NIBB Core Research Facilities

Head
YOSHIDA, Shosei

The NIBB Core Research Facilities support basic biological research conducted at NIBB. They consist of three facilities that develop and provide state-of-the-art technologies aimed at increasing the understanding of biological functions through the application of functional genomics, bioimaging, and bioinformatics. The NIBB Core Research Facilities also act as an intellectual hub to promote collaboration among NIBB researchers and other academic institutions.

Functional Genomics Facility

Professor
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Technical Staff:	*MORI, Tomoko*
	MAKINO, Yumiko
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	AKITA, Asaka
	MATSUMOTO, Miwako
Admin Support Staff:	*ICHIKAWA, Mariko*

The Functional Genomics Facility is a division of the NIBB Core Research Facilities organized jointly by NIBB and NIPS for the promotion of DNA and protein studies. The facility maintains a wide array of core research equipment, ranging from standard machinery (e.g. ultracentrifuges) to cutting edge tools (*e.g.* next generation DNA sequencers), which amount to 90 instruments in total. The facility is dedicated to fostering collaborations with researchers both at NIBB and other academic institutions worldwide through the provision of these tools as well as expertise. Our current focus is functional genomics. We also act as a bridge between experimental biology and bioinformatics by providing close consultation and training.

In 2020, we suffered from the effects of the COVID-19 pandemic that hampered research activities worldwide. While we operated our facility placing the highest priority on users' safety, we provided remote support for the facilities' users and online communication with collaborators to sustain research projects. Such efforts resulted in 17 co-authored papers being published.

Representative Instruments

Genomics

The advent of next-generation sequencing (NGS) technologies is transforming modern biology thanks to ultra-high-throughput DNA sequencing. Utilizing HiSeq, NextSeq and MiSeq (Illumina), Sequel (PacificBio Sciences), and MinION and GridION (Oxford Nanopore Technologies), the Functional Genomics Facility is committed to joint research aimed at exploring new yet otherwise inaccessible fields in basic biology.

During 2019, we carried out 57 NGS projects in collaboration with researchers from academic institutions throughout the world. These projects cover a wide range of species (bacteria, animals, plants, and fungi) including both model and non-model organisms, and various other applications such as genomic re-sequencing, RNA-seq and ChIP-seq.

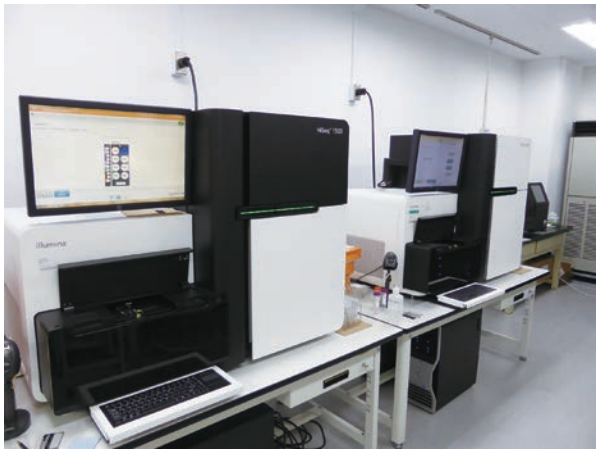

Figure 1. Next-generation sequencer

Proteomics

As is listed below, two types of mass spectrometers and two protein sequencers are used for proteome studies in our facility. In 2019, we analyzed approximately 1000 samples with mass spectrometers and protein sequencers.

- LC-MS (AB SCIEX TripleTOF 5600 system)
- LC-MS (Thermo Fisher SCIENTIFIC Orbtrap Elite)
- Protein sequencer (ABI Procise 494 HT; ABI Procise 492 cLC)

Figure 2. LC-MS/MS system

Other analytical instruments *(excerpts)*

- Cell sorter (SONY SH800)
- Bioimaging analyzer (Fujifilm LAS 3000 mini; GE FLA9000)
- Laser capture microdissection system (Thermo Fisher Scientific Arcturus XT)
- Real-time PCR machine (Thermo Fisher Scientific ABI 7500)
- Ultracentrifuge (Beckman XL-80XP etc.)
- Microplate reader (PerkinElmer Nivo; Hitachi SH-9000Lab)
- Single-cell analysis system (Fluidigm C1)

Publication List on Cooperation:

〔Original Papers〕

- Araki, K.S., Nagano, A.J., Nakano, R.T., Kitazume, T., Yamaguchi, K., Hara-Nishimura, I., Shigenobu, S., and Kudoh, H. (2020). Characterization of rhizome transcriptome and identification of a rhizomatous ER body in the clonal plant *Cardamine leucantha*. Sci. Rep. *10*. DOI: 10.1038/s41598-020-69941-9
- Cui, S., Kubota, T., Nishiyama, T., Ishida, J.K., Shigenobu, S., Shibata, T.F., Toyoda, A., Hasebe, M., Shirasu, K., and Yoshida, S. (2020). Ethylene signaling mediates host invasion by parasitic plants. Sci. Adv. *6*. DOI: 10.1126/sciadv.abc2385
- Feng, Z., Nagao, H., Li, B., Sotta, N., Shikanai, Y., Yamaguchi, K., Shigenobu, S., Kamiya, T., and Fujiwara, T. (2020). An SMU splicing factor complex within nuclear speckles contributes to magnesium homeostasis in Arabidopsis. Plant Physiol. *184*, 428–442. DOI: 10.1104/pp.20.00109
- Shikanai, Y., Yoshida, R., Hirano, T., Enomoto, Y., Li, B., Asada, M., Yamagami, M., Yamaguchi, K., Shigenobu, S., Tabata, R., *et al*. (2020). Callose synthesis suppresses cell death induced by low-calcium conditions in leaves. Plant Physiol. *182*, 2199–2212. DOI: 10.1104/pp.19.00784
- Shimada, T.L., Yamaguchi, K., Shigenobu, S., Takahashi, H., Murase, M., Fukuyoshi, S., and Hara-Nishimura, I. (2020). Excess sterols disrupt plant cellular activity by inducing stress-responsive gene expression. J. Plant Res. *133*, 383–392. DOI: 10.1007/s10265-020-01181-4
- Tominaga, T., Yamaguchi, K., Shigenobu, S., Yamato, M., and Kaminaka, H. (2020). The effects of gibberellin on the expression of symbiosis-related genes in Paris-type arbuscular mycorrhizal symbiosis in *Eustoma grandiflorum*. Plant Signal. & Behav. *15*. DOI: 10.1080/15592324.2020.1784544
- Vu, T.-D., Lwasaki, Y., Shigenobu, S., Maruko Akiko and Oshima, K., Lioka, E., Huang, C.-L., Abe T., Tamaki, S., Lin, Y.-W., Chen, C.-K., Lu Mei-Yeh and Hojo, M., *et al*. (2020). Behavioral and brain-transcriptomic synchronization between the two opponents of a fighting pair of the fish *Betta splendens*. PLoS Genet. *16*. DOI: 10.1371/journal.pgen.1008831
- Fukutomi, Y., Kondo, S., Toyoda, A., Shigenobu, S., and Koshikawa, S. (2021). Transcriptome analysis reveals wingless regulates neural development and signaling genes in the region of wing pigmentation of a polka-dotted fruit fly. FEBS J. *288*, 99–110. DOI: 10.1111/febs.15338
- Gu, N., Tamada, Y., Imai, A., Palfalvi, G., Kabeya, Y., Shigenobu, S., Ishikawa, M., Angelis, K.J., Chen, C., and Hasebe, M. (2020). DNA damage triggers reprogramming of differentiated cells into stem cells in Physcomitrella. Nat. Plants *6*, 1098+. DOI: 10.1038/s41477-020-0745-9
- Li, Y., Omori, A., Flores, R.L., Satterfield, S., Nguyen, C., Ota, T., Tsurugaya, T., Ikuta, T., Ikeo, K., Kikuchi, M., *et al*. (2020). Genomic insights of body plan transitions from bilateral to pentameral symmetry in Echinoderms. Commun. Biol. *3*. DOI: 10.1038/s42003-020-1091-1
- Mondal, S.I., Akter, A., Koga, R., Hosokawa, T., Dayi, M., Murase, K., Tanaka, R., Shigenobu, S., Fukatsu, T., and Kikuchi, T. (2020). Reduced genome of the gut symbiotic bacterium "*Candidatus Benitsuchiphilus tojoi*" provides insight into its possible roles in ecology and adaptation of the host insect. Front. Microbiol. *11*. DOI: 10.3389/fmicb.2020.00840
- Palfalvi, G., Hackl, T., Terhoeven, N., Shibata, T.F., Nishiyama, T., Ankenbrand, M., Becker D., Foerster, F., Freund, M., Iosip, A., Kreuzer, I., *et al*. (2020). Genomes of the Venus flytrap and close relatives unveil the roots of plant carnivory. Curr. Biol. *30*, 2312+. DOI: 10.1016/j.cub.2020.04.051
- Sakuta, H., Lin, C.-H., Hiyama, T.Y., Matsuda, T., Yamaguchi, K., Shigenobu, S., Kobayashi, K., and Noda, M. (2020). SLC9A4 in the organum vasculosum of the lamina terminalis is a [Na^+] sensor for the control of water intake. PFLUGERS Arch. J. Physiol. *472*, 609–624. DOI: 10.1007/s00424-020-02389-y
- Yano, H., Alam, M.Z., Rimbara, E., Shibata, T.F., Fukuyo, M., Furuta, Y., Nishiyama, T., Shigenobu, S., Hasebe, M., Toyoda A., Suzuki, Y., *et al*. (2020). Networking and specificity-changing DNA methyltransferases in *Helicobacter pylori*. Front. Microbiol. *11*. DOI: 10.3389/fmicb.2020.01628
- Kurihara, M., Kato, K., Sanbo, C., Shigenobu, S., Ohkawa, Y., Fuchigami, T., and Miyanari, Y. (2020). Genomic profiling by ALaP-Seq reveals transcriptional regulation by PML Bodies through DNMT3A exclusion. Mol. Cell *78*, 493+. DOI: 10.1016/j.molcel.2020.04.004
- Kanazawa, T., Morinaka, H., Ebine, K., Shimada, T.L., Ishida, S., Minamino, N., Yamaguchi, K., Shigenobu, S., Kohchi, T., Nakano A., Ueda, T. (2020). The liverwort oil body is formed by redirection of the secretory pathway. Nat. Commun. *11*. DOI: 10.1038/s41467-020-19978-1
- Bessho-Uehara, M., Yamamoto, N., Shigenobu, S., Mori, H., Kuwata, K., and Oba, Y. (2020). Kleptoprotein bioluminescence: Parapriacanthus fish obtain luciferase from ostracod prey. Sci. Adv. 6, eaax4942. DOI: 10.1126/sciadv.aax4942

〔Research activity by Shuji Shigenobu〕

- Professor Shuji Shigenobu is the principal investigator of the Laboratory of Evolutionary Genomics. Refer to the laboratory page for details.

SPECTROGRAPHY AND BIOIMAGING FACILITY

Specially Appointed Associate Professor
KAMEI, Yasuhiro

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	TANIGUCHI-SAIDA, Misako
Technical Assistant:	*ICHIKAWA, Chiaki*
	ASAO, Momoko
	NAKAGAWA, Mami

The Spectrography and Bioimaging Facility assists both collaborative and core research by managing and maintaining research tools that use **Light**. The facility, under the guidance of Dr. Kamei, also provides technical support through the management of technical staff assisting in the advancement of collaborative and core research projects, as well as academic support to researchers (please refer to the Collaborative Research Group Research Enhancement Strategy Office section for more information). Among the equipment available are advanced biological microscopes, and the Okazaki Large Spectrograph for photobiology. The Okazaki Large Spectrograph is the world's largest wide spectrum exposure mechanism, and is capable of producing a range of wavelengths from 250 nm (ultraviolet) to 1,000 nm (infrared) along its 10-meter focal curve, thus allowing exposure to strong monochromatic light. The facility's microscopes, which include cutting edge devices such as confocal and multi-photon excitation microscopes, are an indispensable part of core and collaborative projects conducted by both internal and external researchers.

Standard Instruments: Okazaki Large Spectrograph (OLS)

The spectrograph runs on a 30 kW Xenon arc lamp and projects a wavelength spectrum ranging from 250 nm (ultraviolet) to 1,000 nm (infrared) onto its 10 m focal curve with an intensity of monochromatic light at each wavelength more than twice as great as that of the corresponding monochromatic component of tropical sunlight at noon (Watanabe *et al*., Photochem. Photobiol. *36*, 491-498, 1982). The spectrograph is dedicated to action spectroscopical studies of various light-controlled biological processes.

In addition to the other action spectroscopical studies concerning various regulatory and damaging effects of light on living organisms, research involving both biological and artificial organic molecules have been conducted since it has been set up. The NIBB Collaborative Research Program for the Use of the OLS supports about 10 projects every year conducted by both visiting scientists, including foreign researchers, as well as members of NIBB.

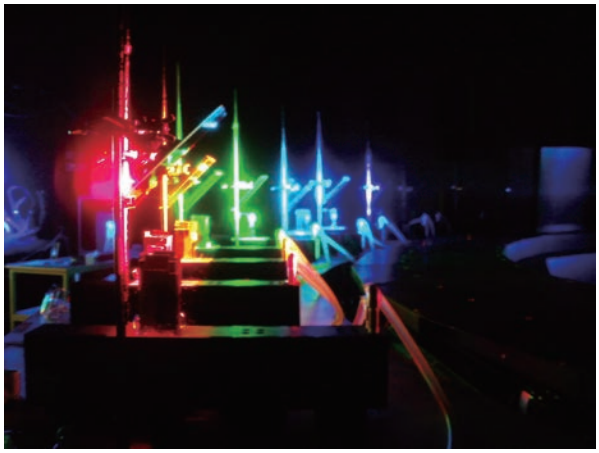

Figure 1. An example of an experiment using the Large Spectrograph. In this photo, various color rays (monochromatic light from right side and reflected by mirrors) are irradiated simultaneously onto samples stored in cooling chambers.

Microscopes

This facility also provides bioimaging machinery, such as wide-field microscopes (Olympus IX-81 and BX-63), confocal microscopes (Leica TCS-SP8, Nikon A1R, Nikon A1Rsi and Yokogawa CSU-X1 with EM-CCD/ CMOS cameras), multi-photon microscopes (Olympus FV1000-MP, FV1200-MPs, Leica TCS-SP8 MPs) and other advanced laser microscopes boasting specialized, cutting edge technology (Light-sheet Microscope and Infrared Laser-Evoked Gene Operator microscope: IR-LEGO), which can be utilized by researchers within NIBB, as well as collaborative guest researchers. Starting from 2016, we have commenced two new types of Collaborative Research Programs. One is a new category within the NIBB Collaborative Research for Integrative Bioimaging program using machinery and bioimage processing/analysis techniques, and the other is the Advanced Bioimaging Support Program (ABiS) which operates under the framework of the Grant-in-Aid for Scientific Research on Innovative Areas.

The light-sheet microscope was developed by Dr. Ernst Stelzer's group at the European Molecular Biology

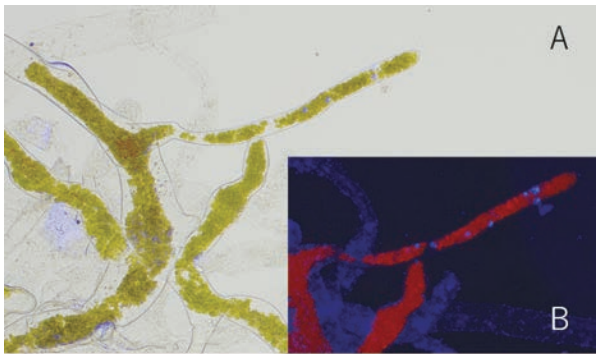

Figure 2. Microscopic images of green algae, *Codium fragile*. This sample was stained by hoechst33342. A) Bright and blue fluorescent marge image. B) Blue fluorescent and red fluorescent marge image. Images were taken by a visitor from a cooperation program with Osaka City University (Seki, Soichiro). *C. fragile* (KU-0654) was provided by KU-MACC, Kobe University.

Laboratory (EMBL). This microscope can realize high-speed z-axis scanning in deeper tissues by illuminating specimens from the side with a light sheet (more information is given in the report submitted by Dr. Shigenori Nonaka's Laboratory for Spatiotemporal Regulations).Subsequently, Dr. Nonaka has conducted and supported roughly 10 Collaborative Research Program projects for Integrative Bioimaging. The IR-LEGO, developed by Drs. Shunsuke Yuba and Yasuhiro Kamei at the National Institute of Advanced Industrial Science and Technology (AIST), can induce a target gene of interest by heating a single target cell *in vivo* with a high efficiency irradiating infrared laser (the details of this are provided in the next section). IR-LEGO was also used for about 10 Collaborative Research projects, including applications aimed at both animals and plants.

Workshop, Symposium and Training course

In 2020, we held the 8th biological image processing training course in cooperation with Drs. Kagayaki Kato, Shigenori Nonaka, Yasuhiro Kamei, Takashi Murata and Hiroshi Koyama. The course was held in an online meeting format (Figure 3) for the first time due to the COVID-19 pandemic.

We have also held annual "Bioimaging Forum" events, which discuss bioimaging from various technical perspectives such as that of microscopy, new photo-technology, and computer science. This year we planned on staging the 14th edition of this event in November with a focus on micro CT technology. Like so many events of its type, this meeting was also to be held in an online format due to the global COVID-19 pandemic.

Figure 3. A scene from the 8th Bio-Imaging Analysis Training Course. The course was held in an online meeting format to prevent COVID-19 infection. The 40 participants were able to interact with lecturers via the use of Zoom and chat applications from their specific locations.

Publication List on Cooperation

〔Original papers (Selected)〕

- Ashida, Y., Honma, Y., Miura, N., Shibuya, T., Kikuchi, H., Tamada, Y., Kamei, Y., Matsuda, A., Hattori, M. (2020) Imaging performance of microscopy adaptive-optics system using scene-based wavefront sensing. J. Biomed. Optics, *25*, 123707.
- Kanazawa, N., Goto, M., Harada, Y., Takimoto, C., Sasaki, Y., Uchikawa, T., Kamei, Y., Matsuo, M. and Fukamachi, S. (2020) Changes in a cone opsin repertoire affect color-dependent social behavior in Medaka but not behavioral photosensitivity. Front. Genetics *11*, Article 801.
- Kawamoto, N., Carpio, D. P., Hofmann, A., Mizuta, Y., Kurihara, D., Higashiyama, T., Uchida, N., Torii, K. U., Colombo, L., Groth, G. and Simon, R. (2020) A peptide pair coordinates regular ovule initiation patterns with seed number and fruit size. Current Biology *30*, 4352–4361.
- Kondow, A., Ohuma, K., Kamei, Y., Taniguchi, A., Bise, R., Sato, Y., Yamaguchi, H., Nonaka, S., Hashimoto, K. (2020) Light-sheet microscopy-based 3D single-cell tracking reveals a correlation between cell cycle and the start of endoderm cell internalization in early zebrafish development. Dev. Growth Differ. *62*, 495-502.
- Sakai, Y., Kato, K., Koyama, H., Kuba, A., Takahashi, H., Fujimori, T., Hatta, M., Negri, A. P., Baird, A. H. and Ueno, N. (2020) A step-down photophobic response in coral larvae: implications for the light-dependent distribution of the common reef coral, Acropora tenuis. Sci Rep. *10*, 17680.
- Sasaki, T., Tome, S. and Takei, T. (2020) Intraventricular IL-17A administration activates microglia and alters their localization in the mouse embryo cerebral cortex. Mol. Brain. *13*, 93.
- Tomoi, T., Kawade, K., Kitagawa, M., Sakata, Y., Tsukaya, H. and Fujita, T. (2020) Quantitative imaging reveals distinct contributions of SnRK2 and ABI3 in plasmodesmatal permeability in Physcomitrella patens. Plant Cell Physiol. *61*, 942-956.
- Yokoi, S., Naruse, K., Kamei, Y., Ansai, S., Kinoshita, M., Mito, M., Iwasaki, S., Inoue, S., Okuyama, T., Nakagawa, S., Young, L. J., Takeuchi, H. (2020) Sexually dimorphic role of oxytocin in medaka mate choice. PNAS, *117*, 4802-4808.

〔Research activity by Yasuhiro Kamei〕

- Specially Appointed Associate Professor Yasuhiro Kamei is the principal investigator of Laboratory for Biothermology. For details, please refer to the laboratory page.

Data Integration and Analysis Facility

Associate Professor
UCHIYAMA, Ikuo

Technical Staff: *NISHIDE, Hiroyo*
NAKAMURA, Takanori
SUGIURA, Hiroki
Technical Assistant: *KOTANI, Keiko*

The Data Integration and Analysis Facility supports research activities based on large-scale biological data analysis, such as genomic sequence, expression data, and imaging data analysis. To achieve this, the facility maintains high-performance computers with large-capacity storage systems. It accordingly supports the development of data analysis pipelines and database construction based on these systems, and also sets up websites to distribute data worldwide as well as providing basic technical support. In addition to computational analysis, the Data Integration and Analysis Facility supports NIBB's information infrastructure, the maintenance of the institute's network systems and provides computer/network consultation for institute members.

Representative Instruments

Our main computer system is the Biological Information Analysis System (BIAS) (Figure 1), which consists of a high-performance cluster system (HPE Apollo r2800, 20 nodes/800 cores, 192 GB memory/node), a shared memory parallel computer (HPE ProLiant DL560, 72 cores, 3TB memory; HP ProLiant DL980 G7, 80 cores, 4TB memory), a high-throughput storage system (DDN SFA7700X, 1.52PB+880TB), and a large capacity storage system (DELL PowerEdge R620, 720TB). All subsystems are connected via a high-speed InfiniBand network, so that large amounts of data can be efficiently processed. Some personal computers and color printers are also available for use. On this system, we provide various biological databases and data retrieval/analysis programs, and support large-scale data analysis and database construction for institute members and collaborative researchers. We have provided support in the construction and maintenance of published databases of various model and non-model organisms in particular. These include XDB (*Xenopus laevis*), PHYSCObase (*Physcomitrella patens*), iNewt (*Pleurodales waltl*), The Plant Organelles Database, MBGD (microbial genomes), and DB-HABs (harmful algal blooms).

Figure 1. Biological Information Analysis System

The facility also provides network communication services. Most of the PCs in each laboratory, as well as all of the above-mentioned service machines, are connected by a local area network, which is linked to the high-performance backbone ORION network connecting the three research institutes in Okazaki. Many local services, including sequence analysis, file sharing, and printer services, are provided through this network. We also maintain a public World Wide Web server that hosts the NIBB home page (https://www.nibb.ac.jp/en).

Research activity by Ikuo Uchiyama

Associate professor Ikuo Uchiyama is the principal investigator of the Laboratory of Genome Informatics. For more details, please refer to the laboratory page.