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	Development of extrachromosomal DNA control system for reprogramming drug-resistant cancer
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	University of California at Davis, USA /Professor **IROAST Visiting Professor*
Kae AKITA	Nihon University /Lecturer

[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 *Current Biology*, and released three press releases from Kumamoto University. Here, we briefly report on three particularly outstanding achievements:

1. Tanaka et al. (2024) *Curr Biol*

We have uncovered the role of HD-ZIP IV transcription factors in establishing the radial axis in Arabidopsis thaliana embryos. Live imaging revealed that these factors, including HDG11, HDG12, and PDF2, are essential for proper periclinal cell division, enabling inner and outer cell differentiation. We found that zygote division initiates apical cell elongation and basal nuclear retention, processes disrupted in *hdg11 hdg12 pdf2* mutants, leading to transverse cell divisions and a failure in radial axis formation. Simulations based on live-cell imaging confirmed that the division plane follows mathematical rules, such as the minimal plane and nucleus-passing principles, dependent on cell shape and nuclear positioning. This precise mechanism highlights how geometric cues guide cell division and axis formation, advancing our understanding of plant morphogenesis. The findings hold potential applications in agricultural research, offering insights into embryo development and trait stability in hybrid crops.

2. Horiuchi et al. (2024) *Protoplasma*

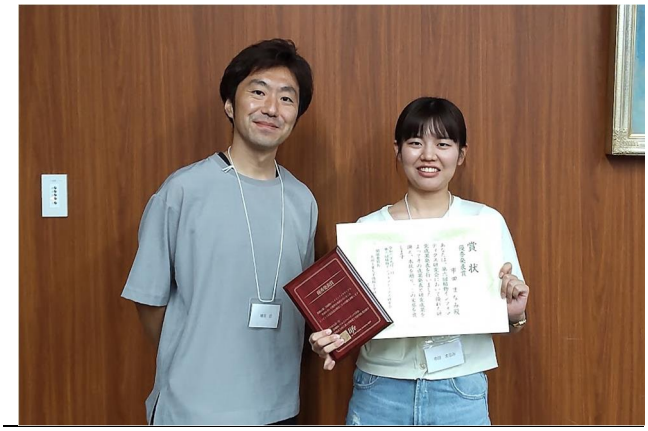
We developed a deep learning-based segmentation method to achieve precise and efficient measurement of cytoskeleton density in plant cells. Using confocal microscopic images of cortical microtubules in tobacco BY-2 cells, we compared the accuracy of this method with conventional approaches. While traditional methods performed adequately for analyzing angles and parallelness, they fell short in accurately measuring cytoskeleton density. In contrast, the deep learning-based method significantly improved the precision of density evaluations. To validate its versatility, we applied the method to physiological processes involving cytoskeleton density changes, such as

stomatal movement in *Arabidopsis thaliana* guard cells and intracellular polarization in elongating zygotes. The method successfully captured these changes, demonstrating its applicability across diverse biological phenomena. This study highlights the utility of deep learning-based segmentation in automating and expediting quantitative analysis of large-scale image datasets, offering a powerful tool for advancing research on cytoskeleton function and associated molecular mechanisms.

3. Ichita et al. (2025) *Plant Mol Biol*

We established a deep learning-based virtual staining technique that enables accurate, label-free analysis of plant cell structures using bright-field microscopy images. This method eliminates the need for traditional fluorescent staining, allowing non-invasive visualization and analysis of cellular features. The model was trained on images of tobacco BY-2 cells stained with fluorescent dyes and successfully identified morphological changes, such as nuclear expansion upon aphidicolin treatment and aspect ratio reduction upon propyzamide treatment. It also accurately characterized the altered phenotypes of *Arabidopsis thaliana* pavement cells in the *bpp125* triple mutant, demonstrating strong correlations with manual measurements of cell area, circularity, and solidity. Furthermore, the method was applied to track chloroplast movement in *Egeria densa* and to classify live and dead BY-2 cells using texture-based machine learning. These findings highlight the versatility and efficiency of virtual staining for dynamic and high-throughput analyses, offering a non-invasive alternative for quantitative plant cell biology research.

Additionally, Manami Ichita, a second-year master's student in my laboratory, presented her research at the 7th Plant Informatics Research Conference, held at Kyushu Institute of Technology, and was honored with the Outstanding Presentation Prize. Similarly, Haruka Ono, a first-year master's student in my laboratory, presented research findings from this project on the mechanisms of cortical microtubule organization at the 33rd Annual Meeting of the Japan Bioimaging Society, held at Tokyo University of Science, where she was honored with the Best Imaging Award for Outstanding Presentation.



Publications

1. Ichita M, Yamamichi H, Higaki H (2025) Virtual staining from bright-field microscopy for label-free quantitative analysis of plant cell structures. *Plant Mol Biol* 115: 29. (Published: 31 Jan 2025)
2. Takeda S, Nishikawa Y, Tachibana T, Higaki T, Sakamoto T, Kimura S (2025) Morphological and transcriptome analysis of the near-threatened orchid *Habenaria radiata* with petals shaped like a flying white bird. *Plants* 14: 393. (Published: 28 Jan 2025)
3. Horiuchi R, Kamimura A, Hanaki Y, Matsumoto H, Ueda M, Higaki T (2024) Deep learning-based cytoskeleton segmentation for accurate high-throughput measurement of cytoskeleton density. *Protoplasma* in press. (Published: 18 Dec 2024) https://doi.org/10.1007/s00709-024-02019-9

4. Hitora Y, Hokaguchi M, Sadahiro Y, Higaki T, Tsukamoto S (2024) Machine learning accelerates screening of osteoclast differentiation inhibitors from natural products. *J Nat Prod* 87: 2393–2397. (Published: 4 Oct 2024) https://doi.org/10.1021/acs.jnatprod.4c00640
5. Notaguchi M, Ichita M, Kawasoe T, Monda K, Kurotani K, Higaki T, Iba K, Hashimoto-Sugimoto M (2024) The PATROL1 function in roots contributes to the increase in shoot biomass. *Planta* 260: 105. (Published: 26 Sep 2024) https://doi.org/10.1007/s00425-024-04526-8
6. Tanaka S, Matsushita Y, Hanaki Y, Higaki T, Kamamoto N, Matsushita K, Higashiyama T, Fujimoto K, Ueda M (2024) HD-ZIP IV genes are essential for embryo initial cell polarization and the radial axis formation in Arabidopsis. *Curr Biol* 34: 4639–4649. (Published: 19 Sep 2024) https://doi.org/10.1016/j.cub.2024.08.038
7. Ezaki K, Koga H, Takeda-Kamiya N, Toyooka K, Higaki T, Sakamoto S, Tsukaya H (2024) Precocious cell differentiation occurs in proliferating cells in leaf primordia in Arabidopsis angustifolia3 mutant. *Front Plant Sci* 15: 1322223. (Published: 16 Apr 2024) https://doi.org/10.3389/fpls.2024.1322223
8. 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*. 87 (4), 1197-1202. https://doi.org/10.1021/acs.jnatprod.4c00158

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 HARTMAN	Institute for Geology, University of Hamburg, Germany / 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-QUIANG	Faculty of Advanced Science and Technology, Kumamoto University / Postdoc		
Kimpei ICHIYANAGI	Faculty of Advanced Science and Technology, Kumamoto Univesity, / 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. 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.

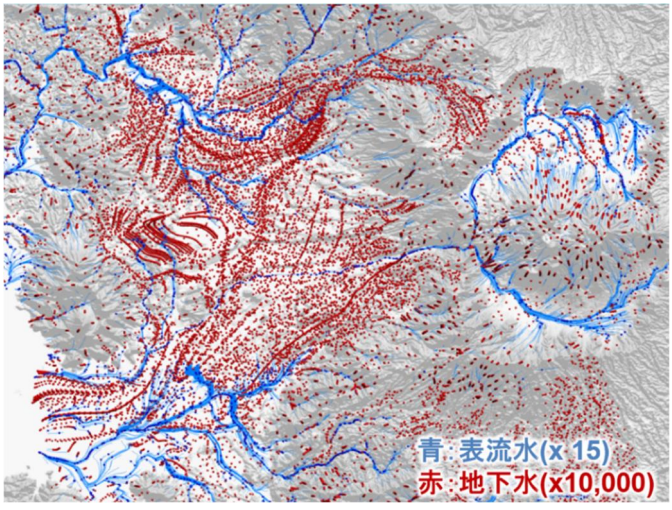


Photo 1. Groundwater simulation model

2. Research progress and results in the fiscal year

Our team 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, 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. Through the work this year we have made progress in constructing a groundwater flow model that can more plausibly explain the trends in material transport (nitrate-nitrogen pollution) by attempting to adjust aquifer conditions.

2) Numerical and statistical study: In order to understand the entire processes of groundwater level changes in response to the Kumamoto Earthquake particularly at an area where abnormal water level rise was recognized, we collected as much long-term data as possible on water levels that can compare for the period between before and after the occurrence of the earthquake. We performed trend and statistical analysis together with tank model analysis and clarified spatiotemporal characteristics of postseismic water level changes and hitherto unknown water level change mechanisms. 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: To evaluate the seasonal changing patterns of groundwater nitrate-nitrogen pollution in Kumamoto, we measured water chemistry and isotope ratios of monthly collected samples during 2024. A part of these samples was further tested for shotgun metagenomic analysis for the first time in Kumamoto under cooperation with team in the Kitasato University (Photo 2). We have obtained unique results and plan to continue the work for upcoming few years. Similar works are ongoing in Okinawa areas too.
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 documents to understand the basic water quality characteristics of 109 first-class rivers nationwide (Photo 3). We completed this sampling in this year for these 109 rivers. In addition, I discussed the concept of this research with Professor Jens Hartmann. We also found importance in continuing similar works expanding the number of rivers to get representative data to explain features on entire regions.
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 on April this year.

Photo 2. Experiment for metagenomic analysis

Photo 3. River water sampling survey

3. Research plan for the next fiscal year

We are planning next semester year's research based on the research results obtained this semester year. First, we will try to improve the reproducibility of the groundwater flow simulation model by considering hydrogeological condition in depths. To make it possible we plan to use deep drill boreholes installed in Onsen sites to obtain samples for determining physicochemical and isotope

ratios and information of depth profiles from the Kumamoto Prefecture. These works are supported by JSPS funding. Also, while achieving publication for the research topic discussing groundwater level changes in response to the Kumamoto Earthquake particularly at an area where abnormal water level rise was recognized, we will extend our research area both in space and time to comprehensively understand entire systems in the next semester year particularly with a scope how post-seismic changes recover or sustain in what timescale using long term water level record continuously obtained even several years after the earthquake. These works with topics regarding isotopic and metagenomic minoring in Kumamoto will continue with a support from TSMC/JASM project, while nation scale river sampling and analysis will continue with a help of IROAST. In addition, we wish completing Kirishima's paper, and enhancing collaboration with international team of our cluster members. The visit of doctoral student from Italy in February April 2025 and Prof. Yingchun Wang from Chengdu University of Technology, China to Kumamoto should be a good opportunity for achieving this aim.

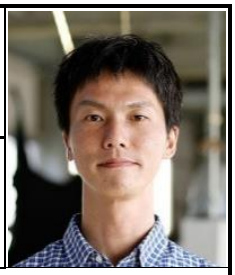
4. List of awards, grants, and patents

- JSPS Grant-in-Aid for Scientific Research A (22H00563), 2022-2025, Leader: Takahiro Hosono
- TSMC/JASM Green Joint Development Project, Multidisciplinary study for comprehensively understanding the groundwater flow systems and environmental dynamics in Kumamoto region, 2024-2026, Leader: Takahiro Hosono

5. List of journal papers

Yu, Z.-Q., **Hosono, T.**, Amano, H., Berndtsson, R., Nakagawa, K., 2024. Groundwater resource assessment by applying long-term trend analysis of spring discharge, groundwater levels, and hydroclimatic parameters. Water Resources Management 38, 4161-4177. https://doi.org/10.1007/s11269-024-03857-1

Wang, Y., Quan, S., Tang, X., **Hosono, T.**, Hao, Y., Tian, J, Pang, Z., 2024. Organic and inorganic carbon sinks reduce long-term deep carbon emissions in the continental collision margin of the southern Tibetan Plateau: Implications for Cenozoic climate cooling. Journal of Geophysical Research: Solid Earth, 129(4), e2024JB028802. https://doi.org/10.1029/2024JB028802

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

Cluster Members	
Name	Affiliation/Title
Motoki AMAGASAKI	Faculty of Advanced Science and Technology/Professor
Masato KIYAMA	Faculty of Advanced Science and Technology/Assistant Professor
Ali ERCAN	Middle East Technical University, Turkey/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

A journal paper based on our collaborative research was finalized and published on an academic journal. In the paper, we developed a new deep learning architecture that combines Convolutional Neural Networks (CNN) and Long Short-Term Memory (LSTM) networks for Rainfall-Runoff modeling. In this approach, long-term hourly meteorological data (precipitation and temperature) are first processed using a CNN. The output of the CNN, along with short-term hourly meteorological data, is then fed into the LSTM. This method successfully improved estimation accuracy. Although the time series structure of the input data is altered in this architecture, the results suggest that this approach allows for more effective transmission of both long-term and short-term information to the final output compared to directly inputting long-term hourly meteorological data into the LSTM. This enhancement likely contributes to the improvement of the estimation performance.

In late August to early September, I visited Middle East Technical University (METU) in Turkey to advance my collaborative research with Professor Erican (Figure 1). As part of the field trip, we explored the topography and rivers around Ankara and visited Lake Tuz (Figure 2), which has been experiencing a decline in water levels due to the effects of global warming.

Furthermore, we focused on preparing a research paper based on the findings of our collaborative study. Since last year, we have been examining global atmospheric data spanning both historical and future periods, utilizing it as input for deep learning models. From this dataset, we have derived various statistical metrics and indices, which we subsequently analyzed. Currently, we are in the process of drafting a manuscript that highlights one of these key results, specifically the aridity index computed from the atmospheric data (Figure 3). To streamline the writing process, we divided the

manuscript into sections and worked on each part individually.

Figure 1. Faculty Dining Hall of METU

Figure 2. Salt Lake near Ankara

In January, I invited Professor Ohara from the University of Wyoming. To gain a better understanding of the water cycle in the Shirakawa River basin, we conducted a field trip of the Aso Caldera's topography, the upstream section of the Shirakawa River, the Kurokawa River, and several spring water sources. Additionally, to comprehend the topographic factors contributing to heavy rainfall in Kumamoto, we surveyed the terrain extending from Mount Kinpo to the Amakusa region, explaining how even relatively small mountains can play a significant role in triggering heavy rainfall. Furthermore, Professor Ohara delivered a lecture on groundwater dynamics as well as snow accumulation and melt processes. We also exchanged research presentations and discussed potential future collaborations. Additionally, we explored the possibility of establishing a Memorandum of Understanding (MOU) between our universities.

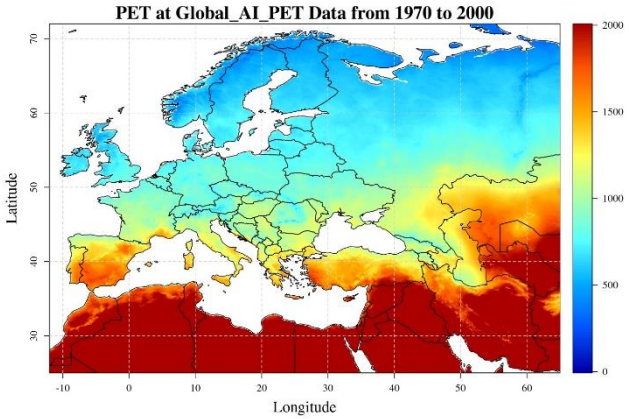


Figure 3. Evapotranspiration over Europe

Figure 4. Field Trip with Prof. Ohara

Additionally, we conducted various studies utilizing deep learning. It is commonly stated that deep learning models can only make predictions within the range of the training data. However, there are no studies in the hydrological field that have quantitatively verified this limitation. Therefore, we conducted a study focusing on Rainfall-Runoff modeling to quantitatively assess this issue. The results indicated that, even when predicting outside the range of the training data, the model was able to achieve a certain level of accuracy.

Handling missing data presents a significant challenge when applying deep learning models. To address this, we proposed a method for Rainfall-Runoff modeling where missing values are first estimated using a separate deep learning model before being interpolated into the dataset. Furthermore, we examined whether incorporating additional data, such as dam operations and snow cover information, could improve the predictive accuracy of Rainfall-Runoff modeling.

3. Research plan for the next fiscal year

As explained above, we conducted several studies applying deep learning to hydrological modeling. One study quantitatively examined the predictive limitations of deep learning models when applied beyond the range of training data in Rainfall-Runoff modeling, revealing that reasonable accuracy could still be achieved. Another study addressed the challenge of missing data by proposing a method that first estimates missing values using a separate deep learning model before interpolation. Additionally, we investigated whether incorporating supplementary data, such as dam operations and snow cover, could enhance predictive performance. We plan to further develop these studies and proceed with manuscript preparation in the next fiscal year.

Our collaborative research with Professor Ercan has yielded various results. Significant progress has been made in studies analyzing the data used as input for deep learning models. As mentioned above, one such study is nearing completion, with a paper almost finalized. Although unexpected data errors delayed our progress, preventing submission within this fiscal year, we aim to publish the paper next fiscal year. For other studies, analyses are ongoing, and figures and visualizations are being prepared. We plan to systematically compile each study into research papers for publication.

4. List of awards, grants, and patents

None

5. List of journal papers in international journal as of the end of December; (excluding conference papers and proceedings)

1) issued with volume numbers from April 2024 to December 2024

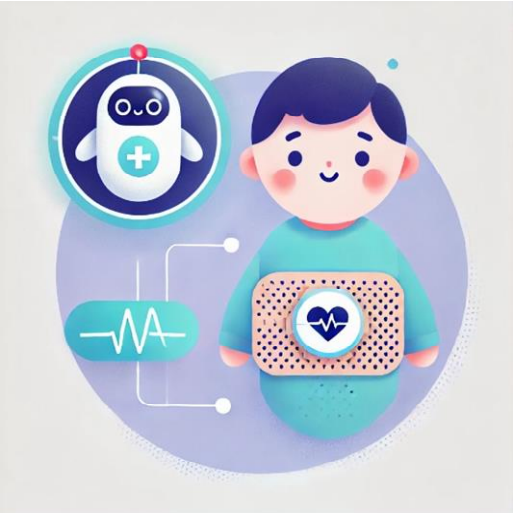
Kei Ishida, Ali Ercan, Takeyoshi Nagasato, Masato Kiyama, Motoki Amagasaki, Use of one-dimensional CNN for input data size reduction in LSTM for improved computational efficiency and accuracy in hourly rainfall-runoff modeling, Journal of Environmental Management, 359, 2024.

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/Affiliate Researcher
Masayuki TANABE	Faculty of Advanced Science and Technology, Kumamoto University/ Assistant Professor
Rajendra Udyavara ACHARYA	University of Southern Queensland, Australia/ Professor **IROAST Distinguished Professor*
Prabal Datta BARUA	University of Southern Queensland, Australia/Adjunct Professor (Graduate School of Science and Technology, Kumamoto University/ Ph. D Student)
Oliver FAUST	Faculty of Science and Engineering, Anglia Ruskin University, UK/ Associate Professor
Ru San TAN	National Heart Centre, Singapore/ Senior Consultant

[Details of Activities]

1. For the sustainable continuity of society, medical DX (Digital Transformation) is indispensable. This necessitates a system incorporating inexpensive wearable sensors that automatically assess the 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 baby simulator 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.

Kobayashi and Tanabe visited a research institution in Australia to reaffirm the research structure and future tasks. Kobayashi will visit Anglia Ruskin University, UK in March. Prof. Baig from University of Southern Queensland will visit Kumamoto University will visit us in March as well.

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. Edge computing research for wearable devices will be held in Anglia Ruskin University, UK.

3. 2025 Kumamoto Tech Planter, Best award

4. List of journal papers (with IROAST as your affiliation) published between April 2024 and December 2024
   - "Fibromyalgia detection and diagnosis: A systematic review of data-driven approaches and clinical implications", A. Atmakuru, S. Chakraborty, M. Salvi, O. Faust, P. D. Barua, M. Kobayashi, R. S. Tan, F. Molinari, A. H.-Baig, U. R. Acahrya, IEEE Access, accepted in Jan. 2024.
   - "Artificial intelligence assisted tools for the detection of anxiety and depression leading to suicidal ideation in adolescents: a review", Prabal Datta Barua, Jahmunah Vicnesh, Oh Shu Lih, Elizabeth Emma Palmer, Toshitaka Yamakawa, Makiko Kobayashi, Udyavara Rajendra Acharya, Cognitive Neurodynamics 18(1) 1-22, Feb. 2024.

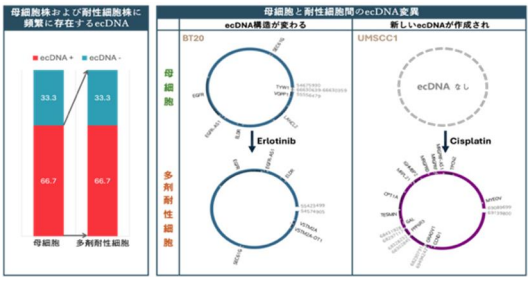
No. 2-4-5	Development of extrachromosomal DNA control system for reprogramming drug-resistant cancer		
Name	Ruda LEE		
Affiliation	Institute of Industrial Nano Materials Email: aeju-lee@kumamoto-u.ac.jp	Title	Associate Professor
Research Field	Biotechnology & healthcare technology		
Cluster Members			
Name	Affiliation/Title		
Hoon KIM	School of Pharmacy, Sungkyunkwan University (SKKU), Korea Associate Professor **IROAST Visiting Professor*		

[Details of Activities]

1. **Research outline and its perspective**

Cancer remains a leading global cause of mortality. Chemotherapy has been used for primary conventional treatment for cancer, but multidrug resistance (MDR) poses a significant challenge in chemotherapy. MDR cancer cells often lead to treatment failure due to their ability to develop ways to evade the effects of chemotherapy. Many advances have been made in identifying somatic alterations inducing and/or enhancing the tumorigenic process. Recent research suggests that oncogenic amplification on extrachromosomal DNA (ecDNA) may play a key role in this rapid tumor evolution and, thus, treatment resistance, in aggressive tumors. However, little research has been conducted on targeting ecDNAs as a new approach to overcoming ecDNA-driving treatment resistance. So, we elucidate finding the specific mechanism of ecDNA maintaining the MDR and reprogramming MDR cancer cells into drug-sensitive cancer cells by controlling the ecDNA-related cell signaling pathway.

2. **Research progress and results in the fiscal year**



Based on the analysis results from the whole genome sequencing (WGS) data of the parental cell lines and chemoresistant tumor cell lines held by Lee LAb on the opposing side, it was confirmed that approximately 67% of the parental cell lines possess ecDNA, which aligns with the existing fact that ecDNA is frequently present in cancer cells. Additionally, among the resistant cell lines, 67% of the cells that possess ecDNA maintain it, suggesting a correlation between ecDNA and drug resistance.

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. Currently, we applied for Korea-Japan collaborative grant and waiting the result.

4. List of journal papers in international journal published between April 2024 and December 2024

(1) Min Woo Kim, Sol Moon, Yong Il Park, Jungho Kim, Seung Il Kim, and **Ruda Lee***. Ultrasound-Responsive Lipid Nanoparticles for Targeted Therapy and Controlled Drug Release in Non-Small Cell Lung Cancer. *Adv. Therap.* 2024, 2400248. DOI: 10.1002/adtp.202400248

(2) Jeon Geun Kim, Hyeon Jung Yu, Ruda Lee*, and Yong Il Park*. Recent Developments in Near-Infrared-II Luminescence Imaging Using Inorganic Nanoparticles: Semiconductor Quantum Dots and Lanthanide Nanoparticles. Korean J. Chem. Eng. 2024; 41, 3603-3619. DOI: 10.1007/s11814-024-00300-4

5. List of awards, grants, and patents

Ruda LEE

1) FY2022 JSPS, Grant-in-Aid for Scientific Research (C)
2) Brain Pool Korea 2022

Hoon KIM

1) STEAM research; Pioneer, FY2022-FY2026
2) Basic Science Research (MRC), FY2019-FY2026
3) Center Research Hospital R&D, FY2024-FY2025
4) Senior Researcher Grant, FY2023-2028
5) Boston-Korea, FY2024-FY2029

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, 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 palm-sized medical devices for cancer detection, postoperative management, medical treatment, and drug discovery based on the characteristics and properties of cells, such as signals (expressed and produced proteins and genes), responses to stimuli, and the morphology and behavior of cells themselves, as well as new technologies to realize these devices.

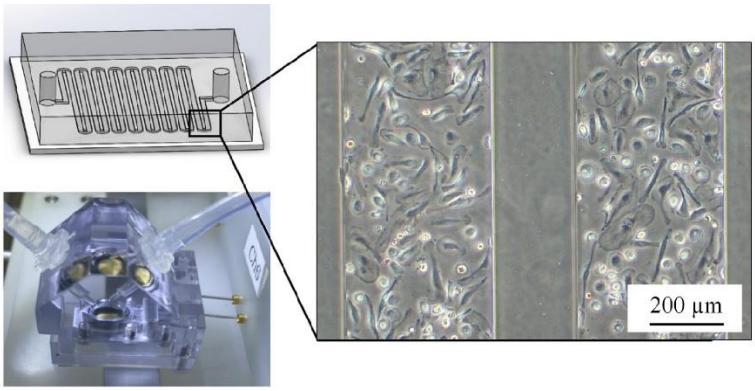


Fig. 1 Schematic of a cancer diagnosis device.

2. Research progress and results in the fiscal year

In this year, we carried out the development of the cancer detection device from cell secretion substances and 3-dimensional cancer organoid creation technique.

(a) Cancer detection technology by cell secretion substances

The objective is to develop a technique to locate the primary tumor by liquid biopsy using the immune

response. Co-culturing various types of cancer cells with immune cells and evaluating the products secreted by the cells revealed that the cellular products differ between cancer types. Based on thes result, we carried out the real time detection of cell secretion substance using the fabricated device. As a results, we succeeded in detect the cell secretions according to the type of cancer (Fig. 2).

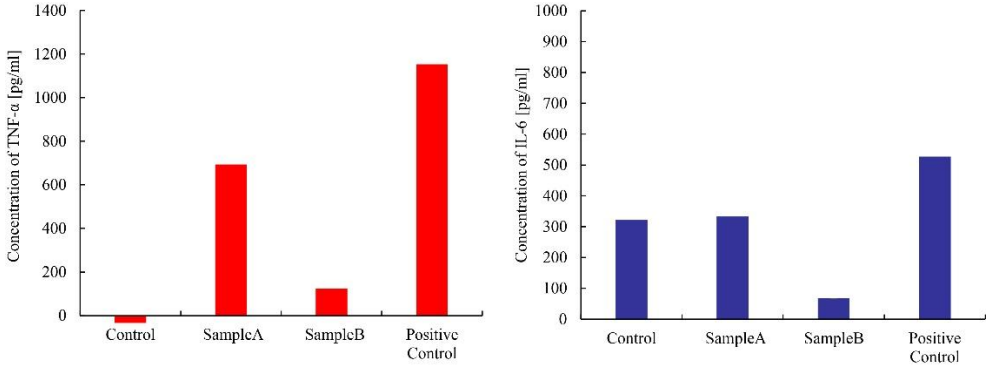


Fig. 2 The fabricated microfluidic device for evaluating cell morphology and migration.

(b) Fabrication of 3-dimentional cancer model

Cell micropattern creation technique was developed based on the photolithography technique. This technique was achieved by processing cell-adhesive or non-adhesive hydrogels using photolithography technique. When the cells were disseminated on the substrate having hydrogel micropattern, cells were adhered to micropattern on the substrate and cell micropattern were created. Then, cultured cells on the micropattern were aggregated each other and formed multiple cell aggregates. After the 336 h, multiple cell aggregates were fused together, the large size there-dimensional cell aggregates were created on the substrate (Fig. 3). The three-dimensional cell aggregates formed can be an organoid model of cancer.

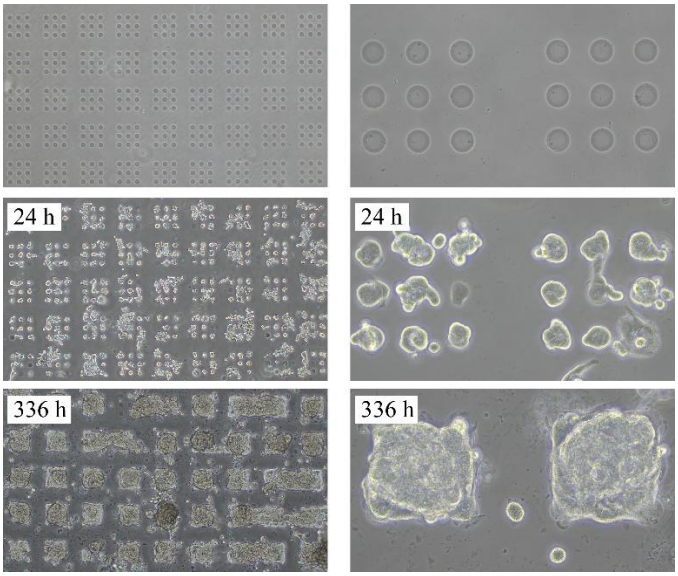


Fig. 3 The fabricated cancer detection (biosensing) device.

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 and demonstrated using biological samples. Based on the results, cancer type will be identified. We will deepen our collaboration with Prof. Lee at NUS and Dr. Iwasaki of AIST on this biosensing technology.

List of journal papers

*Corresponding author

[1] Mami Akaike, Jun Hatakeyama, Yoichi Saito, Yoshitaka Nakanishi, Kenji Shimamura, Yuta Nakashima*, "Microdifferential Pressure Measurement Device for Cellular Microenvironments," Bioengineering, 12(1), 3 (17 pages), 2025.

[2] Ryota Tashiro, Kazushi Miyamoto, Yoshiyuki Kume, Ryo Suzuki, Yukio Fujiwara, Yoshihiro Komohara, Yuta Nakashima, Yoshitaka Nakanishi, "In vitro generation of micro/nano-plastics for biological tests," Journal of Biomechanical Science and Engineering, 19(4), 24-00040, 2024.

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. 2024 – Mar. 2025

[2] Editorial committee
41th Sensorsymposium, IEEJ (The Institute of Electrical Engineers of Japan)
Mar. 2024 – Dec. 31, 2024.

[3] Steering committee
Micro-Nano Science & Technology Division, The Japan Society of Mechanical Engineers
Apr. 2024 – Mar. 2025.

[4] Representative
Micro-Nano Science & Technology Division, The Japan Society of Mechanical Engineers
Apr. 2024 – Mar. 2025.

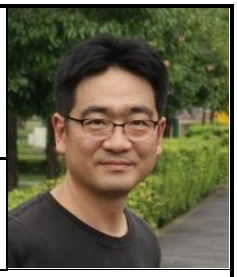
[5] Planning committee member
Bioengineering Division, The Japan Society of Mechanical Engineers
Apr. 2024 – Mar. 2025.

List of patents

[1]発明の名称：標的細胞捕捉フィルター及び標的細胞捕捉方法
登録番号：特許第 7610282 号
登録日：2024 年 12 月 24 日
発明者：中島雄太，北村裕介，安田敬一郎
出願人：株式会社オジックテクノロジーズ

[2]発明の名称：導光ユニットおよび光学測定器
登録番号：特許第 7479590 号
登録日：2024 年 4 月 26 日

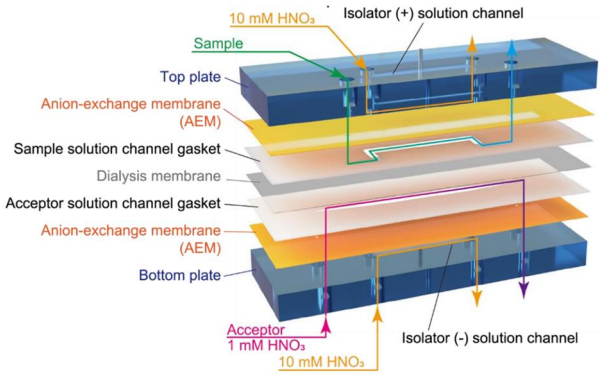
発明者：中島雄太、西川昌平
出願人：国立大学法人熊本大学、西川計測株式会社
[3]発明の名称：標的細胞捕捉装置
出願番号：特願 2024-147082
出願日：2024 年 8 月 29 日
発明者：中島雄太，北村裕介，岩槻政晃，安田敬一郎，中森勇志
出願人：株式会社オジックテクノロジーズ、国立大学法人熊本大学

No. 2-4-7	Separation, synthesis, and detection by means of ionic solutes handling		
Name	Shin-Ichi OHIRA		
Affiliation	Faculty of Advanced Science and Technology 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

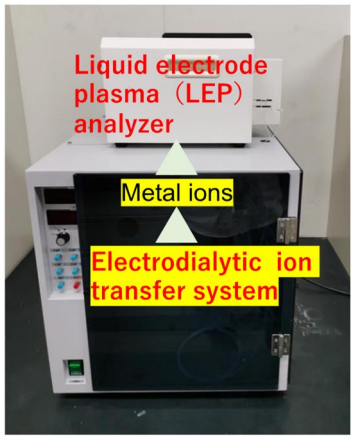
Ion is the key chemical form in environment, human health, and industries. In the present study, the original electrodialytic ion transfer technologies are applying to separation, purification and synthesis of ionic solutes. For the separation to analyze hard matrices samples, electrodialytic ion transfer are studied. The samples such as organic solvents, and sulfuric acid, which are difficult to analyze trace metals with inductivity coupled plasma mass spectrometry (ICP-MS) are studied. These are also important in semiconductor industry. Furthermore, the speciation analysis of silicate, and silica particles are studied. The ionic solute handling will be spread to not only chemical analysis but also many science and technology areas.



Electrodialytic ion handring device

2. Research progress and results in the fiscal year

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

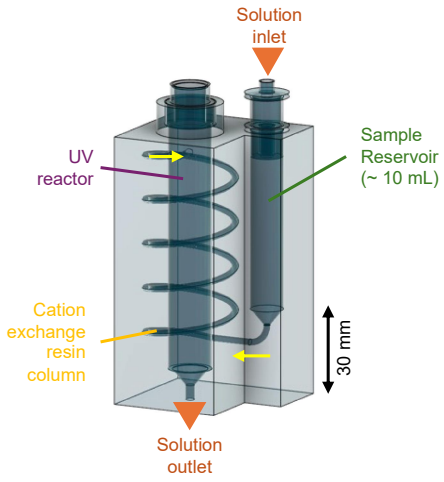


ITD-LEP system

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) Radio isotopes separation for positron emission tomography diagnosis probe. Short half-life radioisotopes generated with a cyclotron required rapid separation from the target. The radioisotopes is presently considered for new generation of positron emission tomography (PET) probe. The separation was achieved with 3D-printed devices.

c) Speciation analysis of silica compounds Silica is difficult to remove on the water purification processes. Furthermore, there are several chemical forms of silicate and silica particles. Thus the speciation analysis is important to produce highly pure water. Presently we have succeeded to proof the concept for the speciation. We will develop automated system for trace silica application.



3D printed device for RI separation

d) 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 is also high enough. The system is developing with the company to commercialize it.

e) 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)

3. Research plan for the next fiscal year

In FY2025, we are going to study followings:

a) **Trace metal analysis in organic solvents with liquid electrode plasma spectroscopy (LEP)**. The sample will be expanded to isopropylalchohol to other solvents to proof the universality of the ion transfer system. Simultaneously the compact and seamless ITD-LEP system will be established. This project is supported by A-STEP (JST, 2023~2025).

b) **Ultra-sensitive ion analysis system**. The key process for the project, quantitative ion exchange at trace levels (~ nmol/L) will be established. Simultaneously the method to supply RI-Ga to the system and the new ion separation concept will be studied. This project is supported by Grant-in-Aid for Scientific Research(B) (2024~2026FY)

c) Selective removal and analysis of urea. Urea is also causing the problems on the ultra-pure water production. Urea is difficult to remove by typical ion exchange. Thus we will develop new removal method for urea and establish the urea analysis method which is enough sensitivity and rapidity.

4. List of awards, grants, and patents

[Patents]

There was not newly applied in this period. However, 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)

Grant-in-Aid for Scientific Research (B)
from Japan Society for the Promotion of Science (JSPS) Grant number 24K01516

“Development of ultra-high sensitivity ion analysis with universal detection” (18,620 kJPY for 3 years)

Collaborative Research with the companies (8 companies, 15,820 kJPY for FY2024)

5. List of journal papers in international journal as of the end of December

Rani, R., Ueda, T., Saeki, K., Toda, K., Ohira, S., Adsorption behavior of zirconium metal-organic frameworks in multicomponent metal-ion solutions
Bulletin of the Chemical Society of Japan, 97(11), uoae113 (2024)

M. Mukai, R. Rani, N. Iwanaga, K. Saeki, K. Toda, S-I. Ohira, Two-step extraction for the evaluation of metal–organic framework impregnated materials, Analytical Sciences, 40, 1793-1797 (2024)

B. B. Sherpa, R. Rani, Advancements in explosive welding process for bimetallic material joining: A review, Journal of Alloys and Metallurgical Systems, 6, 100078 (2024)

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

Cluster Members	
Name	Affiliation/Title
Murat KARAKAS	The University of Adelaide, Australia/Associate Professor
Akira SATO	Faculty of Advanced Science and Technology, Kumamoto University/ Professor
Kazunori NAKASHIMA	Hokkaido University/ Professor
Hiroaki ITO	Kumamoto University/Assistant Professor

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.

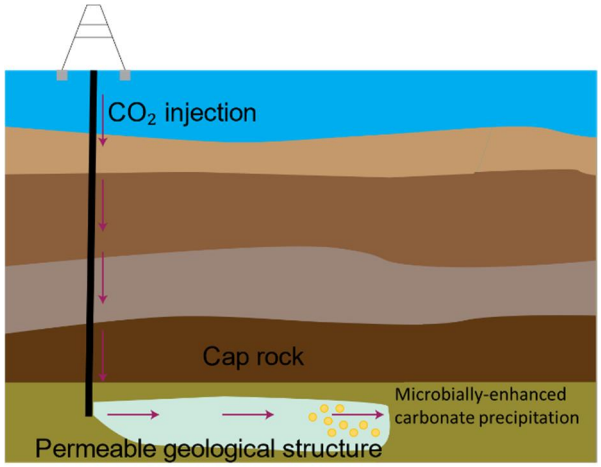


Fig.1: Mitigation of the CO_2 leak-off risk with microbially-enhanced carbon precipitation

2. Research progress and results in the fiscal year

a) EVALUATION OF MINERAL COMPOSITION AND CARBON DIOXIDE INJECTION PRESSURE INFLUENCES ON CARBONATE MINERALIZATION

A series of CO_2 sealing tests have been conducted under various conditions. First, the rock types employed for this year are as follows: serpentinite (Tokushima), Saga basalt, Nagasaki basalt, olivine (Hokkaido), Kimachi sandstone, Ainoura sandstone, and Amakusa mudstone. The injection pressure varies from 6 MPa to 15 MPa, and the temperature ranges from room temperature to 40℃. In addition to that, the effect of carbonic anhydrase generated by Bacillus 168 has been investigated. The experiment without the enzyme of Bacillus 168 indicated that the serpentinite used for this experiment is very reactive with carbon dioxide, generating Magnesium carbonate. Table 1 shows the results of thermogravimetric analysis and indicates the reduction of the weight of the specimen when heated. As carbonate is decomposed when heated more than 800℃, a large amount of weight reduction corresponds to the high reaction with CO_2 and generation of carbonate. According to the table, the sealing test with serpentinite shows approximately 10% increase in the weight reduction percentage compared to the original rock (16.08%), indicating that after the sealing test, 10% of the specimen has been changed to carbonate in weight. In contrast, for other types of rocks, the generation of carbonate rocks was not identified under the condition without microbes. Then, sealing tests with the enzyme of the microbe has been performed to investigate the effect of the enzyme on carbonate generation. The result indicated 9.43 % of weight reduction compared to the original basal.

Table 1: Results of thermogravimetric analysis showing the percentage of weight reduction compared to the original rock

	Serpentinite	**Basalt**	**Olivine**	Sandstone
Original rocks	**16.08**	**1.91**	**1.00**	**7.69**
One week・9MPa	**26.10(+10.02)**	**2.35(+0.44)**	**0.84(-0.16)**	**6.83(-0.86)**
One week・6MPa	**25.80(+9.72)**	**1.47(-0.44)**		
One week・12MPa	**25.02(+8.94)**	**1.24(-0.67)**		
One week・15MPa	**24.73(+8.65)**	**1.35(-0.56)**		
Six weeks・9MPa	**22.47(+6.39)**	**1.77(-0.14)**	**1.45(+0.45)**	**7.24(-0.27)**

b) Fracture stiffness determination for 3D printed specimens

As preparation to elucidate the microscopic strain distribution inside a fractured rock specimen, 3D printed specimens with a single, horizontal fracture were prepared. Triaxial loading tests were performed for the specimens with the triaxial testing machine shown in Figure 1. The machine was nearly developed to perform triaxial loading tests inside the micro-focused X-ray CT owned by X-Earth Center at Kumamoto University. The loading test was performed while increasing the load in a stepwise manner. At each loading stage, the load was kept constant and CT scanning was performed. Figure 2 shows CT images taken during the loading test, where the load was increased from 0 kN to 25 kN in a stepwise manner. 25 kN corresponds to approximately 13 MPa for the specimen with a diameter of 50 mm. Then, after the test, the CT images were analyzed to calculate displacement and strain fields inside the specimen with the digital image correlation method, as shown in Figure 3. Figure 4 shows the displacements averaged every 10 pixels in the z-direction. As shown, the steep line located at 400 corresponds to the location of fracture, based on which fracture stiffness can be estimated.

Figure 1: Newly developed triaxial testing machine for the micro-focused X-ray CT scanner

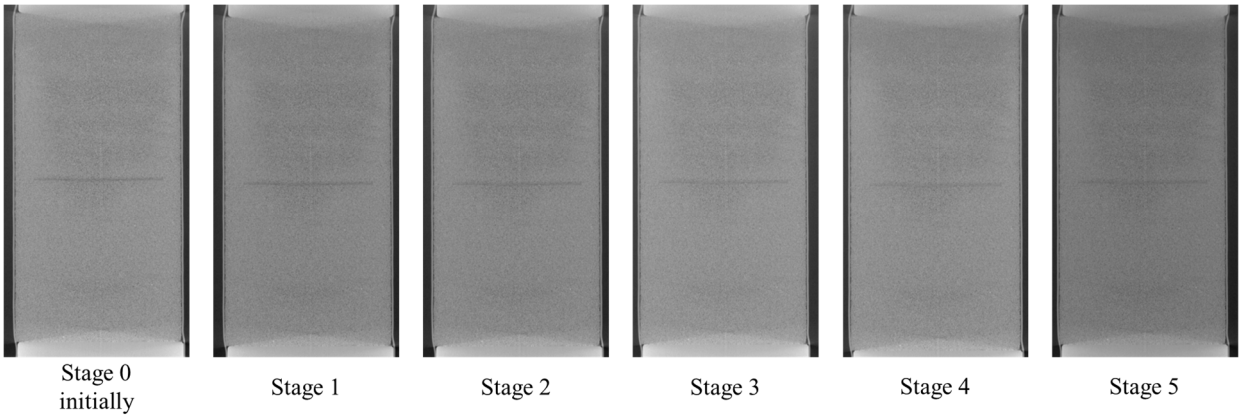


Figure 2: Examples of CT images showing a vertical section of the specimen subjected to a uniaxial load

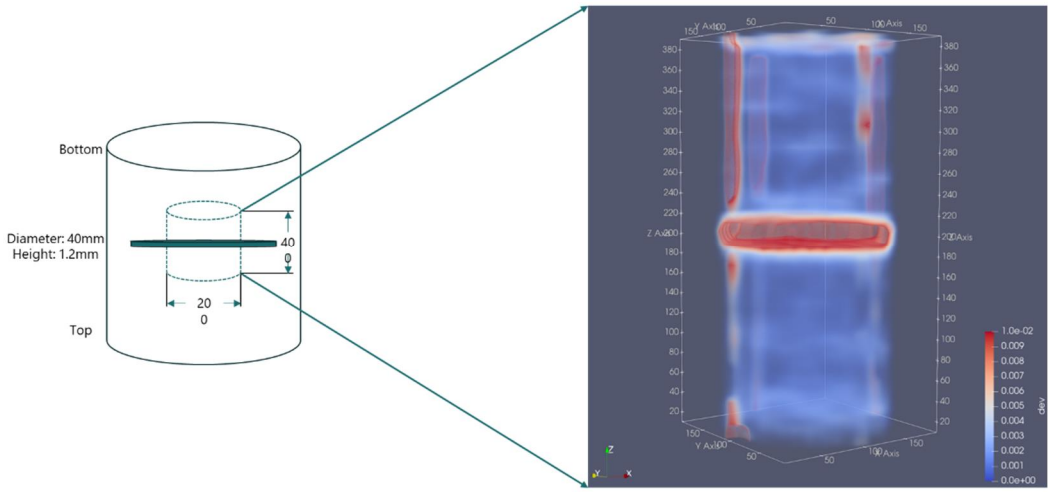


Figure 3: Example of image analysis to calculate the displacement and strain fields inside the specimen

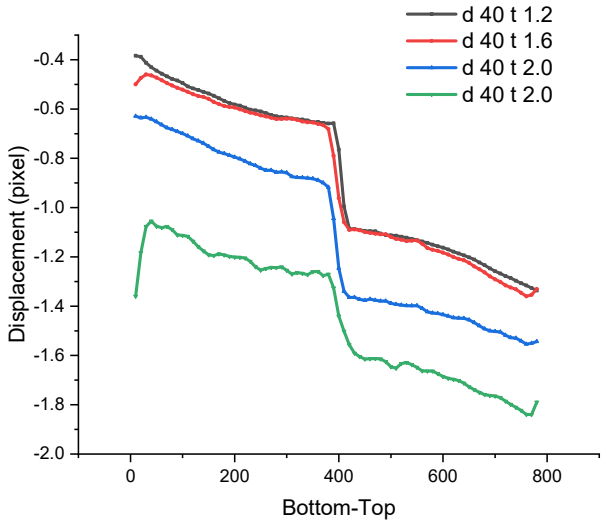


Figure 4: Displacements calculated from the image analysis

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. Last year, we confirmed that the increase in fracture stiffness in a fault damage zone contributes to the decrease in the seismic energy of induced seismicity. This year, we performed a model parametric study to consider various conditions of fault damage zone, such as fracture dip angle, its dip direction, fault depth, fracture density, and the region where fracture stiffness is increased in a fault damage zone. The result is summarized in Figure 5, according to which it is found that for almost all cases, the increase in fracture stiffness effectively control the seismic energy; however, the effectiveness decreases, especially when the region is located away from the fault core where induced seismicity takes place.

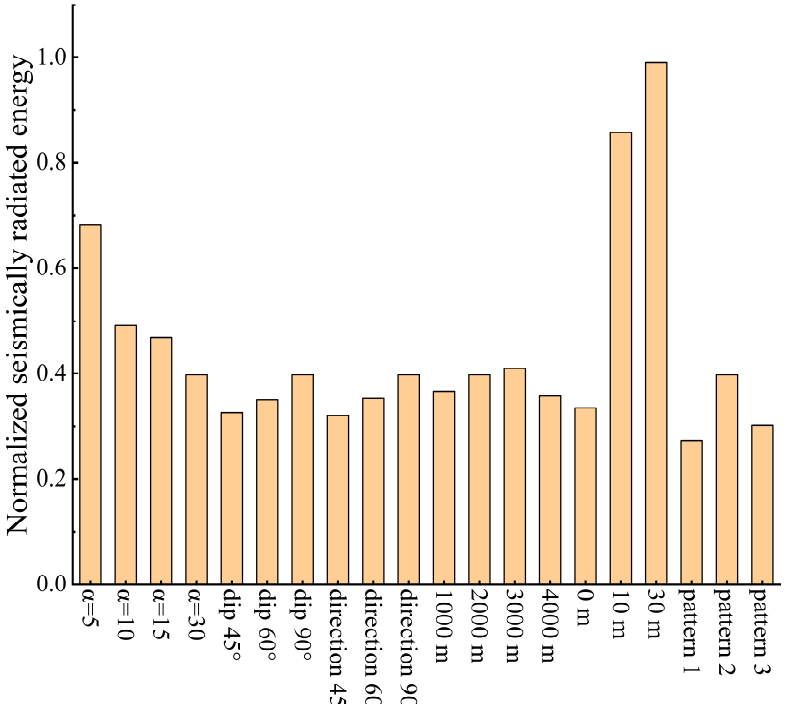


Figure 5: Effect of fracture stiffness in a rock mass on the magnitude of fault-slip

3. Research plan for the next fiscal year

a) CO_2 MINERALIZATION EXPERIMENT

This year, we have confirmed the generation of carbonate with the CO_2-rock chemical reaction. For the next fiscal year, based on the knowledge accumulated this year, the sealing tests will be performed under various temperature range from 40℃ to 60℃ to consider the range of reservoir temperature for CCS. In addition to that, a new type of microbe will be tested as well. This is because bacillus 168 can survive under anaerobic conditions, but the activity decreases. We need a type of bacteria that can grow under anaerobic conditions at great depths. Hence, we need to investigate the effect of pressure and temperature on the growth of the bacteria.

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

For the next fiscal year, 3D printed specimens with internal fractures will be prepared, whereby the triaxial tests will be performed in the micro-focused X-ray CT. Then, based on the image analysis, the microscopic strain distribution will be estimated.

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 2024 and December 2024

- Aliabadian, Z., Sainoki, A. and Sharafisafa, M., 2024. Failure mechanism of transversely isotropic schist under Brazilian test using real-time X-ray nano tomography scanning. *Engineering Fracture Mechanics*, *310*, p.110465.
- Zhang, C., Clement, A.A., Kodama, J.I., Sainoki, A., Fujii, Y., Fukuda, D. and Wang, S., 2024. Effect of the Connectivity of Weak Rock Zones on the Mining-Induced Deformation of Rock Slopes in an Open-Pit Mine. *Sustainability*, *16*(14), p.5974.
- Amagu, C.A., Zhang, C., Sainoki, A., Sugimoto, K., Shimada, H., Dzimunya, N., Sinkala, P. and Kodama, J.I., 2024. Analysis of excavation-induced effect of a rock slope using 2-dimensional back analysis method: a case study for clay-bearing interbedded rock slope. *Geotechnical and Geological Engineering*, *42*(7), pp.6315-6337.
- Wang, X., Jiang, L., Li, Y., Zhang, L., Sainoki, A., Yang, Y. and Peng, X., 2024. Experimental study on the mechanical behavior and failure characteristics of rock analogs with filled internal fractures: A new method by sand powder 3D printing. *Construction and Building Materials*, *427*, p.136261.
- Maha, M.M., Matsuyama, A., Arima, T. and Sainoki, A., 2024. Assessment of Total Mercury Levels Emitted from ASGM into Soil and Groundwater in Chami Town, Mauritania. *Sustainability*, *16*(18) 7992.

5. List of awards, grants, and patents

Encouragement award, The Mining and Materials Processing Institute of Japan

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

[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 FY2024, 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 fabricated a continuous flow-type (or semi-batch type) reaction system that can be used in sub-critical water up to 300^{o}C and 12 MPa.
Also, we carried out hydrothermal treatment of blended cloth at various kinds of temperatures and pressures. As a result, we succeeded to find out good operating conditions where cotton and polyester could be separated.

3) With regards to wastewater treatment, we performed joint research with Prof. Takahashi (Iwate University), Prof. Olivier (Aix-Marseille University, France). Also, I have stayed at Aix-Marseille University for 1 month as a visiting professor and did lots of discussions with some professors as well as Prof. Olivier Boutin for future collaborations between Aix-Marseille University and Kumamoto University. As for research of bisphenol-A decomposition, I have discussed with Prof. Olivier Boutin to complete the preparation of manuscripts.

2. Research progress and results in the fiscal year

From March 7 to March 12, Prof. Olivier Boutin visited Chemical Engineering Laboratory at Kumamoto University and performed the following things during his stay at Kumamoto.

- Presentation about fundamentals of hydrothermal treatment of biomass and wastewater.
- Discussion about preparation of bisphenol-A research,
- Quantification analysis of bisphenol-A decomposition products obtained by pulsed arc discharge treatments.

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 2024 and March 2025.

Mitsuru Sasaki, Kouki Nonaka, Yuka Sakai, Tetsuo Honma, Tomohiro Furusatof and Kunio Kawamura*, “Linear oligopeptide formation from alanine-diketopiperazine in acidic aqueous solutions using interfacial nano-pulsed discharge plasma”, New Journal of Chemistry, 49, 514 - 520 (2025). https://doi.org/10.1039/d3nj05664c

5. List of awards, grants, and patents

(Patents)

We are planning to apply for three patents regarding the separation of cotton and polyester from blended cloth using subcritical water treatment.

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

Cluster Members	
Name	Affiliation/Title
Rachel WEBSTER	Melbourne University, Australia/ Professor
Bart PINDOR	Melbourne University, Australia/ Professor
Takuya AKAHORI	National Astronomical Observatory of Japan/Researcher
Shintaro YOSHIURA	National Astronomical Observatory of Japan/Postdoctoral Researcher
Takeshi FUKUSAKO	Kumamoto University/Professor
Ryo KATO	National Astronomical Observatory of Japan/Research Assistant

[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 and nano-Hz gravitational waves to probe the first astronomical objects. We develope 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, with advanced statistical methods.

2. Research progress and results in the fiscal year

This year, our research focused on advancing pulsar timing and gravitational wave detection, particularly in the context of pulsar timing arrays (PTAs). We analyzed the European Pulsar Timing Array's second data release and Indian Pulsar Timing Array data, providing new insights into the stochastic gravitational wave background and potential astrophysical sources, such as supermassive black hole binaries. We also contributed to cross-PTA comparisons, confirming consistency in recent detections and highlighting the benefits of expanding pulsar datasets.

Additionally, we refined continuous gravitational wave source localization by incorporating realistic pulsar distributions and distance uncertainties. Observationally, we investigated pulse jitter at low radio frequencies, improving our understanding of timing precision for next-generation telescopes.

Our work has strengthened gravitational wave astrophysics by enhancing detection methods, refining

theoretical models, and fostering international collaboration. Moving forward, we aim to further improve localization accuracy and expand observational datasets for deeper insights into the nanohertz gravitational wave universe.

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 improve the measurement of inter-pulsar correlation which is a signal of gravitational-wave background, cooperating with Indian group. We will adopt several advanced statistical methods such as Gaussian Process Regression and Winsorising. 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 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

5. List of journal papers in international journal as of the end of December; (excluding conference papers and proceedings)

[1] Improving DM estimates using low-frequency scatter-broadening estimates
Jaikhomba Singha, Bhal Chandra Joshi, M. A. Krishnakumar, Fazal Kareem, Adarsh Bathula, Churchil Dwivedi, Shebin Jose Jacob, Shantanu Desai, Pratik Tarafdar, P. Arumugam, Swetha Arumugam, Manjari Bagchi, Neelam Dhanda Batra, Subhajit Dandapat, Debabrata Deb, Jyotijwal Debnath, A Gopakumar, Yashwant Gupta, Shinnosuke Hisano, Ryo Kato, Tomonosuke Kikunaga, Piyush Marmat, K. Nobleson, Avinash K. Paladi, Arul Pandian B., Thiagaraj Prabu, Prerna Rana, Aman Srivastava, Mayuresh Surnis, Abhimanyu Susobhanan, Keitaro Takahashi, Monthly Notices of the Royal Astronomical Society, Volume 535, Issue 1, pp.1184-1192, 11/2024, arXiv:2309.16765.

[2] The second data release from the European Pulsar Timing Array V. Search for continuous gravitational wave signals
J. Antoniadis, P. Arumugam, S. Arumugam, S. Babak, M. Bagchi, A. S. Bak Nielsen, C. G. Bassa, A. Bathula, A. Berthereau, M. Bonetti, E. Bortolas, P. R. Brook, M. Burgay, R. N. Caballero, A. Chalumeau, D. J. Champion, S. Chanlaridis, S. Chen, I. Cognard, S. Dandapat, D. Deb, S. Desai, G. Desvignes, N. Dhanda-Batra, C. Dwivedi, M. Falxa, I. Ferranti, R. D. Ferdman, A. Franchini, J. R. Gair, B. Goncharov, A. Gopakumar, E. Graikou, J. M. Grießmeier, L. Guillemot, Y. J. Guo, Y. Gupta, S. Hisano, H. Hu, F. Iraci, D. Izquierdo-Villalba, J. Jang, J. Jawor, G. H. Janssen, A. Jessner, B. C. Joshi, F. Kareem, R. Karuppusamy, E. F. Keane, M. J. Keith, D. Kharbanda, T. Kikunaga, N. Kolhe, M. Kramer, M. A. Krishnakumar, K. Lackeos, K. J. Lee, K. Liu, Y. Liu, A. G. Lyne, J. W. McKee, Y. Maan, R. A. Main, S. Manzini, M. B. Mickaliger, I. C. Nitu, K. Nobleson, A. K. Paladi, A. Parthasarathy, B. B. P. Perera, D. Perrodin, A. Petiteau, N. K. Porayko, A. Possenti, T. Prabu, H. Quelquejay Leclere, P. Rana, A. Samajdar, S. A. Sanidas, A. Sesana, G. Shaifullah, J. Singha, L. Speri, R. Spiewak, A. Srivastava, B. W. Stappers, M. Surnis, S. C. Susarla, A. Susobhanan, K. Takahashi, P. Tarafdar, G. Theureau, C. Tiburzi, E. van der Wateren, A. Vecchio, V. Venkatraman Krishnan, J. P. W. Verbiest, J. Wang, L. Wang, Z. Wu, and EPTA Collaboration; InPTA Collaboration, Astronomy & Astrophysics, Volume 690, id. A118, 14 pp., 10/2024

[3] Comparing recent PTA results on the nanohertz stochastic gravitational wave background
G. Agazie, J. Antoniadis, A. Anumarlapudi, A. M. Archibald, P. Arumugam, S. Arumugam, Z. Arzoumanian, J. Askew, S. Babak, M. Bagchi, M. Bailes, A.-S. Bak Nielsen, P. T. Baker, C. G. Bassa, A. Bathula, B. Bécsy, A. Berthereau, N. D. R. Bhat, L. Blecha, M. Bonetti, E. Bortolas, A. Brazier, P. R. Brook, M. Burgay, S. Burke-Spolaor, R. Burnette, R. N. Caballero, A. Cameron, R. Case, A. Chalumeau, D. J. Champion, S. Chanlaridis, M. Charisi, S. Chatterjee, K. Chatziioannou,

B. D. Cheeseboro, S. Chen, Z.-C. Chen, I. Cognard, T. Cohen, W. A. Coles, J. M. Cordes, N. J. Cornish, F. Crawford, H. T. Cromartie, K. Crowter, M. Curyło, C. J. Cutler, S. Dai, S. Dandapat, D. Deb, M. E. DeCesar, D. DeGan, P. B. Demorest, H. Deng, S. Desai, G. Desvignes, L. Dey, N. Dhanda-Batra, V. Di Marco, T. Dolch, B. Drachler, C. Dwivedi, J. A. Ellis, M. Falxa, Y. Feng, R. D. Ferdman, E. C. Ferrara, W. Fiore, E. Fonseca, A. Franchini, G. E. Freedman, J. R. Gair, N. Garver-Daniels, P. A. Gentile, K. A. Gersbach, J. Glaser, D. C. Good, B. Goncharov, A. Gopakumar, E. Graikou, J.-M. Griessmeier, L. Guillemot, K. Gültekin, Y. J. Guo, Y. Gupta, K. Grunthal, J. S. Hazboun, S. Hisano, G. B. Hobbs, S. Hourihane, H. Hu, F. Iraci, K. Islo, D. Izquierdo-Villalba, J. Jang, J. Jawor, G. H. Janssen, R. J. Jennings, A. Jessner, A. D. Johnson, M. L. Jones, B. C. Joshi, A. R. Kaiser, D. L. Kaplan, A. Kapur, F. Kareem, R. Karuppusamy, E. F. Keane, M. J. Keith, L. Z. Kelley, M. Kerr, J. S. Key, D. Kharbanda, T. Kikunaga, T. C. Klein, N. Kolhe, M. Kramer, M. A. Krishnakumar, A. Kulkarni, N. Laal, K. Lackeos, M. T. Lam, W. G. Lamb, B. B. Larsen, T. J. W. Lazio, K. J. Lee, Y. Levin, N. Lewandowska, T. B. Littenberg, K. Liu, T. Liu, Y. Liu, A. Lommen, D. R. Lorimer, M. E. Lower, J. Luo, R. Luo, R. S. Lynch, A. G. Lyne, C.-P. Ma, Y. Maan, D. R. Madison, R. A. Main, R. N. Manchester, R. Mandow, M. A. Mattson, A. McEwen, J. W. McKee, M. A. McLaughlin, N. McMann, B. W. Meyers, P. M. Meyers, M. B. Mickaliger, M. Miles, C. M. F. Mingarelli, A. Mitridate, P. Natarajan, R. S. Nathan, C. Ng, D. J. Nice, I. C. Niţu, K. Nobleson, S. K. Ocker, K. D. Olum, S. Osłowski, A. K. Paladi, A. Parthasarathy, T. T. Pennucci, B. B. P. Perera, D. Perrodin, A. Petiteau, P. Petrov, N. S. Pol, N. K. Porayko, A. Possenti, T. Prabu, H. Quelquejay Leclere, H. A. Radovan, P. Rana, S. M. Ransom, P. S. Ray, D. J. Reardon, A. F. Rogers, J. D. Romano, C. J. Russell, A. Samajdar, S. A. Sanidas, S. C. Sardesai, A. Schmiedekamp, C. Schmiedekamp, K. Schmitz, L. Schult, A. Sesana, G. Shaifullah, R. M. Shannon, B. J. Shapiro-Albert, X. Siemens, J. Simon, J. Singha, M. S. Siwek, L. Speri, R. Spiewak, A. Srivastava, I. H. Stairs, B. W. Stappers, D. R. Stinebring, K. Stovall, J. P. Sun, M. Surnis, S. C. Susarla, A. Susobhanan, J. K. Swiggum, K. Takahashi, P. Tarafdar, J. Taylor, S. R. Taylor, G. Theureau, E. Thrane, N. Thyagarajan, C. Tiburzi, L. Toomey, J. E. Turner, C. Unal, M. Vallisneri, E. van der Wateren, R. van Haasteren, A. Vecchio, V. Venkatraman Krishnan, J. P. W. Verbiest, S. J. Vigeland, H. M. Wahl, S. Wang, Q. Wang, C. A. Witt, J. Wang, L. Wang, K. E. Wayt, Z. Wu, O. Young, L. Zhang, S. Zhang, X.-J. Zhu, A. Zic, and IPTA Collaboration, The Astrophysical Journal, Volume 966, Issue 1, id.105, 18 pp., 05/2024

[4] The second data release from the European Pulsar Timing Array: IV. Implications for massive black holes, dark matter and the early Universe
J. Antoniadis, P. Arumugam, S. Arumugam, S. Babak, M. Bagchi, A. S. Bak Nielsen, C. G. Bassa, A. Bathula, A. Berthereau, M. Bonetti, E. Bortolas, P. R. Brook, M. Burgay, R. N. Caballero, A. Chalumeau, D. J. Champion, S. Chanlaridis, S. Chen, I. Cognard, S. Dandapat, D. Deb, S. Desai, G. Desvignes, N. Dhanda-Batra, C. Dwivedi, M. Falxa, I. Ferranti, R. D. Ferdman, A. Franchini, J. R. Gair, B. Goncharov, A. Gopakumar, E. Graikou, J. M. Grießmeier, L. Guillemot, Y. J. Guo, Y. Gupta, S. Hisano, H. Hu, F. Iraci, D. Izquierdo-Villalba, J. Jang, J. Jawor, G. H. Janssen, A. Jessner, B. C. Joshi, F. Kareem, R. Karuppusamy, E. F. Keane, M. J. Keith, D. Kharbanda, T. Kikunaga, N. Kolhe, M. Kramer, M. A. Krishnakumar, K. Lackeos, K. J. Lee, K. Liu, Y. Liu, A. G. Lyne, J. W. McKee, Y. Maan, R. A. Main, S. Manzini, M. B. Mickaliger, I. C. Nitu, K. Nobleson, A. K. Paladi, A. Parthasarathy, B. B. P. Perera, D. Perrodin, A. Petiteau, N. K. Porayko, A. Possenti, T. Prabu, H. Quelquejay Leclere, P. Rana, A. Samajdar, S. A. Sanidas, A. Sesana, G. Shaifullah, J. Singha, L. Speri, R. Spiewak, A. Srivastava, B. W. Stappers, M. Surnis, S. C. Susarla, A. Susobhanan, K. Takahashi, P. Tarafdar, G. Theureau, C. Tiburzi, E. van der Wateren, A. Vecchio, V. Venkatraman Krishnan, J. P. W. Verbiest, J. Wang, L. Wang, Z. Wu, and EPTA Collaboration; InPTA Collaboration, Astronomy & Astrophysics, Volume 685, id.A94, 30 pp, 05/2024

[5] Low-frequency pulse-jitter measurement with the uGMRT I : PSR J0437−4715
Tomonosuke Kikunaga, Shinnosuke Hisano, Neelam Dhanda Batra, Shantanu Desai, Bhal Chandra Joshi, Manjari Bagchi, T. Prabu, Keitaro Takahashi, Swetha Arumugam, Adarsh Bathula, Subhajit Dandapat, Debabrata Deb, Churchil Dwivedi, A. Gopakumar, Yashwant Gupta, Shebin Jose Jacob, Fazal Kareem, Nobleson K, Pragna Mamidipaka, Avinash Kumar Paladi, Arul Pandian B, Prerna Rana, Jaikhomba Singha, Aman Srivastava, Mayuresh Surnis, Pratik Tarafdar, Publications of the Astronomical Society of Australia, Volume 41, article id. e036, 05/2024