Research Activities

1. IROAST Researchers

No.	Name	Project Title
1-1	**László Pusztai**	Nanoscale assemblies in hydrogen-bonded liquids and in amorphous materials
1-2	**Mitsuhiro Aida**	Plant developmental biology
1-3	**Gaochuang Cai**	Design for structural safety and sustainability (DfS^3)
1-4	**Takumi Higaki**	Quantitative Bioimaging
1-5	**Takashi Ishida**	Deciphering the molecular basis of the plant morphogenesis
1-6	**Ruda Lee**	Enhanced Nano Drug Delivery System for Overcoming Cancer
1-7	**Hiroki Matsuo**	Development of ferroelectric materials for energy storage and conversion
1-8	**Akiko Nakamasu**	Theoretical modeling for the understanding of plant structure formations
1-9	**Mizuki Yamada**	Analysis of auxin signaling regulation in the apical region development of the plant embryo

No.1-1	Nanoscale assemblies in hydrogen-bonded liquids and in amorphous materials		
Name	László Pusztai		
Affiliation (home)	Wigner Research Centre for Physics, Budapest, Hungary Email: pusztai.laszlo@wigner.hu	Title	Scientific Advisor
Research Field	Nanomaterial Science		
Period of appointment	Month 04, 2021-Month 03, 2022 (I was not able to show up in Japan)		
Host Professor	Ichiro Akai		
Affiliation	Institute of Industrial Nanomaterials, KU Email: iakai@kumamoto-u.ac.jp	Title	Professor

In FY 2021 my activities have been very much determined by the COVID-19 pandemic that prevented me from staying one single day at IROAST. What I can report below is (i) work that has been mostly done before FYI 2020 but has been completed in FY 2021, and (ii) the very much retarded activities that has been possible to maintain remotely.

1. Research achievements

My primary research goal in general may be described in short as '**understanding disordered structures'.** Accordingly, my main activity (still, in general) is the investigation of the microscopic structure of liquids, amorphous materials and disordered crystals. We combine experimental data, such as total scattering structure factors (TSSF) from X-ray and neutron diffraction (XRD and ND, respectively) and EXAFS spectra, with computer modeling tools, such as Reverse Monte Carlo (RMC) and molecular dynamics (MD) simulations. As a result of such an approach, large sets (containing tens of thousands) of atomic coordinates ('particle configurations') in simulation boxes are provided that are consistent (within errors) with experimental data. These configurations are then subjected to various geometrical analyses, so that specific questions concerning the structure of a material may be answered. Below I describe some selected results from the year of 2021 (only publications where the name of IROAST appears).

(i) Temperature dependent structure and dynamics of ethanol-water (CH_3-CH_2-OH)/H_2O) mixtures over a wide concentration range. New X-ray and neutron diffraction experiments have been performed on ethanol−water mixtures as a function of decreasing temperature, so that such diffraction data are now available over the entire composition range. Extensive molecular dynamics simulations show that the all-atom interatomic potentials applied are adequate for gaining insight into the hydrogen-bonded network structure, as well as into its changes on cooling. Various tools have been exploited for revealing details concerning hydrogen bonding, as a function of decreasing temperature and ethanol concentration, like determining the H-bond acceptor and donor sites, calculating the cluster-size distributions and cluster topologies, and computing the Laplace spectra and fractal dimensions of the networks. It is found that 5-membered hydrogen-bonded cycles are dominant up to an ethanol mole fraction $x_{eth} = 0.7$ at room temperature, above which the concentrated ring structures nearly disappear. Percolation has been given special attention, so that it could be shown that at low temperatures, close to the freezing point, even the mixture with 90% ethanol ($x_{eth} = 0.9$) possesses a three-dimensional (3D) percolating network. Moreover, the water subnetwork also percolates even at room temperature, with a percolation transition occurring around $x_{eth} = 0.5$.

Related publication: Pothoczki, S; Pethes, I; **Pusztai, L**; Temleitner, L; Ohara, K; Bakó, I; Properties of Hydrogen-Bonded Networks in Ethanol–Water Liquid Mixtures as a Function of Temperature: Diffraction Experiments and Computer Simulations; *The Journal of Physical Chemistry B*; **125(23),** 6272-6279 (2021); DOI: 10.1021/acs.jpcb.1c03122.

(ii) Temperature-dependent structure of 1-propanol—water (CH_3-CH_2-CH_2-OH)/H_2O) liquid mixtures. — Aqueous mixtures of 1-propanol have been investigated by high-energy synchrotron X-ray diffraction upon cooling. X-ray weighted total scattering structure factors of 6 mixtures, from 8 mol% to 89 mol% alcohol content, as well as that of pure 1-propanol are reported from room temperature down to the freezing points of the liquids. Molecular dynamics simulations have been performed, in order to interpret measured data. The all atom OPLS-AA potential model was used for 1-propanol, combined with both the SPC/E and the TIP4P/2005 water models: both combinations provide a semi-quantitative description of the measured total structure factors at low and high alcohol contents, while the agreement is qualitative for the mixture with 71 mol% of 1-propanol. From the simulated particle configurations, partial radial distribution functions were calculated. Furthermore, detailed description of the hydrogen bonded network is provided, in terms of hydrogen bond numbers, analysis of proton donor–acceptor ratios, size distributions of hydrogen bonded clusters and ring size statistics. Strong temperature dependence of the percolation threshold, as well as of the participation of the number of doubly hydrogen bonded molecules in cyclic entities, has been found for the mixture with 89 mol% of 1-propanol. Above an alcohol content of 20 mol%, 5-fold rings are the most frequent cyclic entities, with a strong temperature dependence in terms of the number of rings.

Related publication: Pethes, I; **Pusztai, L**; Ohara, K; Temleitner, L; Temperature-dependent structure of 1-propanol/water mixtures: X-ray diffraction experiments and computer simulations at low and high alcohol contents; *J. Mol. Liq.*, **340**, 117188 (2021) https://doi.org/10.1016/j.molliq.2021.117188

(iii) Cations in polar solvents. We showed how the dipole moment of a single molecule in a cluster can be calculated and used for describing the polarization effect. Additionally, we reviewed the accuracy of the calculation of the dipole moment of several simple protic and aprotic molecules. It was shown that the dipole moment of polar (water, methanol, formamide, acetone and acetonitrile) molecules in the neighborhood of a cation is increased primarily by polarization from the bare electrostatic charge of the cation, although the effective value of the latter is somewhat reduced by "back donation" of electrons from neighboring polar molecules. In other words, the classical picture may be viewed as if a point charge slightly smaller than the nominal charge of the cation would be placed at the cation site. It was found that the geometrical arrangement of the polar molecules in the first solvation shell is such that their mutual polarization reduces the dipole moments of individual molecules, so that in some cases they become smaller than the dipole moment of the free protic or aprotic molecule. We conjectured, for the first time, that this behavior, namely the roughly 10%–20% decrease of the dipole moment of water in the first shell of cations, with the cation itself removed, is essentially a manifestation of the Le Chatelier–Braun principle. We also remark that if the cation-molecule bond order is too large then the calculated dipole moment for these complexes can be questionable, due to the questionable definition of a single molecule within the "supermolecule"-like cluster.

Related publication: Bakó, I; Csókás, D; Mayer, I; Pothoczki, S; **Pusztai, L**; The influence of cations on the dipole moments of neighboring polar molecules; *Int J. Quant. Chem.*,

(iv) Structural studies of ^{1}H-containing liquids by polarized neutrons. Following a demonstration of how neutron diffraction with polarization analysis may be applied for the accurate determination of the coherent static structure factor of disordered materials containing substantial amounts of proton nuclei (Temleitner et al., Phys. Rev. B 92, 014201, 2015), we now focus on the incoherent scattering. Incoherent contributions are responsible for the great difficulties while processing standard (non-polarized) neutron diffraction data from hydrogenous materials, hence the importance of the issue. Here we report incoherent scattering intensities for liquid acetone, cyclohexane, methanol and water, as function of the ^{1}H/H ratio. The incoherent intensities are determined directly by polarized neutron diffraction. This way, possible variations of the incoherent background due to the changing chemical environment may be monitored. In addition, for some of the water samples, incoherent intensities as a function of the wavelength of the incident neutron beam (at 0.4, 0.5 and 0.8 Å) have also been measured. It is found that in each case, the incoherent intensity can be described by a single Gaussian function, within statistical errors. The (full) width (at half maximum) of the Gaussians clearly depends on the applied wavelength. On the other hand, the different bonding environments of hydrogen atoms do not seem to affect the width of the Gaussian.

Related publication: Temleitner, L; **Pusztai, L**; Cuello, G; Stunault, A; Structural studies of ^{1}H-containing liquids by polarized neutrons: Chemical environment and wavelength dependence of the incoherent background *J. Mol. Liq.*, **350**, 118535 (2022) https://doi.org/10.1016/j.molliq.2022.118535

Talks at meetings, seminars:
No meetings, unfortunately, due to the virus situation.

2. Overview and significance of the research collaboration with Kumamoto University

During FY 2021, I've collaborated mostly with my host professor, Dr. Hosokawa, and his co-worker at the Department of Physics, Dr. Nakajima, a young tenured-track fellow – with both of them, only remotely.

A manuscript with Prof. Hosokawa has been prepared during FY2021.

The joint research work with Dr. Nakajima, on high pressure diffraction measurements of alcohol-water liquid mixtures, produced the first publication in the calendar year 2021. A joint KAKENHI proposal with him has been submitted last Autumn.

3. Prospect for further research collaboration with Kumamoto University

The high pressure work, for which I obtain vital help and assistance from Dr. Nakajima, is still expected to expand – provided that we can conduct proper joint research in the near future. Follow-up publications with Prof. Hosokawa on the structure of amorphous materials will continue to appear for a while (there is one manuscript submitted at the moment).

4. List of co-authored papers published between April 2021 and March 2022

Pothoczki, S; Pethes, I; **Pusztai, L**; Temleitner, L; Ohara, K; Bakó, I; Properties of Hydrogen-Bonded Networks in Ethanol–Water Liquid Mixtures as a Function of Temperature: Diffraction Experiments and Computer Simulations; *The Journal of Physical Chemistry B*; **125(23),** 6272-6279 (2021); DOI: 10.1021/acs.jpcb.1c03122.

Pethes, I; **Pusztai, L**; Ohara, K; Temleitner, L; Temperature-dependent structure of 1-propanol/water mixtures: X-ray diffraction experiments and computer simulations at low and high alcohol contents; *J. Mol. Liq.*, **340**, 117188 (2021) https://doi.org/10.1016/j.molliq.2021.117188

Bakó, I; Csókás, D; Mayer, I; Pothoczki, S; **Pusztai, L**; The influence of cations on the dipole moments of neighboring polar molecules; *Int J. Quant. Chem.*, 2021:e26758 (2021) https://doi.org/10.1002/qua.26758

Temleitner, L; **Pusztai, L**; Cuello, G; Stunault, A; Structural studies of ^{1}H-containing liquids by polarized neutrons: Chemical environment and wavelength dependence of the incoherent background *J. Mol. Liq.*, **350**, 118535 (2022) https://doi.org/10.1016/j.molliq.2022.118535

No.1-2	Plant developmental biology		
Name	Mitsuhiro Aida		
Affiliation	IROAST Email: m-aida@kumamoto-u.ac.jp	Title	Professor
Research Field	Advanced Green Bio		

1. Research achievements

Our research aim is to elucidate molecular and genetic mechanisms that regulate the activity of shoot meristem, a group of dividing cells that is responsible for production of plant aerial organs, such as leaves, stems, and floral organs, which are collectively called shoot organs. In dicotyledonous plants, the shoot meristem is initially formed during embryogenesis in a boundary region between the two cotyledon primordia, and after germination, it maintains a group of stem cells at its center and continuously provides new cells that give rise to shoot organs from its periphery.

This fiscal year we reported the roles of regulatory factors that are expressed in the boundary region between cotyledons. The CUP-SHAPED COTYLEDON (CUC) transcription factors are essential for shoot meristem formation as well as setting up cotyledon primordium boundary region so that the primordia are develop into two distinct organs. We found that the CUC proteins are essential for activation of genes encoding key biosynthetic enzymes for the plant hormone auxin. We also analyzed *EPFL2* encoding a signaling peptide and found that this gene is expressed in the cotyledon boundary region and is required for cotyledon primordium growth as well as proper levels of auxin response in the primordium tips. Together our results highlight the importance of the cotyledon boundary region for auxin-dependent cotyledon development.

2. International research collaboration

International collaborative work was made with Keiko U Torii (Howard Hughes Medical Institute and University of Texas at Austin, USA) and Rüdiger Simon (Heinrich-Heine University, Germany), leading to a publication that describing a role for the *EPFL2* gene during embryogenesis (ref. 5). Other collaborative work has been carried out with Jose Ireapan Reyes Olalde, a research fellow from Consejo Nacional de Ciencia y Tecnología (CONACYT) of Mexico, to elucidate hormonal control of gynoecium development, which led to an oral presentation in the 63rd annual meeting of the Japanese Society of Plant Physiologists, March 22, 2022. A collaboration with Yoshihisa Ikeda from Palacký University (Czech Republic) has led to a publication of the roles of AP2 related transcription factors in the shoot development (ref 3) and details of this activity is described elsewhere (Research Unit “Plant Stem Cells and Regeneration” section).

3. Prospect for further research collaboration

The following collaborative research is in progress in FY2022.

- ✓ Elucidation of mechanisms regulating fruit development in collaboration with Stefan de Folter in CINVESTAV, LANGEBIO, Mexico.
- ✓ Investigation of the roles of the AP2-class transcription factors ESR1 and ESR2 in shoot formation and tissue regeneration in collaboration with Yoshihisa Ikeda in Palacky University in Olomouc.

4. List of journal papers (with IROAST as your affiliation) published between April 2021 and March 2022.

1. Yamada M, Tanaka S, Miyazaki T, Aida M. Expression of the auxin biosynthetic genes *YUCCA1* and *YUCCA4* is dependent on the boundary regulators *CUP-SHAPED*

COTYLEDON genes in the *Arabidopsis thaliana* embryo. **Plant Biotechnol** 39, 37-42, doi: 10.5511/plantbiotechnology.21.0924a.

2. Suzuki, R., Yamada, M., Higaki, T., Aida, M., Kubo, M., Tsai, A.Y-L., Sawa, S. (2021) *PUCHI* regulates giant cell morphology formation during root-knot nematode infection in *Arabidopsis thaliana*. **Frontiers in Plant Science.** 12, 755610. https://doi.org/10.3389/fpls.2021.755610
3. Ikeda Y, Králová M, Zalabák D, Kubalová I, Aida M (2021). Post-embryonic lateral organ development and adaxial–abaxial polarity are regulated by the combined effect of *ENHANCER OF SHOOT REGENERATION 1* and *WUSCHEL* in Arabidopsis shoots. **Int. J. Mol Sci** 22, 10621. doi: 10.3390/ijms221910621
4. Takahama A, Aida M. Visualization and quantification of cortical microtubules in the apical region of the *Arabidopsis thaliana* embryo (2021). **Cytologia** 86, 181-182. doi: 10.1508/cytologia.86.181
5. Fujihara R, Uchida N, Tameshige T, Kawamoto N, Hotokezaka Y, Higaki T, Simon R, Torii KU, Tasaka M, Aida M (2021). The boundary-expressed *EPIDERMAL PATTERNING FACTOR-LIKE2* gene encoding a signaling peptide promotes cotyledon growth during *Arabidopsis thaliana* embryogenesis. **Plant Biotechnol** 38, 317-322. doi: 10.5511/plantbiotechnology.21.0508a
6. Yamamoto K, Tasaka M, *Aida M. "Genetic interactions between the CUP-SHAPED COTYLEDON and the BELLRINGER genes indicate their overlapping functions in carpel boundary development in Arabidopsis thaliana," Plant Morphol, 33 (1), 95-100, 2021

5. List of Awards, Grants, and Patents, if any

Mitsuhiro Aida, Grant-in-Aid for Scientific Research on Innovative Areas (The Japan Society for the Promotion of Science), Principles of pluripotent stem cells underlying plant vitality, "Establishment of plant hormone microenvironment during shoot stem cell formation," April 2020-March 2022.

No.1-3	Design for structural safety and sustainability (DfS^3)		
Name	Gaochuang Cai		
Affiliation	IROAST Email: cai@kumamoto-u.ac.jp	Title	Associate Professor
Research Field	Environmental Science		

Details of activities

1. Research achievements

Since October 2021, the following main achievements have been archived,

(1) Development of reinforced CFT columns toward strong earthquake

The seismic behavior of RCFT columns has been experimentally investigated and the design approach of the CFT columns has been proposed including a simplified model for predicting the load-carrying and deformation capacity of the columns.

(2) Development of assessment methods of bolted precast RC walls under simulated seismic loads

As a demountable and sustainable structure, a steel bolted precast RC wall system has been developed and its seismic performance has been experimentally numerically investigated.

(3) Experimental investigation of seismic performance of high resilient RC beam-column joints

With debonded high-strength steel bars, we have developed an innovative RC beam-column joints with high resilience after being subjected to strong earthquakes. The related calculation models are being developed by analyzing the resistance mechanism and failure modes, and a finite element method analysis.

(4) Seismic performance and key damage control approach of RC frames infilled by masonry walls

The seismic performance of the frames with different filled masonry walls has been experimentally and numerically studied. A capacity and deformation assessment model has been developed for evaluating the seismic behavior of the frames.

2. International research collaboration

International collaboration topics/joint research institution

(1). Properties and modeling of Textile reinforced concrete under aggressive environment/ ENISE, École Centrale de Lyon (ECL), University of Lyon

(2). AI-based performance evaluation and optimization of steel-concrete composite structures under strong earthquake / City, University of London.

(3). Structural performance and design methods of demountable structures Technischen Universität Darmstadt

(4). Seismic performance and design of bolted precast RC walls/Sichuan University

(5). Bond-slip and models of CFRP bars in ultra-high-strength concrete/Zhengzhou University

(6). Seismic performance and design of high resilient RC beam-column joints / Sichuan University

(7). Seismic performance and key damage control approach of RC frames infilled by masonry wall: an experimental and numerical study / Southwest Jiaotong University

(8). Seismic performance and design of concrete columns confined by steel-FRP composite tube / Dalian University of Technology

(9). Basic research on a smart sensor eco-formwork system for I-Construction of concrete structures / Zhejiang university

3. Prospect for further research collaboration

The following mentioned research collaboration in Section 2 will be continued. According to the communication up to now, the prospected NEW collaborations are listed below,

(1) Topic: Resilience of structural systems subject to earthquakes, Digital signal processing, and pattern recognition

PI/Institution: Assoc. Prof. Salvatore Salamone, Smart Structures Research Group (SSRG) at the University of Texas at Austin, U. S.

(2) Topic: Digital and Autonomous Construction (IDAC)
PI/Institution: Professor Kay Smarsly, Institute of Digital and Autonomous Construction, Hamburg University of Technology, Germany.

4. List of journal papers (with IROAST as your affiliation) published between April 2021 and March 2022.
Since the researcher has just joined Kumamoto University in October 2021, the following publications with the affiliation of IROAST have been submitted to several international journals,

(1). **G.C Cai***, T. Fujinaga, A. Si Larbi (2021). Cyclic behaviour of CFT columns reinforced with LBHSRs, *Bulletin of Earthquake Eng.* (Under review).
(2). F. Zhao, F. Xiong, **G.C. Cai***, A. Si Larbi (2021). Experimental and numerical study of full-scale PC wall panels with bolted connections subjected to cyclic loads. *Journal of Building engineering,* (Under review).
(3). Y. Sun, **G.C Cai*** (2021). Lateral capacity and deformation ability of RCCCs under large cyclic loads, *ASCE-J. Struct. Eng.* (Under review).
(4). Y. Wang, G. Chen, **G.C Cai*** et al. (2021) Constitutive models of circular GFRP-steel tube confined concretes under cyclic axial compression, *Engineering Structures*, (Under review).
(5). Q. Su, **G.C Cai***(2021) Damage controlling of infilled RC frames under simulated seismic loads: An experimental study, *Engineering Structures,* (Under review).
(6). Y.L Wang, **G.C Cai*** et al. Seismic performance of square GFRP-steel tube confined concrete columns, *Journal of Building Engineering,* (Under review).

Besides, the following books/papers are under preparation,

(1). **G.C. Cai***, T. Noguchi. By-products reuse in the cement and concrete industry, CRC press. (Contracted, about 350 pages, will be finished in August 2022)
(2). **G.C. Cai***, A. Si Larbi (2021). Cyclic behaviour of XRCFT columns under simulated seismic loads, Target journal: *Structures.*
(3). **G.C. Cai***, W. Liu, A. Si Larbi (2021). Monotonic and cyclic tensile properties and a simplified calculation model of reinforced textile reinforced mortars (RTRM), Target journal: *Buildings*.
(4). H. Zhu, Y. He, **G.C. Cai***, Y. Zhang, L. Chen, Bond performance between CFRP rebars and ultra-high-performance concrete, Target journal: *Construction Buildings Materials*
(5). **G.C. Cai***, Y.J. He (2021). Seismic performance and evaluation of FRP-strengthened RC columns: A critical review, Target journal: *Structures.*

5. List of Awards, Grants, and Patents, if any
Since October 2021, the following JSPS applications have been submitted,

(1). JSPS Invitation Fellowships for research in Japan, AI-based design and optimization of composite structures under strong earthquakes, submitted.
(2). JSPS Postdoctoral fellowships for research in Japan, Resilient RC structures, submitted.
(3). JSPS Postdoctoral fellowships for research in Japan, A smart concrete performance assessment system, submitted.
(4). JSPS Postdoctoral fellowships for research in Japan, AI design and optimization of concrete structures, submitted.

No.1-4	Quantitative Bioimaging		
Name	Takumi Higaki		
Affiliation	IROAST (-Sep 2021); FAST (Oct 2021-) Email: thigaki@kumamoto-u.ac.jp	Title	Professor
Research Field	Advanced Green Bio		

Details of activities

Since I was moved to the Faculty of Advanced Science and Technology from IROAST in October 2021, this report describes my achievements from April 2021 to September 2021.

Recent advances in bioimaging equipment have enabled biological scientists to easily acquire large amounts of bioimage data within a short period of time. Following this influx of information, biologists are now engaging in bioimage informatics, an emerging area of bioinformatics. I worked on the development of a new system to monitor and measure the plant epidermal cell shapes with metal-nano-ink and artificial intelligence (AI). It is known that most epidermal cells in dicotyledonous plant leaves change from a simple brick shape to a complex jigsaw puzzle shape as they grow. This distinctive jigsaw puzzle cell shape transformation has attracted attention not only in the field of basic biology, but also in applied research fields such as the design of artificial structures. The technique of monitoring cell shape changes by tracking cells over time is a fundamental and important technique in the study of cell shape changes. Until now, tracking and measuring shape changes in leaf epidermal cells required special techniques and expensive microscopy equipment. In this study, we found that by applying metal-nano-ink to the leaf surface, the shape of cells can be clearly visualized even with a relatively inexpensive metallurgical microscope. Furthermore, they developed an AI system that automatically recognizes cells from microscope images and measures their shapes (Figure). The widespread use of this technology is expected to dramatically advance our understanding of the shaping mechanism of plant cells. This work was published in 'Frontiers in Plant Science' in September 2021.

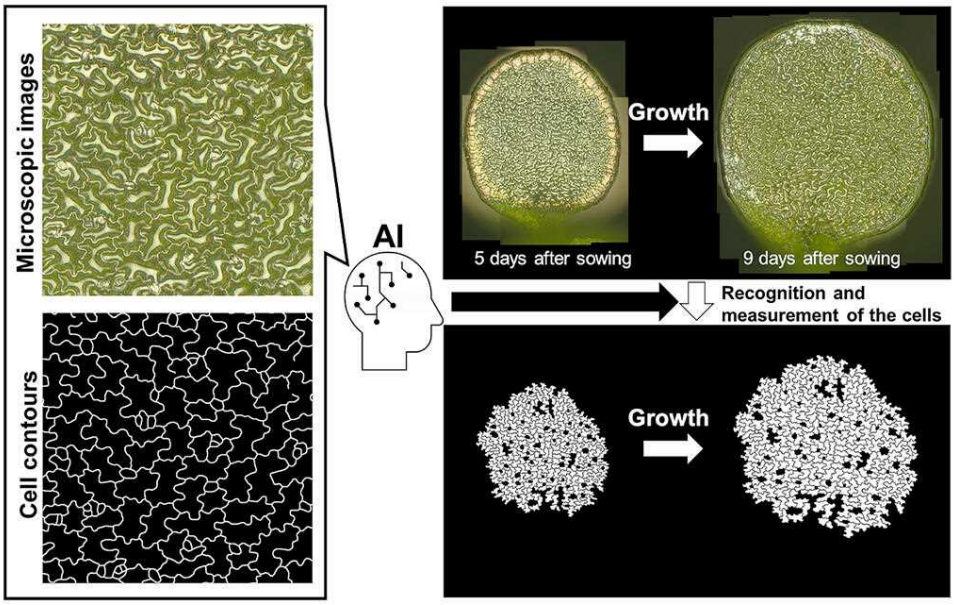


Figure. Conceptual diagram of the developed technology. Cell recognition and measurement were automated using microscopy images and the corresponding AI model (lower right). This image was provided for the following website; https://sj.jst.go.jp/news/202111/n1130-03k.html.

Publications between April 2021 and September 2021

Suzuki R, Yamada M, Higaki T, Aida M, Kubo M, Tsai AY, Sawa S,"PUCHI regulates giant cell morphology during root-knot nematode infection in Arabidopsis thaliana" Front Plant Sci 12, 755610. (Published: 06 Oct 2021) https://doi.org/10.3389/fpls.2021.755609

Kikukawa K, Sato R, Iwamoto M, Higaki T, "Wide-range segmentation of cotyledon epidermal cells for morphometrical analysis and mechanical simulation," Cytologia, 86: 189-194. 2021. (Published: 25 Sep 2021) https://doi.org/10.1508/cytologia.86.189

Kikukawa K, Yoshimura K, Watanabe A, Higaki T (2021) Metal-nano-ink coating for monitoring and quantification of cotyledon epidermal cell morphogenesis. Front Plant Sci 12: 745980. (Published: 21 Sep 2021) https://doi.org/10.3389/fpls.2021.745980

Kamon E, Noda C, Higaki T, Demura T, Ohtani M (2021) Calcium signaling contributes to xylem vessel cell differentiation via post-transcriptional regulation of VND7 downstream events. Plant Biotech 38: 331-337 (Published: 18 Sep 2021) https://doi.org/10.5511/plantbiotechnology.21.0519a

Fujihara R, Uchida N, Tameshige T, Kawamoto N, Hotokezaka Y, Higaki T, Simon R, Torii KU, Tasaka M, Aida M (2021) The boundary-expressed EPIDERMAL PATTERNING FACTOR-LIKE2 gene encoding a signaling peptide promotes cotyledon growth during Arabidopsis thaliana embryogenesis. Plant Biotech 38: 317-322. (Published: 18 Sep 2021) https://doi.org/10.5511/plantbiotechnology.21.0508a

Sato F, Iba K, Higaki T (2021) Involvement of the membrane trafficking factor PATROL1 in the salinity stress tolerance of Arabidopsis thaliana. Cytologia 86: 119-126. (Published: 25 June 2021) https://doi.org/10.1508/cytologia.86.119

Higaki T, Sato F, Iba K (2021) Environmental responses of the membrane trafficking factor PATROL1 in the Arabidopsis stomatal complex. Cytologia 86: 101-102. (Published: 25 June 2021) https://doi.org/10.1508/cytologia.86.102

Kimura T, Haga K, Nomura Y, Higaki T, Nakagami H, Sakai T (2021) Phosphorylation of NONPHOTOTROPIC HYPOCOTYL3 affects photosensory adaptation during the phototropic response. Plant Physiol 187: 981–995. (Published: 17 June 2021) https://doi.org/10.1093/plphys/kiab281

Matsumoto H, Kimata Y, Higaki T, Higashiyama T, Ueda M (2021) Dynamic rearrangement and directional migration of tubular vacuoles are required for the asymmetric division of the Arabidopsis zygote. Plant Cell Physiol 62: 1280–1289. (Published: 02 Jun 2021) https://doi.org/10.1093/pcp/pcab075

Kunita I, Morita MT, Toda M, Higaki T (2021) A three-dimensional scanning system for digital archiving and quantitative evaluation of Arabidopsis plant architectures. Plant Cell Physiol 62: 1975-1982. (Published: 22 May 2021) https://doi.org/10.1093/pcp/pcab068

No.1-5	Deciphering the molecular basis of the plant morphogenesis.		
Name	Takashi Ishida		
Affiliation	IROAST (-May 2021); FAST (Jun 2021-) Email: ishida-takashi@kumamoto-u.ac.jp	Title	Assistant Professor → Associate Professor
Research Field	Advanced Green Bio		

Details of activities

1. Research achievements

The mitotic cell cycle is a crucial cellular process to ensure cell proliferation in eukaryotes. Although the general mechanisms of the cell cycle progression are conserved across the kingdoms of fungi, animals and plants, the regulatory machinery and its underlying molecular basis likely differ in some ways.

In order to decipher the molecular insights into the plant cell cycle progression, we analyzed an Arabidopsis mutant *high ploidy 2* (*hpy2*) and found that *HPY2* encodes a homolog of NSE2/MMS21, a component of a widely conserved Structural Maintenance of Chromosomes protein (SMC) 5/6 complex (Ishida et al., Plant Cell 2009). Literature has revealed that yeast and animal NSE2/MMS21 mutants were defective in chromosome segregation, maintenance of chromosome structure and DNA damage responses. Consistent with this, we observed by live imaging analyses that fluorescent protein-tagged HPY2 co-localizes with chromosomes during mitosis. Further, we found that the lesion of HPY2 induced atypical pattern of chromosome movement during mitosis. These results obtained in this FY suggest that HPY2 is involved in the regulation of chromosome segregation

We prepared additional alleles of *hpy2* mutants and characterized them in the context of cell cycle regulation and genome integrity. Among the new mutants, an allele, namely *hpy2-cr1*, contained a frame-shift mutation lacking >80% of HPY2 protein displayed comparable phenotypes that were observed in *hpy2-1* stronger mutant allele. Moreover, we found a usefulness of *hpy2-cr1* that the sequence is easily distinguished from that of wild-type. Owing to this, we think that the use of the allele will be advantageous for the multiple types of genetic analyses and that the investigations will shed light to the functional analyses of SMC5/6 complex.

Coordinated cell proliferation and cell differentiation are essential processes in multicellular organisms. To achieve these functions, organisms have developed scrupulously designed cell-to-cell communication systems over the course of evolution. Plants have established unique ligand-receptor-based signaling modules, such as the CLAVATA (CLV) pathway, which comprises the CLV3 peptide hormone and leucine-rich repeat (LRR) domain-containing transmembrane receptors. In the shoot meristem of *Arabidopsis thaliana*, the CLV signaling modules play roles in the repression of cell proliferation. However, the molecular basis for the CLV-like signaling systems functioning in the root is to be elucidated. In order to decipher the molecular mechanisms revolving around the CLV-like systems in root, we have conducted genetic analyses using multiple mutants for the components of the signaling pathway to assess the genetic relationships. Resulted in the pharmacological analyses, we found a novel genetic pathway that modulate the root growth. Further, we found multiple molecules that are involved in the signaling. In this FY, we prepared a manuscript reporting above findings and submitted it (under revision).

In addition, we conducted functional analyses to clarify the biological relevance of plant-specific peptide hormones. CLE family peptides are representative plant peptide hormone and some of the members are known to be involved in multifaceted biological processes including developmental

and physiological regulations in the plant life cycle, though most of the members are not yet to be elucidated. A major problem in research on small peptide hormone-encoding genes is the limited number of loss-of-function mutants available due to their small gene size. We have previously generated and published a collection of mutants for CLE-peptide-encoding genes in *Arabidopsis thaliana* generated by CRISPR/Cas9-Mediated gene targeting (Yamaguchi et al., Plant Cell Physiol 2017).

In this FY, we asked biological relevance of several CLE peptides using the mutant collection. For instance, we discovered that a CLE peptide are involved in novel functions related to plant durability. We have submitted a manuscript related to this study and the manuscript is under revision (accepted in April 2022). In addition, we discovered a group of CLE peptides are involved in the regulation of stem cell function and organogenesis, whose manuscript has been submitted to Frontiers in Plant Science (Accepted in April 2022). These studies were done as international collaborative researches,

The functional characterization of peptide family that are composed of multiple number of highly redundant sequence and function is particularly challenging. We have tried to generate higher-order multiple mutants using CRISPR-Cas9 technology and succeeded it. Our analyses using the mutants revealed that the group of CLE peptides is involved in multifaceted biological processes including developmental regulation and response to biotic/abiotic stimuli. Manuscripts reporting these findings are currently under reviewing or in preparation.

Media

2021 年 4 月 9 日　熊本大など　菌由来の除草剤開発化学工業日報
(Related to a publication: Ishida et al., Scientific Reports 2021)

Patents

Oct. 12, 2021 United States Patent, Patent number: US 11,142,497 B2, Plant growth suppression agent, and plant growth suppression method using same
Hayato Ishikawa, Tokio Tani, Shinichiro Sawa, **Takashi Ishida**, Yusuke Fukushima, Jun Inagaki

2. International research collaboration

Although there is no publication accomplished by international collaboration in this FY, several international collaborative studies are ongoing. In particular, 5 manuscripts reporting the functional analyses regarding small genes in plants that are performed as international collaboration are under review (2 out of 5 are accepted in April 2022).

3. Prospect for further research collaboration

In FY2021, I have started several international collaborations on genome editing in plants. Our pipeline for rapid generation of Arabidopsis mutant will provide us opportunities for further collaborations; in particular, generation of the higher-order loss-of-function mutants using the technique will solve the redundancy problem in the molecular genetic studies. Our ongoing project using the method (related to the 3rd section of research achievements) will be published in the near future and will proved the usefulness of the method.

No.1-6	Enhanced Nano Drug Delivery System for Overcoming Cancer		
Name	Ruda Lee		
Affiliation	IROAST (-Dec 2021); IIna (Jan 2022-) Email: aeju-lee@kumamoto-u.ac.jp	Title	Associate Professor
Research Field	Advanced Green Bio		

1. Research achievements

The growing interest in applying nanotechnology to cancer is largely attributable to its uniquely appealing features for drug delivery, diagnosis, and imaging. Along with enormous progress in the field of cancer nanomedicine, we have also gradually realized the challenges and opportunities that lie ahead. To overcome the limitations, I focused on active targeted drug delivery materials for enhancing target ability and stimuli-responsive drug delivery systems for controlled drug release.

In FY2021, my lab focused on 1) developing nucleus targeted drug delivery system, 2) overcoming multi-drug resistance, 3) multi-receptor targeted drug delivery, and 4) in situ click chemistry. Depending on the target diseases, we designed a tailored nano-drug delivery system. Various NPs were successfully developed and evaluated in vitro and in vivo. Each NPs showed its characteristics and worked adequately under a specific environment (low pH, reactive oxygen species). Primarily, we developed a nucleus target delivery carrier and proved the efficacy to know down the gene expression. There was some research on which nucleus can target delivery. However, most of them were small inorganic NPs. It causes various side effects and shows high organ toxicity. My research group developed a non-toxic and minimized the off-target rate compared to previous research. One undergraduate student developed a stable polymer-based liposome. It showed high stability as well as overcoming multi-drug resistance effects. The manuscript is under preparation, and the NPs will upgrade during the student's master's periods. Furthermore, we found another strategy for increasing drug delivery efficacy. We hypothesized the possibility of enhancing treatment effects by dual ligand targeted modified NPs. AS we expected, dual ligand delivery efficacy was higher than the single ligand modified NPs. The manuscript is under preparation for submission.

2. International research collaboration

In FY2021, I suggested making strong collaboration with US and Korea. First, I applied for a CRDF grant with a new collaborator of Dana Faber Cancer Institute, and we were granted it for two years. It's a new project for developing virus-neutralized vaccines and real-time imaging sensors to monitor treatment responses. We are now working on creating/evaluating new imaging sensors and searching for the following grant applications. Second, the IROAST visiting Professor, Dr. Ick Chan Kwon, and I collaborate to develop a new click chemistry-mediated drug delivery system. One undergraduate student took this project and finalized the chemical synthesis. For a year, we actively discussed the project and troubleshot the materials. From FY2022, the student entered the graduate school semester and will perform in vitro experiments.

Furthermore, different countries' researchers and I were preparing to begin a new research collaboration. Dr. Chinmaya (India) and Dr. Manash (USA) had good knowledge of organoid research, and I started to join the research team. We published a review pare and began to organize

grant applications. Following the FY2020, the research exchange was not easy because of COVID-19. However, I got permission from IROAST and went to Korea to perform collaborative research. The results were under analysis and expected to publish in FY2022.

3. Prospect for further research collaboration

In FY2022, I have the plan to collaborate with a researcher at the Department of Biomedical Engineering, University of Utah (USA). The researcher had various experiences in microfluidics, so we have a plan to design the proper types of microfluidic chips for single-cell imaging. It will be a new collaborative project which I organize with Korea-Japan-US to apply for grants in FY2023. In July, I have a plan to apply for JSPS FOSTERING (A) grant with the US team for learning microfluidic technology. This opportunity can give me a chance to upgrade my research at single-cell level analysis. Furthermore, the IROAST Research Unit team members (Korea-Japan-Australia) will apply for Australia Ideas Grant. We failed the grant but the reviewer's comments sound right on the edge of getting it. So, we will modify the proposal and will apply again in May 2022.

4. List of journal papers (with IROAST as your affiliation) published between April 2021 and March 2022.

① Chinmaya Mahapatra, **Ruda Lee**, Manash K. Paul. Emerging role and promise of nanomaterials in organoid research. Drug Discovery Today, 27, 890-899. March 2022.

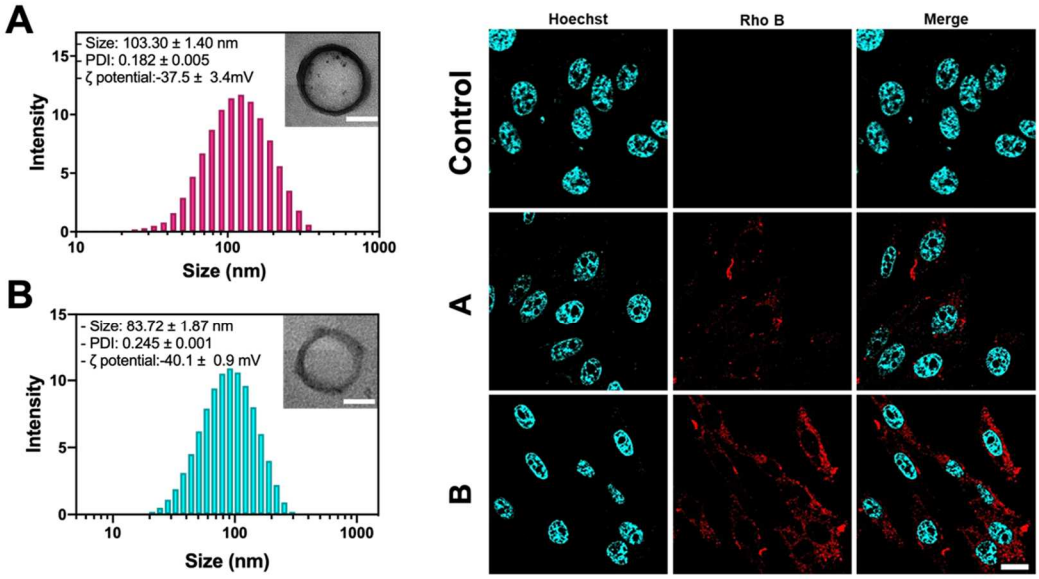
② Kang Pa Lee, Suji Baek, Myeong Sik Yoon, Ji Soo Park, Bok Sil Hong, Sang Ju Lee, Seung Jun Oh, Seung Hae Kwon, **Ruda Lee**, Dae Ho Lee, Kang-Seo Park, Byung Seok Moon. Potential anticancer effect of aspirin and 2'-hydroxy-2,3,5'-trimethoxychalcone-linked polymeric micelles against cervical cancer through apoptosis. Oncology Letters, 23, 31, November 2021.

③ Sajid Fazal, **Ruda Lee***. Biomimetic Bacterial Membrane Vesicles for Drug Delivery Applications. Pharmaceutics, 13, 1430. September 2021.

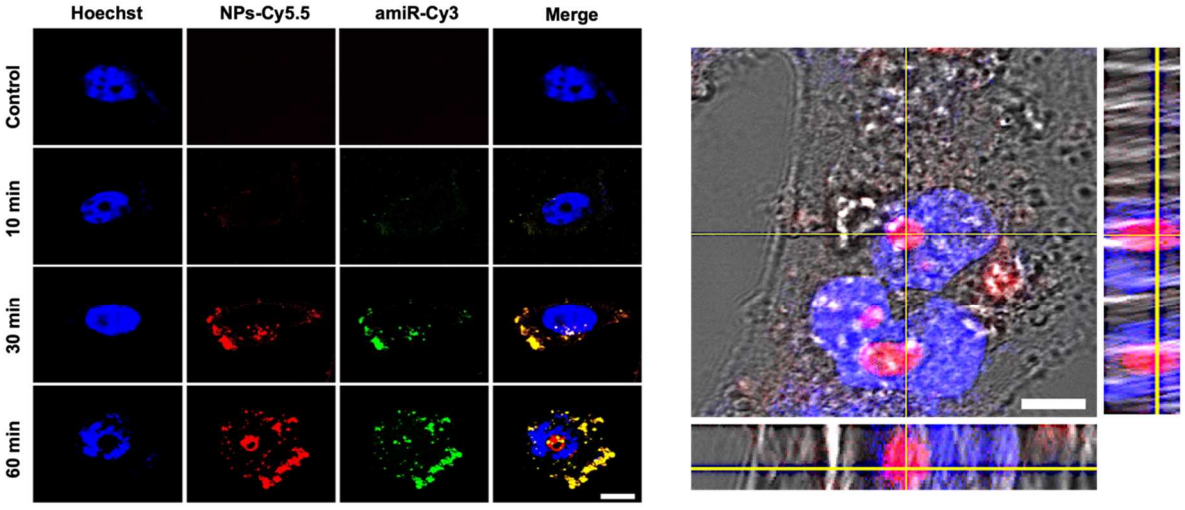
5. List of Awards, Grants, and Patents

① PI: Ruda Lee

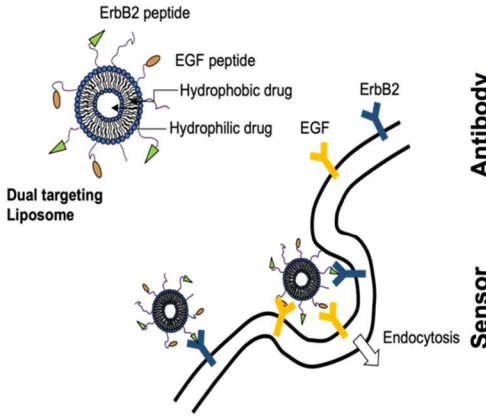
Japan Agency for Medical Research and Development (AMED), FY 2021 Infectious Diseases and Immunology Research: U.S.-Japan Cooperative Medical Sciences Program Collaborative Awards, "Investigating DNA origami vaccine efficacy in infectious diseases", FY2021-FY2023

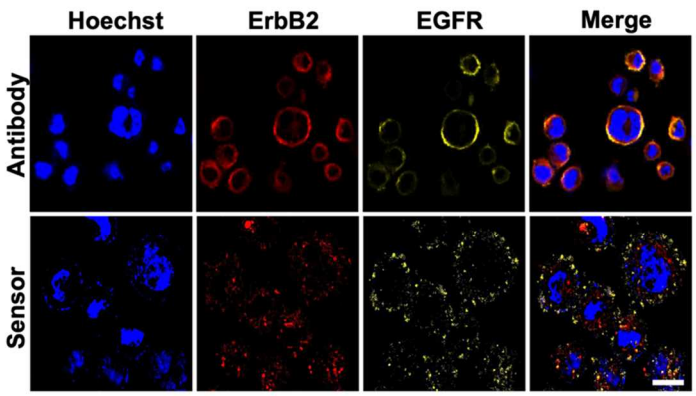


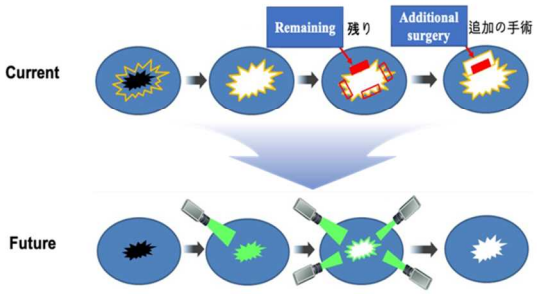
1. Overcoming multi-drug resistance

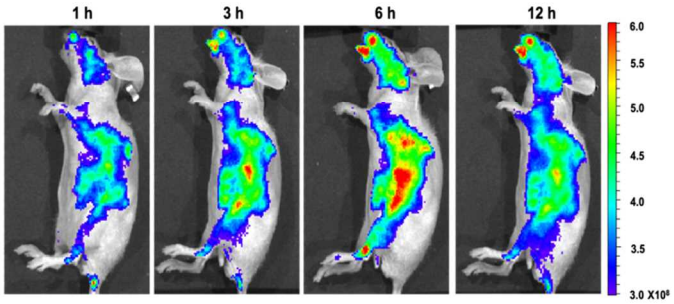


2. Nucleus targeted gene delivery carrier









3. Students supervising

No.1-7	Development of ferroelectric materials for energy storage and conversion		
Name	Hiroki Matsuo		
Affiliation	IROAST Email: matsuo_h@cs.kumamoto-u.ac.jp	Title	Associate Professor
Research Field	Nano Material Science / Green energy		

1. Research achievements

The goal of our research is to develop ferroelectric materials that are applicable to efficient energy storage and conversion. In this term, we conducted research focusing on a) the development of ferroelectrics for high energy-density ceramic capacitors and b) the photovoltaic effect of heavily doped ferroelectrics.

a) Development of ferroelectrics for high energy-density ceramic capacitors

We worked on the development of ferroelectric materials with a high energy storage density that utilize an interaction between lattice defects and spontaneous polarization. We found that Cu-doped $BaTiO_3$ ceramics after an appropriate thermal annealing and an electrical treatment exhibited a shifted polarization (P)-electric field (E) hysteresis loop as a result of the formation of strong an internal electric field. Our density functional theory calculations revealed that the internal electric filed was derived from a strong interaction between the ferroelectric polarization and defect dipoles comprised with Cu atom and oxygen vacancy. A resultant effective dielectric permittivity was about two times as large as that of typical undoped $BaTiO_3$ ceramics and the energy density was significantly improved without sacrificing an energy storage efficiency. A paper reporting these results has been published to a preprint server (DOI: doi.org/10.21203/rs.3.rs-1429045/v1).

b) Photovoltaic effect of heavily doped ferroelectrics

Ferroelectric materials exhibit a characteristic photovoltaic effect that can generate photovoltage far exceeding their bandgap energy and light polarization-dependent photocurrent. In this term, photovoltaic properties of 5% Mn-doped $BiFeO_3$ (Mn-BFO) ferroelectric thin films were analyzed. The Mn-BFO thin films exhibited a large photovoltaic response compared with undoped $BiFeO_3$ thin films under visible light irradiation. We found the Mn-BFO thin films exhibited an open circuit voltage of 14 V which was much higher than bandgap energy of the material and a large photocurrent anisotropy depending on incident light polarization. Quantitative analyses for the photovoltaic polarization dependent photocurrent suggested that the photocurrent anisotropy can be furtherly enhanced by increasing a ferroelastic domain wall density. I believe the enhanced ferroelectric photovoltaic effect will be applicable to light polarization detect applications. A paper on this achievement is in preparation.

Besides, the research on the ferroelectric photovoltaic effect in Mn-doped $BaTiO_3$ (Mn-BT) ferroelectric single crystals has also stared as a collaboration research with a Korean single-crystal production company.

2. International research collaboration

An international collaboration research on ferroelectric photovoltaics was launched in Nov. 2021. This collaboration research is comprised with researchers of Kumamoto University and Ceracomp Co. Ltd. which is a Korean company producing ferroelectric oxide single crystals by a special growth method called solid-state crystal growth. The goal of this collaboration is to develop ferroelectric materials with a large photovoltaic response. Ceracomp Co. Ltd., supplied heavily transition metal-doped ferroelectric single crystals to Kumamoto University. Structural analyses and photovoltaic measurements for the single crystals have been performed in Kumamoto University.

3. Prospect for further research collaboration

For deeper understanding of photocarrier dynamics in the ferroelectric photovoltaic effect, further collaboration with researchers who specialize in spectroscopic measurements is being planned. The new collaboration will provide information of photocarrier lifetime in ferroelectric materials and role of the impurity levels in the photovoltaic effects.

4. List of journal papers

Hiroki Matsuo*, Masashi Utsunomiya, Yuji Noguchi* "Utilizing ferrorestorable polarization in energy storage ceramic capacitors" (2022). Preprint (under review)
DOI: https://doi.org/10.21203/rs.3.rs-1429045/v1

Yuji Noguchi*, Hiroki Matsuo* "Polarizaion and Dielectric Properties of $BiFeO_3$-$BaTiO_3$ Superlattice-Structured Ferroelectric Films"
Nanomaterials, 11, 1857 (2021).
DOI: https://doi.org/10.3390/nano11071857

5. List of Awards

The 18th JACG Best Presentation Awards
"Gap-State Engineering for Ferroelectric Photovoltaic Effect in $BiFeO_3$ Epitaxial Thin Film"
Japanese Association for Crystal Growth (JACG). Dec. 13 2021.

No.1-8	Theoretical modeling for the understanding of plant structure formations		
Name	Akiko Nakamasu		
Affiliation	IROAST Email: akikonakamasu@gmail.com	Title	Project Assistant Professor
Research Field	Advanced Green Bio		

1. Research achievements

In this fiscal year, I tried following themes and grasped the directions of them. I'd like to thank to IROAST members for helping of these achievements.

1) Collaborative research for modeling of pattern formation phenomena.
2) Modeling for understanding of morphogenesis (especially in leaf formations).
3) Mathematical modeling and analysis of dynamical pattern.

1) is about pigmentation pattern formation on zebrafish body trunk. I explained the effects of connexon defects in *leopard* mutant by a mathematical model constructed more than ten years ago. Then 2) is treated about combination of positional information for morphogenesis. In this theme, the difference in proportions of leaf shape was explained by an algorithm for expansive growth with bias. Then loss and gain of shaping robustness could be observed depending on the characteristics of the pattern as positional information on the blade. 3) is about dynamics observed by a combination of spatial inhomogeneity with spatial pattern.

2. International research collaboration

I participated to an international webinar series "From Cellular Dynamics to Morphology II" as a speaker, and to an international meeting Pacifichem @ Honolulu (hybrid event on site and virtual) to give a contribution talk. Though, it was difficult to conduct new international collaborative researches for my over-capacity.

3. Prospect for further research collaboration

I'd like to collaborate with experimental biologists in the fields of pigmentation pattern formation in animal, and plant biology.

4. List of journal papers published between April 2021 and March 2022.

1) "Correspondences between parameters in a reaction-diffusion model and connexin functions during zebrafish stripe formation." to an open access journal Frontiers in Physics 18 January 2022.
2) "An algorithm for expansive growths with uniaxial gradients can explain different proportions in two-dimensional leaf shapes" to Frontiers in Cell and Developmental Biology. (under preparation of resubmission)
3) "Dynamics obtained by an equally spaced pattern interacting with spatial inhomogeneity" (under preparation)

5. List of Awards, Grants, and Patents, if any

It was a last year of following grants

1) Grant-in-Aid for Scientific Research on Innovative Areas (The Japan Society for the Promotion of Science), Periodicity and its modulation in plant and "Theoretical understandings of leaf diversity caused by modulations of spatial periodicity on leaf peripherals." June 2020-March 2022.
2) Research grant from Shimazu Science Foundation "Study on displacable effects of spatial inhomogeneity on Turing pattern." February 19, 2020- December 2021.
   *This is for a collaborative research with T.Higaki and H. Izuhara.

I regret that potential collaborative researches could not conducted in the former Research Area, even though modeling techniques and mathematical analysis for pattern formations have been required in this area.

No.1-9	Analysis of auxin signaling regulation in the apical region development of the plant embryo		
Name	Mizuki Yamada		
Affiliation	IROAST Email: myamada@kumamoto-u.ac.jp	Title	Postdoctoral Researcher
Research Field	Advanced Green Bio		

Details of activities

1. Research achievements

The aerial organs of plants (leaves, stems, flowers etc..) are generated from the shoot meristem, which is initially established during embryogenesis. In dicotyledonous plants, the apical region of the embryo is separated into two cotyledon primordia, and shoot meristem is formed in boundary region between cotyledon primordia. The *CUP-SHAPED COTYLEDON* (*CUC*) genes are known to act as key regulators of this process and are required for it. Besides the *CUC* genes, phytohormone auxin, which is involved in various developmental processes in plants, also plays important roles in embryogenesis. However, the relationship between *CUC* genes and auxin is not well understood. I am investigating the regulation of auxin signaling patterns by *CUC* genes in the apical region of the *Arabidopsis thaliana* embryos.

In FY2021, I reported the relationship between *CUC* genes and auxin biosynthetic genes (Yamada et al., 2022). Among auxin biosynthetic genes, the expression levels of *YUC1* and *YUC4*, which are expressed in the cotyledon boundary of the embryo, were reduced in the *cuc* loss-of-function mutant embryos. This result suggested that the auxin biosynthesis in cotyledon boundary of the embryos is dependent on the activity of the *CUC* genes. Moreover, I found that the *CUC* genes also regulate the spatial pattern of auxin response in the apical region of the embryo and that this regulation contributes to normal development. These results were reported at the 85th annual meeting of BSJ and will be published in a paper in FY2022.

Besides the auxin related genes, I am trying to find additional genes which work as *CUC* downstream factors by the transcriptome analysis (collaboration with Dr. Minoru Kubo; NAIST). In FY2021, I listed the several new candidate genes in comparison with other published datasets. Our group and I have already started to investigate some of candidate genes.

2. International research collaboration

NA

3. Prospect for further research collaboration

From the results of embryo transcriptome analysis, I will find the various new candidate genes involved in the tissue differentiation in the apical region of embryo. I will have to collaborate with researchers who are expert in those genes.

4. List of journal papers

Yamada M, Tanaka S, Miyazaki T, Aida M (2022). Expression of the auxin biosynthetic genes *YUCCA1* and *YUCCA4* is dependent on the boundary regulators *CUP-SHAPED COTYLEDON* genes in the *Arabidopsis thaliana* embryo. Plant Biotechnol 39, 37-42. doi: 10.5511/plantbiotechnology.21.0924a

Suzuki R, **Yamada M**, Higaki T, Aida M, Kubo M, Tsai AY, Sawa S (2021). *PUCHI* regulates giant cell morphology during root-knot nematode infection in *Arabidopsis thaliana*. Front Plant Sci 12, 755610. doi: 10.3389/fpls.2021.75561

5. List of Awards, Grants, and Patents, if any

NA