and etc. and the novel techniques for achieving these devices. In this research project, we focused the cell-based biomarker such as cell morphology, cell expressed proteins, cell signaling, cell behavior, and responses to stimulation for achieving the above objective. Through these, we challenge to create comprehensive cancer medical systems.

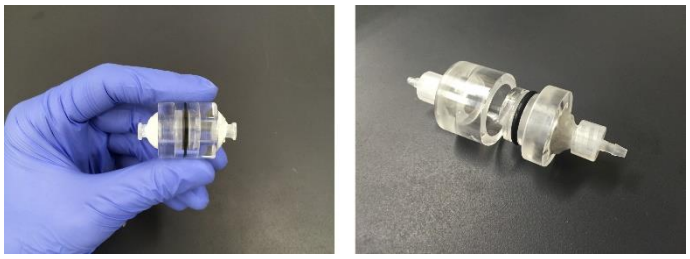


Fig. 1 Schematic of a cancer diagnosis device.

2. Research progress and results in the fiscal year

In this year, we carried out the development of the technologies for the detection and analysis of biological substances.

(a) Cancer detection technology by cell morphology change and migration

The morphological changes, amount and direction of migration of cells affected by cancer cells were evaluated by culturing cancer cells and normal cells in the same

environment. In this experiment, a microfluidic device was used for creating the concentration gradient of cancer cell production substance (Fig.2). The morphology of cells cultured in the various cancer cell environments were changed to flattened or pointed shapes, depending on the type of cancer. Also, migration induction and amount were changed depending on cancer type. These results suggest that the type of cancer can be identified by evaluating cell shape and migration using the fabricated device.

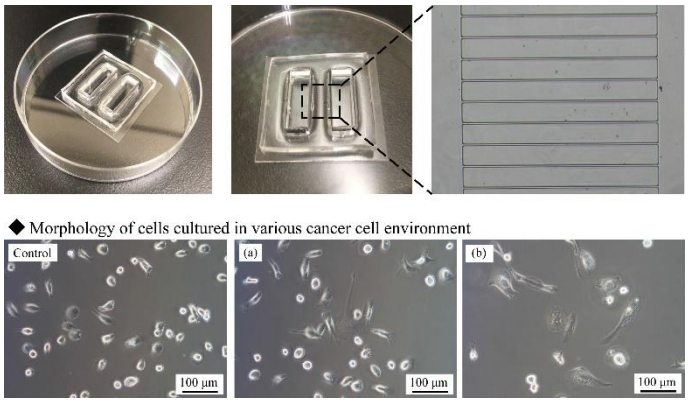


Fig. 2 The fabricated microfluidic device for evaluating cell morphology and migration.

(b) Cancer detection technology by immune response

The objective is to develop a technique to locate the primary tumor by liquid biopsy using the immune response. At first, we evaluated the immune response of immune cells co-cultured with the various types of cancer cells. As a results, we clarified the immune response that occur specifically in different cancer types. In addition, we succeeded in fabricating a device that can detect immune response substance in real time by combining a microfluidic device for immune response with QCM (quartz crystal microbalance) (Fig.3).

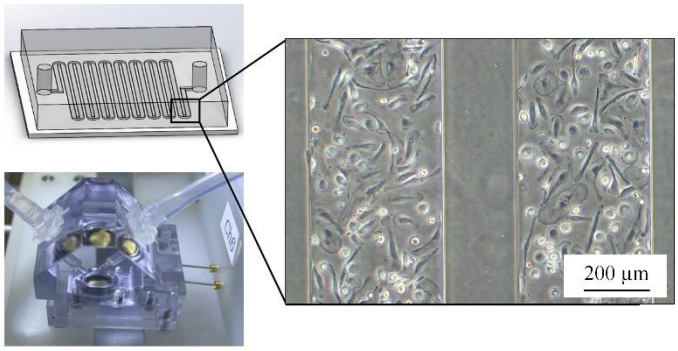


Fig. 3 The fabricated cancer detection (biosensing) device.

3. Research plan for the next fiscal year

In the next year, the microfluidic device that can detect primary cancer cells using liquid samples will be validated. Specifically, supernatants of three types of cancer cells will be prepared, and the response behavior of the cells when the supernatants are applied to will be evaluated using biosensor. Based on the results, the three types of cancer will be identified. We will deepen our collaboration with Prof. Lee at NUS on this biosensing technology. In addition, we will also deepen collaboration with Dr. Iwasaki of AIST on analytical techniques, especially paper-based analytical techniques.

List of journal papers

*Corresponding author

[1] Haruhiko Takemoto, Keito Sonoda, Kanae Ike, Yoichi Saito, Yoshitaka Nakanishi, Yuta Nakashima*, "Development of Cell Micropatterning Technique Using Laser Processing of Alginate Gel," Journal of Robotics and Mechatronics, 35(5), 1185-1192, 2023.

[2] Yuta Kishimoto, Sachiko Ide, Toyohiro Naito, Yuta Nakashima, Yoshitaka Nakanishi, Noritada Kaji, "Development of a Microfluidic Ion Current Measurement System for Single-Microplastic Detection," Journal of Robotics and Mechatronics, 35(5), 1193-1202, 2023.

[3] Yoshitaka Nakanishi, Yukio Fujiwara, Yuta Nakashima, "Generation of Nano/Microplastics for Immunological Assessments," Biotribology, 33-34, 100235, 2023.

[4] Yoshitaka Nakanishi, Yukio Fujiwara, Yuta Nakashima, Yoshihiro Komohara, Kazunori Hino, Hiromasa Miura, Hidehiko Higaki, "Microchamber device for studying phagocytosis of ultra-high molecular weight polyethylene particles by human monocyte-derived macrophages," Wear, 523, 204749, 2023.

[5] Yoichi Saito, Yukio Fujiwara, Yuji Miyamoto, Koji Ohnishi, Yuta Nakashima, Yasuhiko Tabata, Hideo Baba, Yoshihiro Komohara, "CD169+ sinus macrophages in regional lymph nodes do not predict mismatch-repair status of patients with colorectal cancer," Cancer Medicine, 12(9), 10199-10211, 2023.

List of awards

[1] New Field Development Award, Micro-Nano Science and Technology Division, Japan Society of Mechanical Engineering, 2023.

List of grants

[1] KAKENHI (Grant-in-Aid for Challenging Research (Exploratory)), ¥ 4,900,000-, Apr. 2023 – Mar. 2025. (Principal Investigator)

[2] KAKENHI (Grant-in-Aid for Scientific Research (B)), ¥ 3,000,000-, Apr. 2020 – Mar. 2023. (Co-Investigator)

[3] Go-Tech (成長型中小企業等研究開発支援事業), ¥ 7,498,400-, Apr. 2023 – Mar. 2024. (Sub-Leader)

[4] Go-Tech (成長型中小企業等研究開発支援事業), ¥ 3,092,949-, Apr. 2023 – Mar. 2024. (Co-Investigator)

[5] JST FOREST, ¥ 20,000,000-, Apr. 2022 – Mar. 2025. (Principal Investigator)

[6] KAKENHI (Grant-in-Aid for Scientific Research (C)), ¥600,000-, Apr. 2022 – Mar. 2025. (Co-Investigator)

[7] KAKENHI (Fund for the Promotion of Joint International Research (Fostering Joint International Research (B))), ¥ 2,500,000-, Apr. 2019 – Mar. 2025. (Co-Investigator)

List of social contributions

[1] Committee Member
Research for Innovation & Synthesis of Technology in Kumamoto
Apr. 2023 – Mar. 2024

[2] Editorial committee
40th Sensorsymposium, IEEJ (The Institute of Electrical Engineers of Japan)
Mar. 2023 – Dec. 31, 2023.

[3] Steering committee
Micro-Nano Science & Technology Division, The Japan Society of Mechanical Engineers
Apr. 2023 – Mar. 2024.

[4] Representative
Micro-Nano Science & Technology Division, The Japan Society of Mechanical Engineers
Apr. 2023 – Mar. 2024.

[5] Committee member
The 14th Symposium on Micro-Nano Science and Technology

Apr. 2023 – Mar. 2024.

[6] Committee member

Cheminas48, Society for Chemistry and Micro-Nano-Systems

Apr. 2023 – Mar. 2024.

List of patents

[1]発明の名称：光学測定器用サンプルホルダおよび光学測定器

登録番号：特許第 7425428 号

登録日：2024 年 1 月 23 日

発明者：**中島雄太**，西川昌平

出願人：国立大学法人熊本大学，西川計測株式会社

No. 2-4-7	Separation, synthesis, and detection by means of ionic solutes handling		
Name	Shin-Ichi OHIRA		
Affiliation	FAST, IROAST Email: ohira@kumamoto-u.ac.jp	Title	Professor
Research Field	Environment-friendly technology		

Cluster Members	
Name	Affiliation/Title
Jian MA	Xiamen University, China / Professor
C. Phillip SHELOR	University of Texas at Arlington, USA / Assistant Professor
Ganjar FADILLAH	Universitas Islam Indonesia, Indonesia / Lecturer

[Details of Activities]

1. Research outline and its perspective

Ion is the key chemical form in environment, human health, and industries. In the present study, electrodialytic ion transfer technologies are applying to separation, purification and synthesis of ionic solutes. For the separation, one of the most difficult separation, chiral separations are studied. The method may be suited for pharmacy purification. For the purification, the ionic solutes as contaminants can be removed effectively and is suited for semiconductor industry. The synthesis is also suited for many areas which required highly pure chemicals. The ionic solute handling will be spread to not only chemical analysis but also many science and technology areas.

2. Research progress and results in the fiscal year

In FY 2023, we have succeeded the following applications:

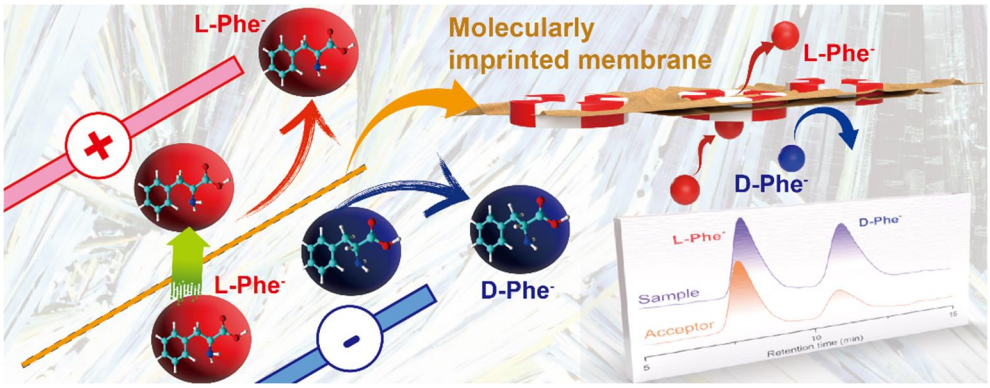
a) **Electrodialytic removal of ionic solutes for a purification of particulate solutions**. The ionic solutes contained in the particle solutions are interfering for analyzing the distribution of metal particles. The developed method can decrease the size limit of the detection for the metal particle analysis. (The results are applied to the patent.)

b) **Selective ion adsorption with ion imprinted membrane for trace metal in sea water analysis**. The ion imprinted membrane was successfully synthesized after the parameter optimizations. The membrane showed strong selectivity to the targeted Zn^{2+}. The membrane was used as the trap of Zn^{2+} from artificial sea water then desorbed with the electric field. (The manuscript is under preparation.)

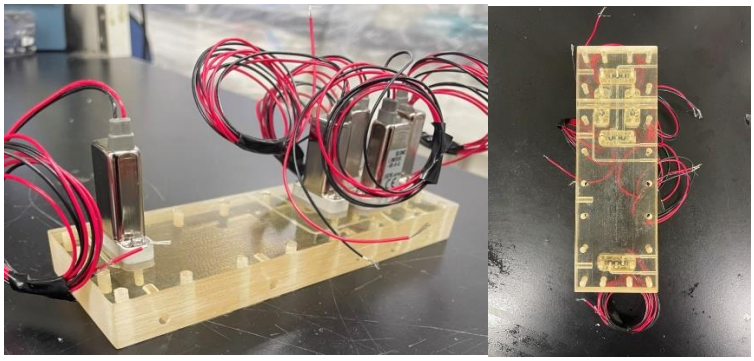
c) **Chiral separation for amino acids.** The molecular imprinted membrane was successfully prepared to transfer one of the chiral compounds from racemic solutions.

d) **Radio isotopes separation for positron emission tomography diagnosis probe.** Short half life radio isotopes generated with a cyclotron required rapid separation from the target. This separation was achieved with 3D-printed flow devices.

e) **Synthesis of ionic compounds**. Not only an ionic liquid but also ionic compounds are successfully synthesized with our electrodialytic flow synthesis. The purity of the synthesized compounds are also high enough.



Chiral separation for amino acids


3D-printed flow device for radioisotope separation

3. Research plan for the next fiscal year

In FY2024, we are going to study followings:

a) **Trace metal analysis in organic solvents with liquid electrode plasma spectroscopy (LEP)**. Highly pure organic solvents are strongly recommended in semiconductor industry. Simultaneously, the evaluation method is recommended. Furthermore, the onsite monitoring is strongly required to keep the solvents highly pure. Thus, our electrodialytic ion transfer is applied to transfer and enrich the metal ions in organic solvents into the matrices which is suited to determine with LEP. LEP is plasma gas free elemental analyzer. This project is supported by A-STEP (JST, 2023~2025).

b) **Ultra-sensitive ion analysis system**. Ion chromatography is powerful tool to determine major ionic solutes. However, the sensitivity is not enough to evaluate ultra-pure water quality without high degrees of enrichment. In this study, we are effectively use radio isotope, which can be detected at very low concentration (~ pmol/L). This project is supported by Grant-in-Aid for Scientific Research(B) (2024~2026FY)

c) **Selective ion extraction for rare-metal recovery and elemental analysis in a sea water**. Electrodialytic ion transfer with ion imprinted membrane will be expanded to other kinds of metals. Some of the metals are effective for recovering the key elemental material, or matrices isolation for sea water analysis.

d) **Selective chiral compounds extraction for separation and analysis**. One of the difficult separations is chiral separations. We are going to approach to achieve chiral separation for many types of compounds.

Some of the finished study will be published in FY2024.

4. List of journal papers (with IROAST as your affiliation) published between April 2023 and March 2024.

Rahmat Hidayat, Ganjar Fadillah*, Shin-Ichi Ohira, Febi Indah Fajarwati, Dian Ayu Setyorini , Anggi Saputra, Facile green synthesis of Ag doped TiO_2 nanoparticles using Maple leaf for bisphenol-A degradation and its antibacterial properties, *Materials Today Sustainability* (IF: 7.8), **26**, 100752 (2024). ***Scheduled to be issued in June, 2024***
**Co-authored Paper with IROAST Internship student*

Kei Toda*, Vladimir Obolkin, Shin-Ichi Ohira, Kentaro Saeki, Abundant production of dimethylsulfoniopropionate as a cryoprotectant by freshwater phytoplanktonic dinoflagellates in ice-covered Lake Baikal, *Communications Biology* (IF: 5.9), **6**, Article number: 1194 (2023).

Rahmat Hidayat*, Ganjar Fadillah, Shin-Ichi Ohira, Glass tube-coated TiO_2 nanostructure for degradation of methylene blue: an experimental and design of column photocatalytic reactor, *Indonesian Journal of Chemical Analysis (IJCA)*, **6**(1), 52–62 (2023).
Published in the previous fiscal year, but included in this report due to being unlisted in the previous year.
**Co-authored Paper with IROAST Internship student*

5. List of awards, grants, and patents

[Patents]
Registered patents
・Analysis system and method for ionic solutes in ultra-pure water JP7310867 (2023/7/10).
・System and method for Separation and purification of radio isotopes JP7288261 (2023/05/30).

Total 2 patents are newly applied in this period. Furthermore, some of the patents are applied to foreign countries.

[Grants]
Adaptable and Seamless Technology transfer Program through Target-driven R&D (A-STEP) from Japan Science and Technology Agency (JST) Grant Number JPMJTR23RH.
"Development of ultra-trace elemental analysis by means of ion-exchange with liquid electrode plasma" (15,000 kJPY for 2.5 yrs)

Collaborative Research with the companies (8 companies, 10,000 kJPY for FY2023)

No. 2-4-8	Development of Microbially-Aided Carbon Sequestration Technology		
Name	Atsushi SAINOKI		
Affiliation	Faculty of Advanced Science & Technology Email: atsushi_sainoki@kumamoto-u.ac.jp	Title	Associate Professor
Research Field	Environment-friendly technology		

Cluster Members	
Name	Affiliation/Title
Murat KARAKUS	The University of Adelaide, Associate Prof.
Akira SATO	Kumamoto University, Prof.
Kazunori NAKASHIMA	Hokkaido University, Associate Prof.
Hiroaki ITO	Kumamoto University, Assistant Prof.

[Details of Activities]

1. Research outline and its perspective

The purpose of this project is to develop a technology to cause microbially-aided carbon precipitation by injecting CO_2 into a rock in deep underground with anaerobic bacteria, elements, and nutrients. The proposed technology is deeply related to the prevention of global warming with carbon capture and sequestration generally abbreviated as CCS. CCS is one of technologies being developed around the world to reduce the amount of CO_2 released into the atmosphere by injecting carbon dioxide into deep underground with a depth of more than 2000 m. The technology is deemed necessary to achieve the Paris Agreement, but there are several concerns to be addressed, one of which is CO_2 leak-off to the ground surface through pre-existing rock mass fractures and geological structures such as faults and fractured zones. The microbially-aided carbon precipitation can contribute to mitigating the risk for CO_2 leak-off by transforming injected CO_2 into a precipitated carbonate with the help of microbes.

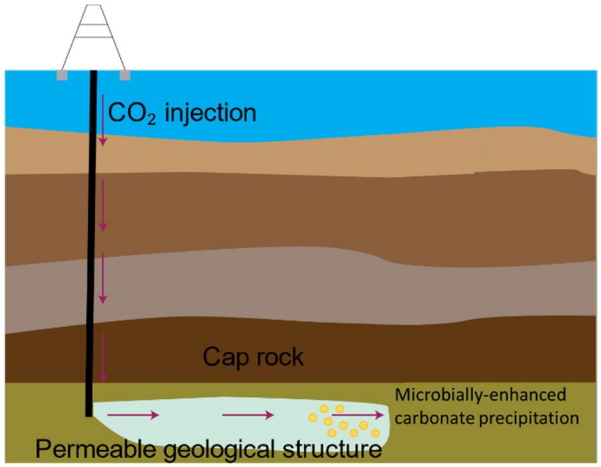


Fig.1: Mitigation of the CO_2 leak-off risk with microbially-enhanced carbon precipitation

2. Research progress and results in the fiscal year

a) EVALUATION OF MINERAL COMPOSITION AND CARBON DIOXIDE INJECTION PRESSURE INFLUENCES ON ANAEROBIC MICROORGANISMS

In order to evaluate the potential of CO_2 mineralization for different types of rocks in carbonated water, we investigated the effects of minerals contained in sandstone, serpentine, peridotite, basalt, and andesite on the immobilization of CO_2 with anaerobic microorganisms considering C_O2 injection pressure, mineral elution and incubation period. Overall, it was shown that the cations required for carbonate mineralization were eluted in about one week. In addition, DNA analysis revealed that although there were likely to be no microorganisms that promoted mineralization in the samples tested for this year, two microorganisms, Esherichia-chelia and Cutibacterium, were resistant to CO_2 and that sandstone-derived microorganisms preferentially lived under low pressure. In the elemental analysis, the culture of coal in sandstone did not lead to mineralization, but in the elution test of serpentine, it was found that carbonate was precipitated in about one week without the cultivation of microorganisms.

Table 1: Analysis of elemental concentration derived from immersion tests of different types of rocks in a pressurized carbonated water

Rock Types	Blank		Sandstone		Serpentinite		Peridotite	
Period	Immediately after	7 days	Immediately after	7 days	Immediately after	7 days	Immediately after	7 days
[Na]	332	1027	14116	120723	433	2068	439	4250
[Mg]	1291	11792	46	1692	11214	3378098	1055	325247
[Al]	3	27	17	27	3	17	97	10
[K]	<0.000	793	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000
[Ca]	84	1819	1191	56712	403	431	297	9285
[V]	0	0	19	7	0	0	1	0
[Cr]	1	67	1	11	1	15	1	7
[Mn]	3	1663	7	1245	<0.000	229	8	1987
[Fe]	<0.000	7125	3	<0.000	<0.000	1537	338	60238
[Co]	0	25	<0.000	8	<0.000	79	0	173
[Ni]	7	1437	<LOD	49	3	8208	5	3014
[Cu]	1	14	4	15	5	8	2	2
[Zn]	17	196	<0.000	101	1	1	20	87
[As]	0	0	1	0	<0.000	0	<0.000	0

[Se]	<0.000	<0.000	1	<0.000	<0.000	<0.000	<0.000	<0.000
[Mo]	2	57	2	1	0	1	<0.000	11
[Ag]	<0.000	0	<0.000	<0.000	<0.000	<0.000	<0.000	<0.000
[Cd]	<0.000	0	0	0	<0.000	<0.000	0	0

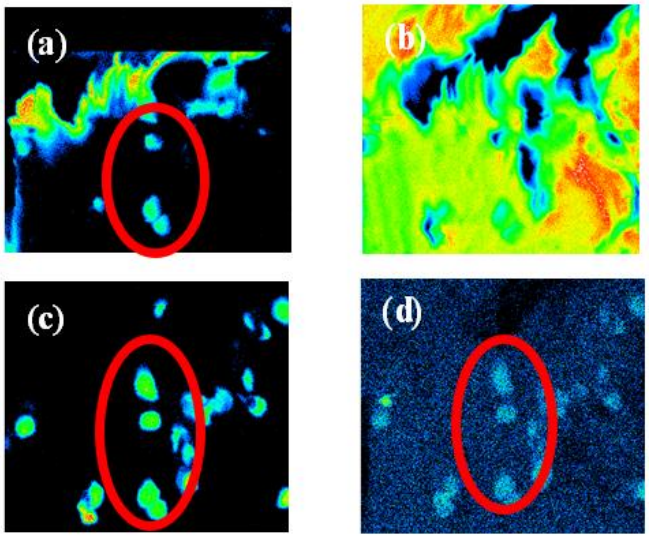


Figure 1: CO_2 mineralization taking place in the immersion test of serpentinite using carbonated water under CO_2 pressure of 0.06 MPa: (a) C, (b) O, (c) Fe, and (d) Ca

b) DEVELOPMENT OF 3D PRINTING TECHNOLOGY FOR CO2 MINERALIZATION TEST IN AN X-RAY CT

3D printing technology with sand powders is a novel and promising method in the field of rock mechanics because of its consistent mechanical properties, not affected inherent heterogeneity and anisotropic characteristics that natural rocks have. However, no fundamental studies have been conducted on the 3D printed specimen, although its mechanical properties are affected by the size distribution of the sand particle. In order to gain a deep understanding on the effect, we performed a series of uniaxial compressive tests in an X-ray CT scanner at Kumamoto University, followed by image analysis to examine strain distribution during the loading stage and its implication for the final rupture. As a result, we successfully delineated an incipient rupture plane. This implies that this technology can be used to detect strain distribution and localization in a fractured 3D printed specimen, thus allowing us to evaluate the effect of CO_2 mineralization in a 3D printed specimen on the strain distribution and development of fractures in the specimen in the future.

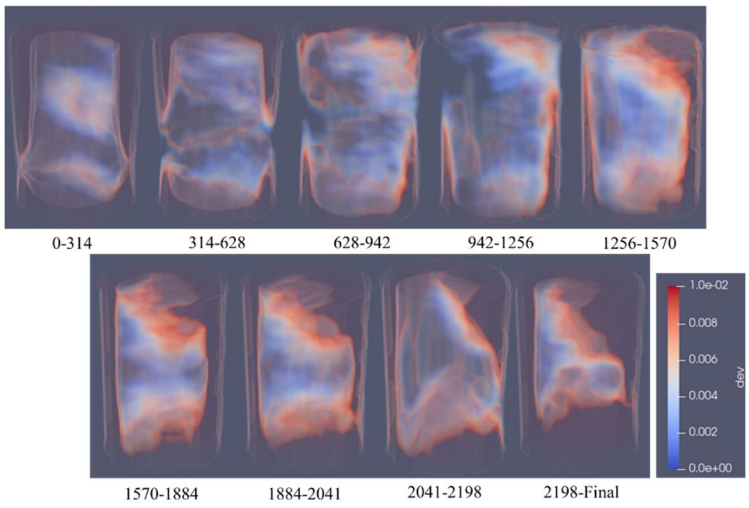


Figure 2: Strain distribution inside a 3D printed specimen derived from image analysis based on CT scanning

c) EFFECT OF FRACTURE STIFFNESS IN A FAULT DAMAGE ZONE ON SEISMIC SOURCE PARAMETERS OF INDUCED FAULT-SLIP

It is well recognized that inherent stress concentration within a fault damage zone may lead to unexpected fault-slip with a large magnitude, resulting in severe damage to underground openings. Previous studies indicate that the intensity of fault-slip is affected by not only the mechanical properties of the fault core but also the stiffness of the surrounding rock mass, implying that fracture stiffness in a fault damage zone could be an important factor that needs to be studied. Hence, in the present study, the effect of the fracture stiffness on seismic source parameters of induced fault-slip is investigated using a mine-wide scale heterogeneous continuum model. The model is constructed based on a discrete fracture network within a fault damage zone, utilizing the crack tensor theory and boundary traction method[1]. The fault core is simulated as a discontinuous plane with interface elements at the center of the model. Furthermore, fault-slip is induced by gradually decreasing the effective normal stress on the fault plane. Lastly, seismic source parameters under different fracture stiffness conditions are computed and analyzed. Seismically radiated energy is defined as the work done by the stress perturbation across a closed surface located at a distance from the earthquake source, whereas seismic moment is calculated by the moment tensor of a seismic source in an anisotropic medium. The fracture stiffness investigated in this study is increased while maintaining the normal-to-shear stiffness ratio of three. Results obtained from the dynamic analysis indicate that seismically radiated energy and seismic moment are notably affected by the fracture stiffness. Both of them significantly decrease with the increase in the fracture stiffness. These results imply that CO_2 mineralization technology could contribute to the control of seismic energy released from induced fault-slip.

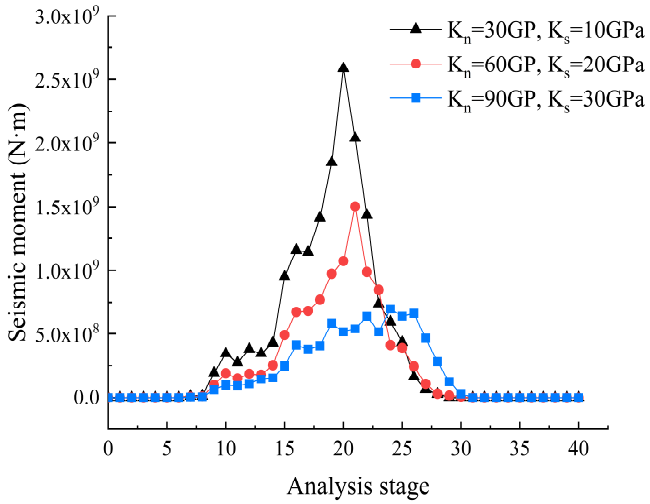


Figure 3: Effect of fracture stiffness in a rock mass on the magnitude of fault-slip

3. Research plan for the next fiscal year

a) CO_2 MINERALIZATION EXPERIMENT

For the next fiscal year, based on the knowledge accumulated this year, a new idea will be tested to enhance CO_2 mineralization by varying CO_2 pressure from a very high value to a lower value, which is intended to decrease pH first and then increase pH. The decrease in pH will enhance the dissolution of minerals into the carbonated water, producing cation. Then, the increase in pH by decreasing CO_2 pressure will cause CO_2 mineralization with the presence of carbonate ion and cation. In addition to this, anaerobic microorganisms that can produce carbonic anhydrase will be tested in order to investigate its potential to enhance CO_2 mineralization. Temperature and pressure will be parameters for the study. As CO2 injection will be performed at depths more than 1000 m,

high pressure more than 10 MPa will be tested while increasing the temperature up to 50 Celsius degrees.

b) TRIAXIAL TESTS OF 3D PRINTED SPECIMEN IN AN X-RAY CT

For the next fiscal year, 3D printed specimens with pre-existing fractures will be generated, which will be then used for small-scale triaxial tests in an X-ray CT. Then, image analyses will be performed to identify strain distribution and localization in the 3D printed specimens. The difficulties expected is the generation of pre-existing fractures in a 3D printed specimen. It is important that the artificially generated fractures should behave in the same way as the natural fractures. Hence, the first step would be to investigate the mechanical behaviour of the fracture and evaluate its stiffness. It is also important to examine accuracy of the self-developed triaxial testing machine. After validating the aforementioned things, the micro-scale strain distribution inside the fractured 3D printed specimen will be investigated.

c) EFFECT OF FRACTURE STIFFNESS ON THE SEISMIC SOURCE PARAMETERS OF INDUCED FAULT-SLIP

This year, the effect of fracture stiffness on the magnitude of induced fault-slip was examined with an equivalent continuum model. For the next fiscal year, the discrete element method will be employed to generate fractures in a fault damage zone, and more accurate simulations will be performed.

4. List of journal papers (with IROAST as your affiliation) published between April 2023 and March 2024.

Feng, Hao, Lishuai Jiang, Qingwei Wang, Peng Tang, Atsushi Sainoki, and Hani S. Mitri. "Effect of surface retaining elements on rock stability: laboratory investigation with sand powder 3D printing." *International Journal of Coal Science & Technology* 10, no. 1 (2023): 46.

Schwartzkopff, Adam K., Atsushi Sainoki, Thomas Bruning, and Murat Karakus. "A conceptual three-dimensional frictional model to predict the effect of the intermediate principal stress based on the Mohr-Coulomb and Hoek-Brown failure criteria." *International Journal of Rock Mechanics and Mining Sciences* 172 (2023): 105605.

Zhang, Zhe*, Lishuai Jiang, Chunang Li, **Yang Zhao***, Atsushi Sainoki**, and Xuanlin Gong. "Characteristics and mechanism of time on sand powder 3D printing rock analogue: a new method for fractured rock mechanics." *Geomechanics and Geophysics for Geo-Energy and Geo-Resources* 9, no. 1 (2023): 166.

**Internship students (1st: ①2022.12.6-2022.12.7/②2023.5.22-2023.6.16, 2nd: 2023.07.24-2023.10. 27) in IROAST Research Internship Program ***Host professor*

5. List of awards, grants, and patents

JSPS Postdoctoral Fellowship (PI: Sainoki)
Kaken B (PI: Professor Sato)

No. 2-4-9	Environmentally promising processes for medical and skincare nanomaterials		
Name	Mitsuru SASAKI		
Affiliation	Institute of Industrial Nanomaterials (IINa) Email: msasaki@kumamoto-u.ac.jp	Title	Associate Professor
Research Field	Biotechnology & healthcare technology / Environmental bioscience / Environment-friendly technology		

Cluster Members

Name	Affiliation/Title
Elisabeth BADENS	Full Professor, Laboratory M2P2, UMR CNRS 7340, Department of Chemistry, Aix Marseille University, France
Olivier BOUTIN	Professor, Dr. Eng., M2P2 Laboratory, Aix Marseille University, France
Hamid HOSANO	Professor, Biomaterials and Bioelectrics Division, Institute of Industrial Nanomaterials (IINa), Kumamoto University, Japan
Marleny D.A. SALDAÑA	Professor in Food/Bio-Engineering Processing, Department of Agricultural, Food and Nutritional Sciences, Faculty of Agricultural, Life and Environmental Sciences, University of Alberta, Canada
Bushra AL-DURI	Reader in Hydrothermal Processing, School of Chemical Engineering, College of Engineering and Physical Sciences, University of Birmingham, UK
Rodolfo MORALES IBARRA	Associate Professor, Faculty of Mechanical and Electrical Engineering Universidad Autonoma de Nuevo Leon, Mexico
Cinthya Soreli CASTRO ISSASI	PhD, Faculty of Mechanical and Electrical Engineering Universidad Autonoma de Nuevo Leon, Mexico

[Details of Activities]

1. Research outline and its perspective

In this research cluster, we would like to propose new processes for producing medically available materials and skincare products with environmentally benign and economically feasible manners. In FY2023, we have got obtained the following findings.

1) Hydrothermal liquefaction experiments of some kinds of citrus peels, wood chips, cotton/polyester blended shirt, and silkworm cocoon were carried out in terms of hydrothermal autoclave at various temperatures, treatment times and biomass/water ratio. As the results, we succeeded to fractionate each component exist in the residual biomass.

2) For improving selectivity of component as well as the degree of liquefaction of the biomass resource, we tried to fabricate a continuous flow-type (or semi-batch type) reaction system that can be used in sub and supercritical water. As for March in 2024, we finished the leak test at up to 10 MPa at room temperature and then confirmed tunability of temperatures in the apparatus.

We would like to complete fabricating the system by the end of this May and will start continuous treatment of citrus peel, blended cloth, silkworm cocoon.

3) With regards to wastewater treatment, we performed joint research with Prof. Takahashi (Iwate University), Prof. Olivier (Aix-Marseille University, France) and two short-term internship students (Emma from France, and Kenneth from Malaysia) and did lots of experiments for decomposition of bisphenol-A at various kinds of operating conditions on the pulsed-arc discharge experiments. Based on the results, we managed to prepare the draft of manuscript.

2. Research progress and results in the fiscal year

I was invited as keynote speakers at the following two international conferences and greatly communicated with researchers and young students in Spain and other European countries.

- The 3rd Global Conference on Polymers, Plastics, and Composites, Barcelona, Spain, and
- International Solvothermal and Hydrothermal Association Conference, Valladorid, Spain

In addition, I was invited by a Japanese film company and the Environmental Division of Kumamoto prefecture to deliver results of our research and developments using sub- and supercritical water and under pulsed discharge condition this January and February, respectively.

3. Research plan for the next fiscal year

The next fiscal year, we will complete preparing manuscripts and submit to international peer-reviewing journals so that all the manuscripts can be accepted and published as much as possible. Also, we start the continuous flow-type reaction system as soon as possible to develop new fractionation method for citrus peels, silkworm cocoon, and blended cloth samples.

I would like to challenge to apply for proposals to JST, Ministry of Environment, MEXT Kakenhi.

4. List of journal papers (with IROAST as your affiliation) published between April 2023 and March 2024.

Mei Matsumura, Jun Inagaki, Ryo Yamada, Natsuko Tashiro, Katsuya Ito, Mitsuru Sasaki*, "Recycling of Cotton/Polyester Blend Fabrics Using Hydrothemal Treatment", *Seikei-Kakou*, **36**(4), 158-160 (2024). https://doi.org/10.4325/seikeikakou.36.158

Mei Matsumura, Jun Inagaki, Ryo Yamada, Natsuko Tashiro, Katsuya Ito, and Mitsuru Sasaki*, "Material Separation from Polyester/Cotton Blended Fabrics Using Hydrothermal Treatment", ACS Omega, 9(11), 13125–13133 (2024). https://doi.org/10.1021/acsomega.3c09350

5. List of awards, grants, and patents

(Patent)
WO 2024/024571 A1 (PCT/JP2023/026272),
"METHOD FOR RECYCLING HIGH-MOLECULAR-WEIGHT MATERIAL, AND FIBER PRODUCT",
TOYOBO Co. LTD. and NATIONAL UNIVERSITY CORPORATION KUMAMOTO UNIVERSITY,
MATSUMURA, Mei; INAGAKI, Jun; ITO, Katsuya; SASAKI, Mitsuru.

No. 2-4-10	Study of First-Generation Objects in the Universe with Radio Telescopes		
Name	Keitaro TAKAHASHI		
Affiliation	FAST Email: keitaro@kumamoto-u.ac.jp	Title	Professor
Research Field	Data science and AI		

Cluster Members

Name	Affiliation/Title
Rachel WEBSTER	Melbourne University Professor
Bart PINDOR	Melbourne University Professor
Takuya AKAHORI	National Astronomical Observatory of Japan Researcher
Shintaro YOSHIURA	National Astronomical Observatory of Japan JSPS Fellow
Takeshi FUKUSAKO	Kumamoto University Professor
Ryo KATO	Kumamoto University Researcher

[Details of Activities]

1. Research outline and its perspective

We aim to detect the 21 cm-line radio waves from neutral hydrogen atoms in the early stage of the universe to probe the first astronomical objects. We develop algorithms and practical software for extracting information from large amounts of observational data precisely and efficiently. In particular, we address the removal of foreground radiation and RFI, which are the most significant obstacles to signal detection.

2. Research progress and results in the fiscal year

Our research group, by a collaboration with Indian groups, made long-term, precise observations of milli-second pulsars with a state-of-the-art radio interferometer named uGMRT [2,6]. Data analysis of pulsar timing array provided, by a combination with data from European group, strong evidence that gravitational waves with nano-hertz frequencies are travelling to Earth from all directions in the Universe [4,5]. The gravitational waves can be thought to come from multiple supermassive black-hole binaries at distant galaxies. The results of this research were released in the press and covered by various media outlets, including newspapers and scientific magazines.
https://www.kumamoto-u.ac.jp/whatsnew/sizen/20230629

Further, we studied the potential usefulness of precise pulsar-distance measurements in the determination of the sky location of a single GW source [3]. We showed that only a few pulsars with a distance precision of 1 pc will improve the precision of the source location by more than 1 order in the presence of white noise of 10 ns.


radio interferometer uGMRT in India


artist impression of gravitational-wave background from supermassive black-hole binaries and pulsar timing array. CREDIT-Danielle Futselaar, MPIfR

3. Research plan for the next fiscal year

We will continue to observe milli-second pulsars and upgrade software to mitigate RFI to improve the calibration and to estimate intrinsic pulsar noise called jitter noise, cooperating with Indian group. Further, we will try to combine observation data from other groups around the world to enhance the statistical significance of gravitational-wave background signal. International cooperation is essential for the above research, and we plan to actively exchange researchers with India and Europe.

4. List of journal papers (with IROAST as your affiliation) published between April 2023 and March 2024.

[1] “A search for auroral radio emission from β Pictoris b”
Yuta Shiohira, Yuka Fujii, Hajime Kita, Tomoki Kimura, Yuka Terada, Keitaro Takahashi,

Monthly Notices of the Royal Astronomical Society, Volume 528, Issue 2, pp.2136-2144, 02/2024

[2] “Multi-band Extension of the Wideband Timing Technique”
Avinash Kumar Paladi, Churchil Dwivedi, Prerna Rana, Nobleson K, Abhimanyu Susobhanan, Bhal Chandra Joshi, Pratik Tarafdar, Debabrata Deb, Swetha Arumugam, A Gopakumar, M A Krishnakumar, Neelam Dhanda Batra, Jyotijwal Debnath, Fazal Kareem, Paramasivan Arumugam, Manjari Bagchi, Adarsh Bathula, Subhajit Dandapat, Shantanu Desai, Yashwant Gupta, Shinnosuke Hisano, Divyansh Kharbanda, Tomonosuke Kikunaga, Neel Kolhe, Yogesh Maan, P K Manoharan, Jaikhomba Singha, Aman Srivastava, Mayuresh Surnis, Keitaro Takahashi,
Monthly Notices of the Royal Astronomical Society, Volume 527, Issue 1, pp.213-231, 01/2024

[3] “Precision of localization of single gravitational-wave source with pulsar timing array”
Ryo Kato and Keitaro Takahashi,
Physical Review D, Volume 108, Issue 12, article id.123535, 12/2023

[4] “The second data release from the European Pulsar Timing Array. III. Search for gravitational wave signals”
EPTA Collaboration; InPTA Collaboration,
Astronomy & Astrophysics, Volume 678, id.A50, 22 pp., 10/2023

[5] “The second data release from the European Pulsar Timing Array. II. Customised pulsar noise” models for spatially correlated gravitational waves
EPTA Collaboration; InPTA Collaboration,
Astronomy & Astrophysics, Volume 678, id.A49, 20 pp., 10/2023

[6] “Noise analysis in the Indian Pulsar Timing Array Data Release I”
Aman Srivastava, Shantanu Desai, Neel Kolhe, Mayuresh Surnis, Bhal Chandra Joshi, Abhimanyu Susobhanan, Aurélien Chalumeau, Shinnosuke Hisano, Nobleson K., Swetha Arumugam, Divyansh Kharbanda, Jaikhomba Singha, Pratik Tarafdar, P Arumugam, Manjari Bagchi, Adarsh Bathula, Subhajit Dandapat, Lankeswar Dey, Churchil Dwivedi, Raghav Girgaonkar, A. Gopakumar, Yashwant Gupta, Tomonosuke Kikunaga, M. A. Krishnakumar, Kuo Liu, Yogesh Maan, P K Manoharan, Avinash Kumar Paladi, Prerna Rana, Golam M. Shaifullah, Keitaro Takahashi,
Physical Review D, Volume 108, Issue 2, article id.023008, 07/2023

5. List of awards, grants, and patents

科学研究費補助金基盤研究 B（2021 年度～2024 年度）代表・直接経費：13,200,000 円
科学研究費補助金学術変革領域 A 公募研究（2024 年度～2025 年度）代表・直接経費：6,000,000 円
二国間交流事業（2023 年度～2024 年度）代表・直接経費：2,000,000 円