The Spectrography and Bioimaging Facility assists both collaborative and core research by managing and maintaining research tools that use “Light”. The facility also provides technical support through management of technical staff assisting in the advancement of collaborative and core research projects, as well as academic support to researchers. Among its tools are confocal microscopes and the Okazaki Large Spectrograph. The Okazaki Large Spectrograph is the world’s largest wide spectrum exposure mechanism, capable of producing a range of wavelengths from 250 nm (ultraviolet) to 1,000 nm (infrared) along its 10 meter focal curve; allowing exposure to strong monochromatic light. The facility’s microscopes, which are cutting edge devices such as confocal and two-photon excitation microscopes, are used by both internal and external researchers as vital equipment for core and collaborative research projects.

Representative Instruments:

Okazaki Large Spectrograph (OLS)

The spectrograph runs on a 30 kW Xenon arc lamp and projects a wavelength spectrum 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 much 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.

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 those in NIBB.

Action spectroscopical studies for various regulatory and damaging effects of light on living organisms, biological molecules, and artificial organic molecules have been conducted.

Microscopes

This facility also has Bioimaging machines such as widefield microscopes (Olympus IX-81, BX-63 and KEYENCE BZ-8000), confocal microscopes (Olympus FV1000, Leica TCS SP2 and Nikon A1R, Carl Zeiss Duos 5) and other custom-made laser microscopes (Digital Scanned Light-sheet Microscope: DSLM and Infrared Laser-Evoked Gene Operator microscope: IR-LEGO) for users in NIBB and collaborative guest researchers. We began Collaborative Research Programs using these machines since 2010.

The DSLM was developed by Dr. Ernst Stelzer’s group at the European Molecular Biology Laboratory (EMBL). This microscope can realize high-speed z-axis scanning in deeper tissue by illuminating from the side of a specimen with a light sheet (more information is described in Dr. Nonaka’s section: Lab. for Spatiotemporal Regulators). Dr. Nonaka conducted and supported 7 projects of the Collaborative Research Program for the Use of the DSLM.

The IR-LEGO was developed by Drs. Shunsuke Yuba and Yasuhiro Kamei at the National Institute of Advanced Industrial Science and Technology (AIST). This microscope can induce a target gene of interest by heating a single target cell in vivo with a high efficiency irradiating infrared laser (Kamei et al. Nat. Methods, 2009). Details are described in the next section. The IR-LEGO was also used for 8 Individual Collaborative Research projects, including applications for higher plants and small fish.

Publication List on Cooperation

Original papers

To investigate a gene function in each cell we have to express the gene in the cell \textit{in vivo}, ideally the expression must be limited only to the single cell. Tissue or cell specific promoters were used to reveal gene functions, however promoter-driven gene expression was governed by cell fate or environment, therefore we could not control the timing of gene expression. To achieve timing-controlled gene expression we employed one of the stress responses, the heat shock response. The heat shock promoter is the transcription regulation region of heat shock proteins and all organisms have this mechanism. Positioning the target gene downstream of the promoter, we can induce the target gene expression by heating.

In infrared (IR) beams can heat water molecules, which are the main constituent of cells, hence, we can heat a single cell by irradiating IR to a target cell using a microscope. We have developed a microscope, IR laser evoked gene operator (IR-LEGO), specialized for this purpose (Figure 1). The IR-LEGO microscope can irradiate an IR laser to a single cell \textit{in vivo} such as \textit{C. elegans}, medaka and \textit{Arabidopsis}, to induce the heat shock response at a desired timing.

Optimal heating induces the heat shock response and subsequent gene expression, while an excess results in cell death. Hence, we must precisely control laser heating; however, there was no way to measure temperature in a microenvironment under microscopic observation. To achieve this we employed green fluorescent protein (GFP) as a thermometer. Since fluorescent matter has the common property of temperature dependent decrease of emission intensity, we can estimate temperature shift by emission intensity change. GFP expressing \textit{E. coli} was used to measure temperature as a micro thermometer. Using this probe, we evaluated heating properties of IR-LEGO such as time course of temperature rise and 3-dimensional distribution of temperature during IR irradiation. In a model tissue which contained GFP expressing bacteria in polyacrylamide gel, temperature rose rapidly with IR irradiation and kept a constant level dependant on IR laser power (Figure 2 left). On the other hand, the heated area was limited to a small volume about as large as a typical cell (Figure 2 right).

With this in mind, we tried to induce gene expression in various species. At first, we reported an IR-LEGO experiment in living \textit{C. elegans}. Target gene expression in a target cell could be induced with only 1 s-IR irradiation. Whereas the optimal power range which can induce gene induction without cell damage was limited. Excess laser power resulted in cell death or cessation of cell division. We confirmed that an optimal irradiation, e.g. 11 mW for 1 s, induced physiological gene expression in the target cell and subsequent cell division or morphogenesis underwent normal development. Next, we tried the experiment in fishes, medaka and zebrafish, and the higher plant, \textit{Arabidopsis}, since all organisms have a heat shock response system. We succeeded in local gene induction in the species as expected.

Studies of cell fates, cell-cell interaction, or analysis of non-cell autonomous phenomena require a fine control system of gene expression in experiments. IR-LEGO will be a powerful tool for these studies in combination with molecular biological techniques, such as the cre-loxP system (Figure 3). By Applying IR-LEGO to a mutant and its rescue transgenic strain; using hsp-cre with a rescue gene which is sandwiched...
by loxP sequences, we will achieve single-cell knockout experiments in living organisms, and reveal fine interaction between the cells. We are now testing this system using medaka. We have already constructed a medaka TILLING library and a screening system for reverse genetic mutant screening, furthermore we have started establishment of a cre-loxP system in medaka.

Publication List

[Original paper]


[Original paper (E-publication ahead of print)]


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The Data Integration and Analysis Facility supports research activities based on large-scale biological data analysis, such as genomic sequence analysis, expression data analysis, and imaging data analysis. For this purpose, the facility maintains high-performance computers with large-capacity storage systems. On the basis of this system, the facility supports development of data analysis pipelines, database construction and setting up websites to distribute the data worldwide. In addition to computational analysis, the Data Integration and Analysis Facility supports NIBB’s information infrastructure, the maintenance of the network system in the institute and 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 shared memory parallel computer (DELL PowerEdge R905; 4 nodes/16 cores, 256GB memory), a high-performance cluster system (DELL PowerEdge M1000e+M610; 32 nodes/256 cores, 768GB memory) and a large-capacity storage system (DELL Equallogic; 35TB SAS, 26TB SATA, 750GB SSD). All subsystems are connected via a high-speed InfiniBand network so that large amounts of data can be processed efficiently. Some personal computers and color/monochrome printers are also available. 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. Especially, we have supported the construction and maintenance of published databases of various model organisms including XDB (*Xenopus laevis*), PHYSCObase (*Physcomitrella patens*), DaphniaBASE (*Daphnia magna*), The Plant Organelles Database, and MBGD (microbial genomes).

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 network ORION connecting the three research institutes in Okazaki. Many local services, including sequence analysis services, file sharing services, and printer services, are provided through this network. We also maintain a public World Wide Web server that hosts the NIBB home page (http://www.nibb.ac.jp/).

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**Research activity by I. Uchiyama**

Assistant professor I. Uchiyama is the principal investigator of the Laboratory of Genome Informatics, which currently focuses on microbial comparative genomics studies. For details, please refer to the laboratory page (p. 66).