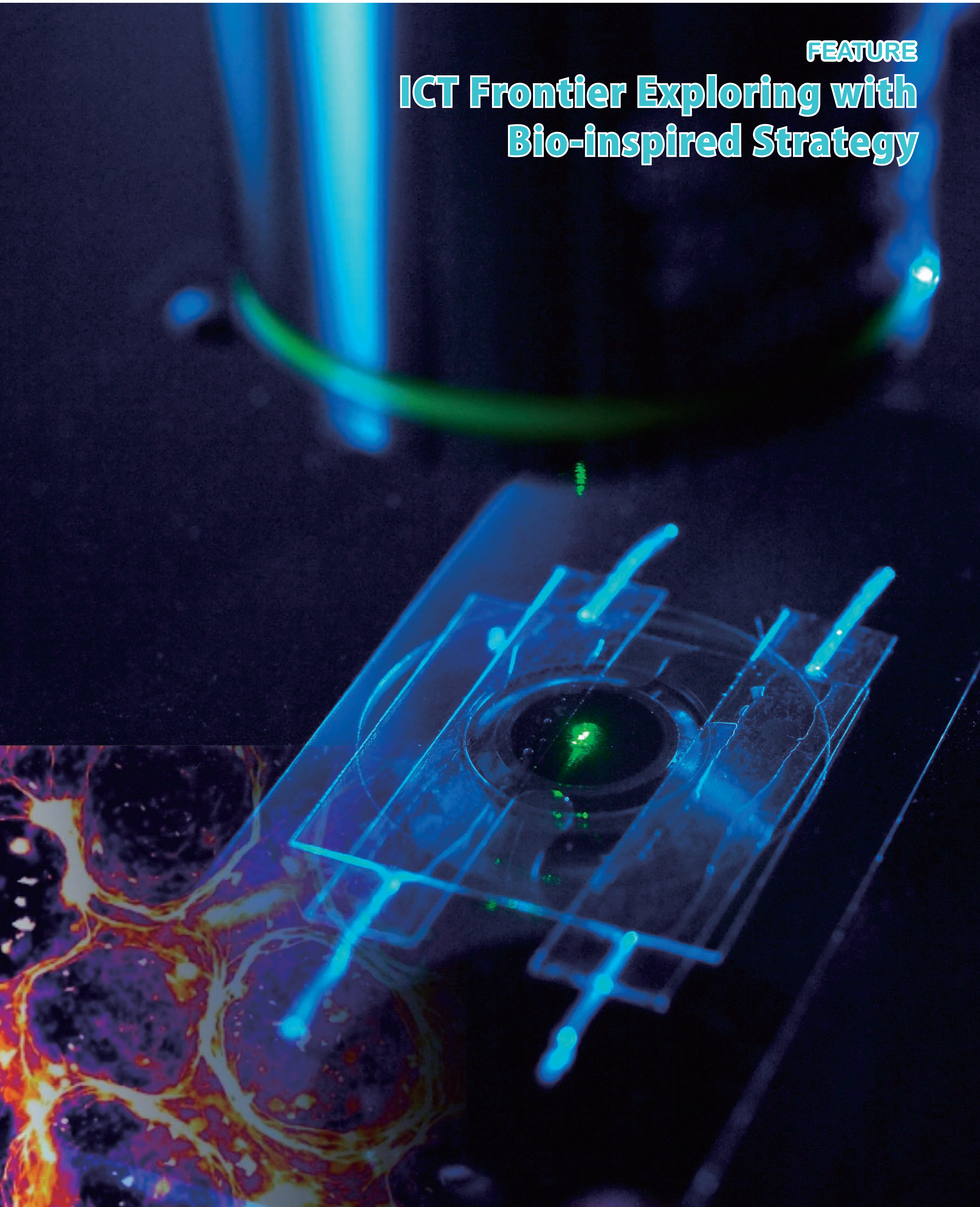
National Institute of Information and Communications Technology

NICT NEWS

2018 No.4 Vol. 470



NICT NEWS 2018 No.4 Vol.470
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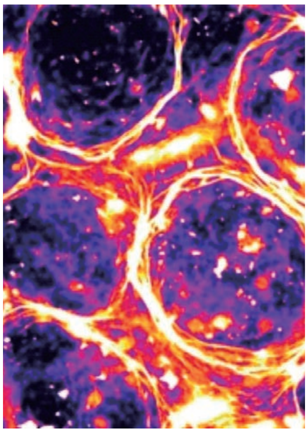

Cover photo: "Observation of microtubule motion utilizing a total reflection fluorescence excitation system"
This photo is a total reflection fluorescence excitation system superimposed with an observed image of microtubule motion. Microtubules execute a sliding motion on a glass substrate surface that has adsorbed a protein motor. If we use the exuding of light that occurs in total reflection (evanescent light) to excite a fluorescent substance marking a microtubule, we can see the movements of each individual microtubule.

Upper left photo: "Observed image of a collective motion of microtubule motion"
Furthermore, we learned that when microtubules engage in collective motion with a high density, a millimeter-sized vortex is created.

INTERVIEW

What is the "BioICT" that will open up the future of information and communications?

Kazuhiro OIWA
Distinguished Researcher
Advanced ICT Research Institute

After gaining a Ph.D, Kazuhiro Oiwa worked as an associate professor at the Teikyo University. In 1993, he joined the National Institute of Information and Communications Technology (NICT, former Communications Research Laboratory) in Kobe. Since then, as a staff scientist and as a group leader, he applied his knowledge and techniques to understanding the properties of protein motors as nanometer-scale machines. After serving as the Director General of the Advanced ICT Research Institute, he is the Distinguished Researcher and the Fellow of NICT. He was awarded the 23rd Osaka Science Prize in 2005.

The progress of technology has ups and downs. There are times when it grows rapidly and there are times when it stagnates. We can perhaps conclude that currently information and communications technology is stagnating somewhat. Moore's law stating that the components per chip will double every 18 months is also breaking down. There are no longer sufficient resources for processing the volume of information which continues to increase dramatically.

What has the potential to drive the wedge of a major breakthrough here are the mechanisms of living things. I asked Kazuhiro OIWA, Distinguished Researcher, Advanced ICT Research Institute and NICT Fellow, about the current state of the research and future prospects.

— I often hear the initialism "ICT" which stands for Information and Communications Technology, but what kinds of things does "BioICT" indicate?

OIWA: BioICT is the approach of knowing living things, learning from living things, using the characteristics of living things, and applying them directly or indirectly to ICT (information and communications technology).

Living things have repeated a process of trial-and-error during a history of nearly four billion years of evolution and adapted to their environment and have survived. During the process, living things have gained outstanding characteristics.

Areas in which these kinds of characteristics of living things can be applied technologically are called biomimetics. These are ideas that are often used as breakthroughs when there are technological dead-ends in a variety of areas, and this means that "we should learn from living things when we are in trouble."

For example, the nose cone which is the tip of the Nozomi 500-series bullet train was made with reference to the shape of the beak of the common kingfisher. This is because it optimized air resistance when entering a tunnel. Furthermore, resistance in water is being reduced by making the surface of swimsuits for competitive swimmers like the skin of sharks.

Long ago, the details of the structure of the skin of sharks were not well understood. However, as biology progressed, details of the structure of the skin of sharks (small protuberances lined up regularly) became well understood. However, this alone would not lead to a breakthrough. This is because the technology to make the tiny protuberances is necessary. When technology progresses, development advances suddenly. It became possible to mimic the structure of the skin of sharks technologically.

— What is your image of biomimetics in information and communications technology?

OIWA: For example, a porifera or sponge called *Euplectella* (Venus's flower basket) with a skeleton that uses fibrous glass for a mesh lives in the deep sea. The structure of this glass fiber is the same structure as the optical fiber made by humans.

How were living things able to make this kind of outstanding optical fiber? When we make quartz optical fiber, we add heat to melt the quartz and then draw out the optical fiber from it. However, these living things are not doing anything like this. They are synthesizing optical fiber under the ordinary temperatures and pressures in which they live.

If we utilize this finding, we can achieve things that were thought to be difficult until now. For example, suppose we try to add metal ions such as sodium, etc. in order to improve the properties of the optical fiber. It is difficult to add metal ions under high temperature conditions, but if we use the mechanism possessed by this porifera or sponge, that becomes possible. Furthermore, it is reported that it contains organic materials such as proteins, etc. so it is possible to make optical fiber that is pliable and difficult to break.

Learning from "Intellect in nature"

— What BioICT research are you attempting to do?

OIWA: NICT is a national research institution for ICT, so of course we should engage in

INTERVIEW

What is the "BioICT" that will open up the future of information and communications?

research in cutting-edge areas of information and communications, but I believe we must also conduct high-risk research that companies would hesitate to do. One kind of research like this is bio research for ICT. As I stated above, there are many examples of researchers gaining hints from living things. However, the question of how to apply this to information and communications technology is the issue going forward.

Firstly, it is important to come into contact with the functions of organisms and realize that "this could be used as a new idea." I intend to combine these kinds of organism-derived technological concepts under the term "Intellect in nature."

Now technology of artificial intelligence is developing rapidly. On the other hand, a large number of phenomena can be found in the natural world which also seem to behave with a high level of intelligence. What is behind the intelligence possessed by nature? We can conclude that it is the information possessed by nature and the information processing of "Intellect in nature." In the same way as an algorithm (computational procedure) in the information processing of computers, some form of algorithm is also functioning inside "Intellect in nature."

— What is "Intellect in nature" specifically?

OIWA: For example, there is the collective motion of schools of fish and flocks of birds. They move around as if the overall group possesses one intelligence. A group of white-cheeked starlings is not run by centralized control but somehow they fly in a beautiful formation. The birds are not looking at the overall situation. They are only observing what the bird next to them is doing. A model stating that kind of collective behavior emerges when the birds maintain position, distance, and velocity with the bird next to them has been proposed. Similar collective movements to this are found in simple and small biomolecules such as protein filaments, etc. It is conceivable that the findings obtained here could be applied to control technology in self-driving cars, to ensure that they do not crash into other cars or objects.

— The information science of living things is simple, isn't it?

OIWA: Living things use their own unique information devices such as information systems and sensors, etc. in order to survive. These correspond to the IC chips and sensors used in general computer systems.

The digital devices we use were developed based on the idea of using a lot of energy to reduce noise. In contrast to that, the information systems of living things are in the middle of noise. Despite that they work properly, it seems that living things do not use much energy to reduce noise. If we learn from this technique, perhaps we will be able to efficiently draw out significant information from information containing a lot of noise, and the huge amount of electricity for reducing noise will probably become unnecessary.

There is still another way in which the systems of living things are superior. I mean their sensitivity with respect to a particular substance, or in other words their specificity, is extremely high. Furthermore, we can conclude that even if each individual device is small and moves in a different way, they have the robustness (stability with respect to a disturbance) of functioning stably because they

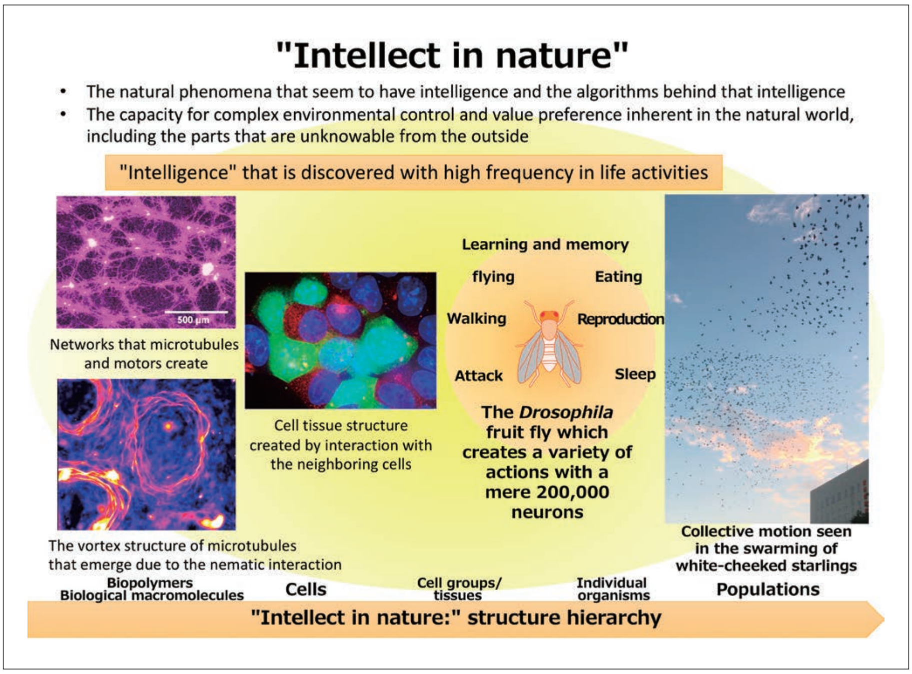

Figure 1 "Intellect in nature" — the "intelligence" that is discovered in all structure hierarchies of organisms

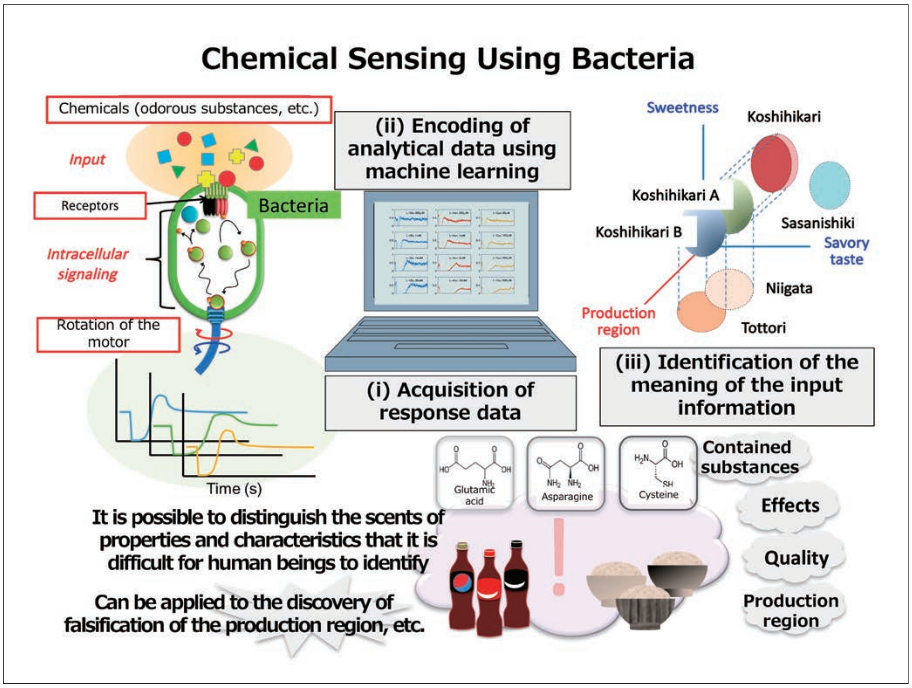

Figure 2 Overview of bacteria chemical sensing that combines chemotaxis mechanism of bacteria with machine learning based on Bayesian inference

function as a group.

— In what kinds of areas can the information systems of living things be applied?

OIWA: One is application as chemical sensors. We humans have used the characteristics of living things for the sensing of chemicals for some time. For example, it is reported that in the old days coal miners took canaries with them when they entered the mine shaft. A canary detects poisonous gas and lack of oxygen and reliably reacts sooner than a human being, so if the canary stopped chirping the miners knew they were in a dangerous situation.

Chemical sensors using *Escherichia coli* were developed by obtaining hints from this kind of sensing of chemicals by living things. We can conclude that the modern canary was made using *Escherichia coli*. This technology can be used in the quality control of food, etc., so collaboration with companies is proceeding.

The role of BioICT

— At the Advanced ICT Research Institute, how do you proceed with human resources development and collaboration with outside organizations?

OIWA: This Research Institute also offers graduate school education through partner graduate school agreements with universities, so many students come here. The advantage for the students is that this Research Institute offers a wide range of research areas. They can have an interdisciplinary perspective. There is nanotechnology, device engineering, and biology; moreover, within biology there is biology using individual organisms like the *Drosophila* fruit fly and research targeting cells and protein molecules. There are researchers specializing in information science and in neuroscience too.

We are also actively advancing technology development in partnership with companies. We exhibit in exhibitions, etc. too. I think the important thing is to learn about what kinds of needs there are in society by deepening collaboration with outside organizations. Basic research that pursues truth is also important, but I believe that it is also important for us as a national research institution to conduct research that meets the needs of society.

— How do you think you would like to develop this Research Institute?

OIWA: There is an institute that I imagine to be the model. That is the Woods Hole Marine Biological Laboratory in Massachusetts, the United States. That is the workplace of Osamu SHIMOMURA, who was awarded the Nobel Prize in Chemistry in 2008. This Laboratory is in a summer retreat area, and in the summer it runs a summer school in an environment in which researchers and students gathered together from many places can all hold open-minded discussions. Researchers in different areas can conduct information exchanges there, and as a result academic exchanges progress further, leading to new discoveries.

I believe that we should learn from Woods Hole and invite many researchers and students who are the future of our institute to create a forum enabling discussions and information exchanges.

Living things are a treasure house of knowledge. I intend to further increase knowledge regarding living things and at the same time I intend to introduce to many people about the research activities at this Research Institute to make it a place which spreads knowledge further. In addition, I intend to go beyond the world of biology and, with the help of researchers in a wide range of areas, utilize the rich world of living things in information and communications technology.

Designing a Real Artificial Intelligence through Imitating Memory of a Fruit Fly, *Drosophila*

Motojiro YOSHIHARA
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Advanced ICT Research Institute

He received a Human Frontiers Science Program fellowship to do postdoctoral work at the City of Hope, California. After working as a visiting scientist at MIT, he joined the Department of Neurobiology at the University of Massachusetts Medical School as a faculty member in 2006. In 2013, he left University of Massachusetts Medical School to work as a visiting professor at MIT. In 2014, he moved back to Japan, and joined NICT. Ph.D. (Science).

We frequently observe the misunderstanding that "artificial intelligence" using deep learning, etc., which is extremely popular currently, functions in the same way as the original "intelligence" produced by the brain, but actually the human race still does not know much about how "intelligence" functions in the brains of human beings and other animals. This is because the mechanism of the original "memory" in the brain is not yet understood, in spite of the fact that the "memory" (a katakana word without an accent at the beginning) of computers is used freely. It is this "memory" that is the foundation of intelligence, and knowing how memory is created and stored is understanding the essence of intelligence.

However, there was no method of knowing how nerve cells, an element of the brain, create memory, so understanding the mechanisms of memory was always impossible until now. There is no alternative but to create an entirely new, original method to attempt to solve a mystery that no one in the world has been able to get close to solving. In this paper, I present the methodology of memory research that we developed over many years in the United States before I joined NICT, its development at the Advanced ICT Research Institute, the concept of "real artificial intelligence" which began to bear fruit when combined with the technology of NICT, and the limitless possibilities of the methodology which started from nothing.

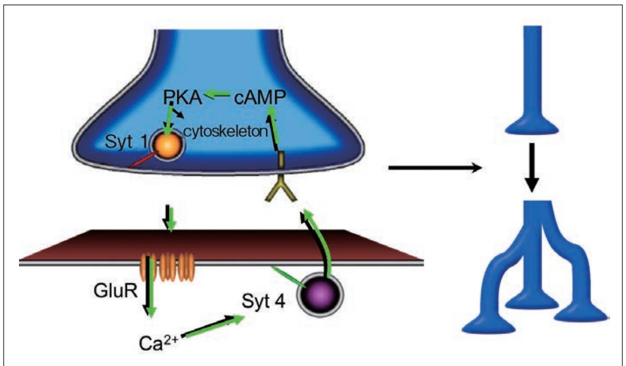

Figure 1 "Local feedback hypothesis" (*Science*, 2005)

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＊2 Flood, Iguchi, Gorczyca, White, Ito, & Yoshihara (2013) *Nature*, 499:83-87.

＊3 Yoshihara (2012) *JoVE* 62: e3625.

＊4 Motojiro Yoshihara (2013) Life Science First Authors (http://first.lifesciencedb.jp/archives/7415#more-7415)

＊5 Sakurai, Littleton, Kojima, Yoshihara, currently being prepared for publication

New hypothesis about memory

It is believed that memory is possible due to the changes in the connections between nerve cells (neurons). Therefore, firstly, I used a simple experimental system to investigate the mechanisms of that synaptic plasticity.

That experimental system consisted of the activity-dependent changes in the synapses connecting the motor neurons and muscle cells of the fruit fly, *Drosophila*, and I investigated that system in detail and proposed in *Science* a hypothesis of memory based on a completely new idea, the "local feedback hypothesis" (Figure 1).＊[1] I thought that positive feedback is generated between two cells connected in the brain being reciprocally strengthened by releasing transmitters to each other, resulting that memory is retained in a single synapse, furthermore causing morphological changes in the synapse that change it into a persistent memory. It is a "physiological condition" rather than molecules that retains short-term memory. This is a new way of thinking that did not exist before, and it explained the many experimental results reported until then well, so I expect that this was the general "micro" mechanism that forms memory. However, there was no experimental system for connecting this to macro memory for testing, so this hypothesis was no more than a desktop theory. If this was really occurring in the brain, then it would be a basic principle of brain function, so I thought of an experimental system for testing this any way I could, and deployed all the resources of the laboratories I was presiding over at the University of Massachusetts and MIT to attempt to create an experimental system for verifying this hypothesis from nothing.

Original experimental system connecting micro to macro — "Feeding" command neurons

In order to connect the micro-mechanism between cells postulated in the local feedback hypothesis to memory, it is necessary to get micro cell changes to correspond to the memory that is manifested as macro behavior. The new strategy I thought of for that purpose was to use the concept of a "command neuron" established

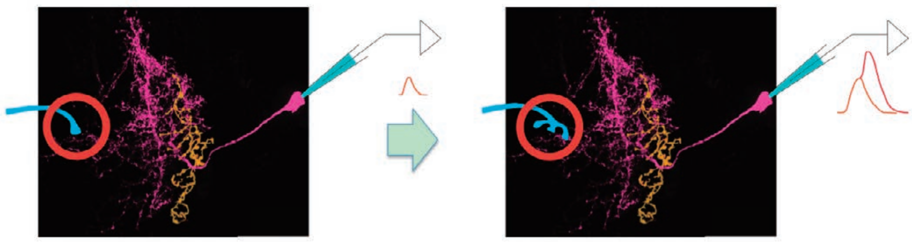

Figure 2 Witnessing memory being achieved with the feeding neurons in the brain of the fruit fly, *Drosophila* in real time. (*Nature*, 2013)

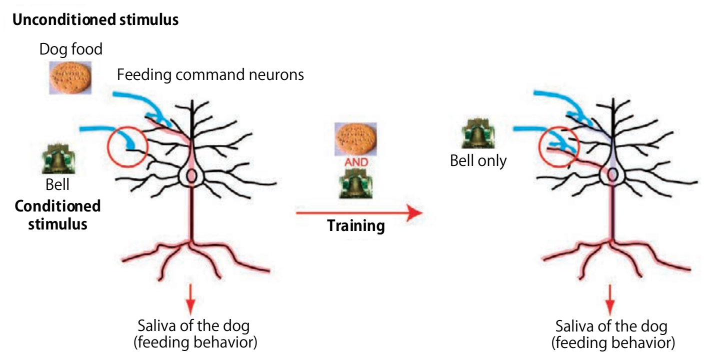

Figure 3 Pavlov's classical conditioning

by Dr. Kazuo IKEDA, the mentor of this author, in the 1960s as a neuron that commands behavior. If memory is formed by changes in micro synapses on this neuron then that should be manifested as macro behavior changes, and due to that we can make micro and macro correspond. Therefore, firstly, we started by developing a method for finding command neurons. We discovered the "Feeding neuron" commanding eating behavior which is optimal (refer to the next page) for memory research (Figure 2) and reported this to *Nature*.*2 Furthermore, I independently developed an experimental system which we could observe the micro activity of cells in the brain while at the same time observing macro eating behavior.*3 Due to these technical developments, we completed preparations for getting micro cell phenomena to correspond to macro behavior.

Pavlov's fly

I thought of conducting an experiment similar to the classical conditioning of Pavlov, which is well known as a model of memory, in order to create memory in the Feeding neurons. It is predicted that the inside of the brain of a dog would change through this "conditioning" as the sound of a bell activates the neural circuits equivalent to the Feeding neurons. Therefore, if we conduct a similar experiment to the "Pavlov's dog" experiment using a fly, I expected that we could track the "single cell level" memory created in the Feeding neurons*4 (Figure 3). Together with Dr. Akira SAKURAI, who had come to study at the laboratory of the author in the United States as a post-doc student (currently Senior Researcher at NICT), we developed an experimental method for classical conditioning of a fly based on Pavlov's experiment. In the Memory Neurobiology Project of the Frontier Research Laboratory in the NICT Advanced ICT Research Institute, it was confirmed that due to conditioning, the reactivity of the Feeding neurons changes; in other words, new connections are formed in the neural circuits.*5

Currently, we are using a two-photon microscope that can see deep brain parts to attempt to detect the changes in the connections in the Feeding neurons when a fly remembers through classical conditioning. Using the methodology that we built up uniquely, we can observe a scene that the human race has never seen before, "the moment in which memory is achieved," in real time. We have traveled a roundabout route, but I am excited to say that, as indicated by the saying "seeing is believing," after this series of technical developments, it has finally become possible to verify our original hypothesis (Figure 1), and we are able to reach the principles of memory at last.

Future prospects — Designing real artificial intelligence by implementing, on a device, the changes in a single cell when memory is formed

This research goes beyond basic research on the principles of memory to an analysis of the single cell level when memory is formed, which enables us to understand for the first time in history the mechanism by which each and every element of the brain stores memories, or in other words the functioning of "memory" in the brain.

These obtained findings, by themselves, make it possible to directly develop devices that imitate the functions of nerve cells, so in this project we are already planning to carry this out jointly with the group of Advanced ICT Research Institute Director General Iwao HOSAKO, which is developing high-performance semiconductor devices. By connecting many of these devices together to make a network (Figure 4), we can create "real artificial intelligence" for the first time by truly imitating the functions of the brain.

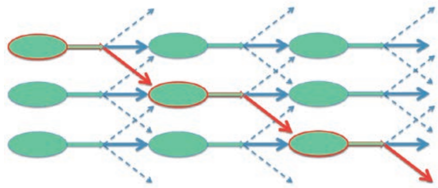

Figure 4 Many devices imitating the input-output change of the feeding neurons are connected in order to artificially recreate the neural circuits that retain memory. Blue arrows: input-output of each device (ellipse); red: the circuit that retains the memory.

There is a possibility that this will lead to the development of computers with much greater energy efficiency like the brain. In addition, we can expect that creating silicon circuits modeled on neural circuits will conversely become a new methodology that gives insights into the information processing of the brain and also to outstanding problems of the brain such as consciousness, etc. Going forward, the dream will expand even further to new information and communications technology / new biology of the brain.

Development of a Chemical Substance Identification Device Using Microorganisms

Understanding the "meaning" of chemical substances to living creatures while constructing and using bio-devices

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After completing a doctoral course in the graduate school, became a Research Scientist of JST and then joined NICT in 2009 as Senior Researcher. Has been engaging in research and development of chemical substance identification technologies. Ph.D. (Science).

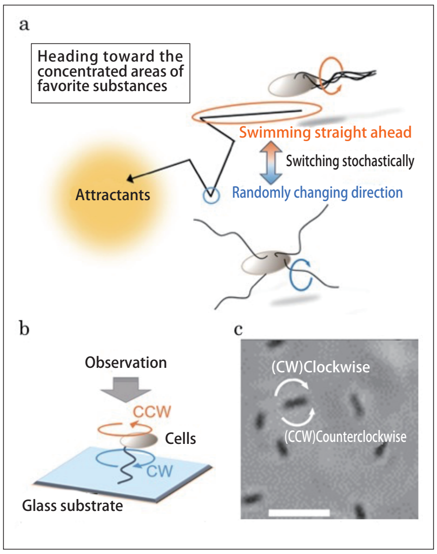

Figure 1 The chemotaxis response of *Escherichia coli* and an example of the observed images
a. Chemotaxis response b. Tethered assay
c. Image observed under the microscope (phase difference image, scale bar :5μm)

Chemical substances possess a variety of effects including taste, aroma, pharmaceutical benefits, toxicity, etc. These kinds of effects have "meaning" due to the "existence of living creatures." One type of microorganism, the bacteria *Escherichia coli (E. coli)*, detects chemical substances in order to live, and cells of *E. coli* change their behavior deperding on the properties of the chemical substance. We use the behavioral changes of cells of *E. coli* to "visualize" the chemical substance properties, and constructed a foundational technology for "identifying chemical substances" from the properties visualized via behavioral change by using statistical techniques. In this article, we present the background to the technology development, the details of our efforts, and the prospects going forward.

Background to the development research

Due to technological progress, there are now a variety of chemical substances in the environment surrounding us. The chemical substances that exist in this kind of environment affect living creatures in a variety of ways.

The effects of chemical substances on living creatures are wide-ranging, so chemical substance evaluation and identification (categorization) of their bioactivity have become more and more important in recent years.

We thought, "if living creatures are affected by chemical substances, could we use the functions of those living creatures for chemical substance sensing?" and studied the possibilities for being able to use microorganisms for the sensing of chemical substances. The bacteria *Escherichia coli*, one type of microorganism, detects the chemical substances in its environment and swims in the direction with a high concentration of substances it likes (attractants) (chemotaxis response, Figure 1a). *Escherichia coli* are unicellular organisms with a size of several microns, and we can say they are a chemical substance detection device that engages in "packaging from the chemical substance signal input to the response output." We worked on the development of a chemical substance identification device by applying this chemotaxis response of cells of *E. coli*.

"Quantitative measurement of the response of microorganisms" that visualizes the properties of chemical substances using microorganisms

Using living creatures as chemical substance detectors consists of the detection of chemical substances which have interactions with living creatures (bioactivity). *Escherichia coli* changes the way it swims depending on the types and concentration of diverse chemical substances, so many chemical substances can be detected. In order to correctly determine what the chemical substances detected from this chemotaxis response are, it is necessary to accurately quantify subtle differences in the response. With the chemotaxis response, "swimming straight ahead" and "changing direction" switch stochastically. This swimming switch reflects the rotation direction switch of the flagellar motor (a molecular motor at the base of the roots of flagellum). What we focused on here was the changes in the chemotaxis response over time (the adaptation process). Here, we used a tethered assay (Figure 1b, c, one of conventional techniques for research into the *Escherichia coli* chemotaxis response) to acquire data by photographing the rotational motion of the cell body (reflecting the rotation of the flagellar motor) for ten minutes at a frame rate of 100 Hz. For this data acquisition, we improved the micro-channels in order to reduce effects of the speed of flow when a solution containing a chemical substance was exchanged (Figure 2a, b), and developed an image analysis program to quantify the motion of the several hundred cell bodies that existed on the photographic screen and computed the ensemble average in order to reduce fluctuation of the individuality of the cells (Figure 2c). Due to the development of this measurement system, we were able to acquire large amounts of data related to the chemical substance input and response output and it became possible to use the behavior of the bacteria *Escherichia coli* as a detector.

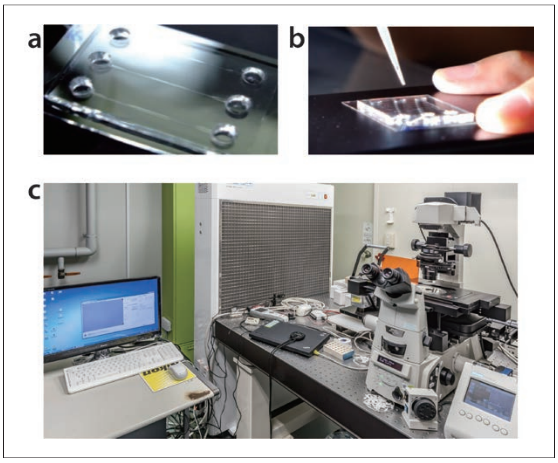

Figure 2 Quantitative measurement of the response of microorganisms
a. Measurement chamber b. Infusion of the test solution
c. Measurement system (mainly comprised of a microscope, high-speed camera, and computer)

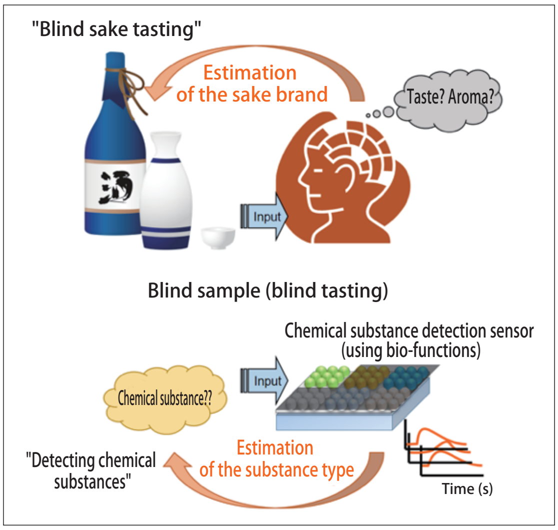

Figure 3 "Detecting chemical substances" using the bacteria *Escherichia coli*

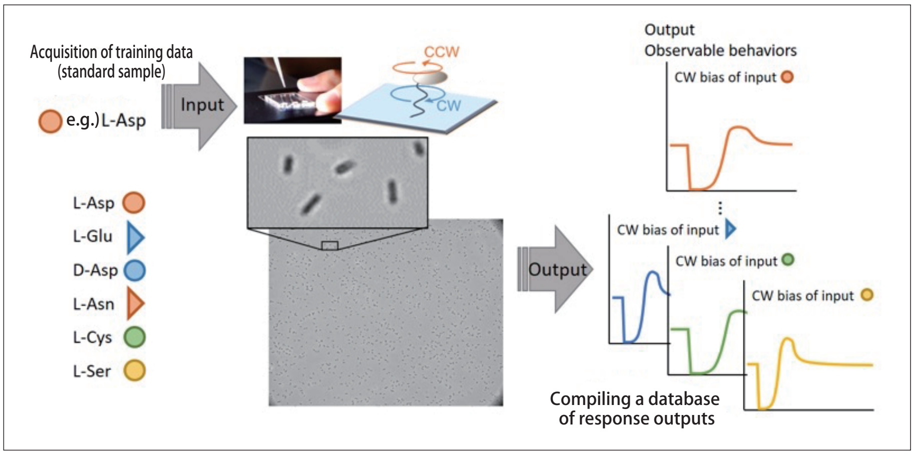

Figure 4 Compiling a database of standard samples using *Escherichia coli*

Extraction of chemical substance information using statistical processing "From detection to identification of chemical substances"

If the swimming of cells of *E. coli* changes, we know that some kind of chemical substance is present there (detection), but identifying that chemical substance is difficult. In order to overcome this difficulty, we used statistical techniques. Conceptually, like "blind sake testing" carried out by humans, by statistically speculating (identifying) the type of chemical substance with "detecting chemical substances" by *Escherichia coli* and statistical techniques (Figure 3), we can say that we have truly developed a "method (device) for identifying chemical substances using microorganisms" based on a measurement system and statistical analysis techniques. For basic research, we tested amino acids as a standard sample (training data). We applied a solution containing one type of chemical substance from the standard sample to cells of *E. coli* inside micro-channels, and recorded the changes in the chemotaxis response over time. In order to test the possibilities for chemical substance identification including the response fluctuation for each measurement, we decided to dispose the cells and the micro-channels after each single measurement (response measurement for ten minutes). We carried out this measurement repeatedly while changing the type and concentration of the amino acids, acquired a large amount of data, and evaluated identification performance (Figure 4). Due to the properties of *Escherichia coli*, in the case of the identification of amino acids, the identification rate changed depending on the combination, but with the combination of L-glutamic acid and L-asparagine, the identification rate of the blind sample exceeded 90%. The results of this test showed that our techniques functioned well and a solution containing a chemical substance can be identified by combining living creatures and statistical techniques.

Future prospects

As we stated at the beginning, it is conceivable that the technologies presented in this article could be used as sensors for monitoring chemical substances present in the environment, etc. Furthermore, there is the possibility that they could be used as evaluation devices for drinks and food by applying their "identification device" aspects. Regarding identification of chemical substances from the output response of the bacteria *Escherichia coli* (above, "detecting chemical substances"), we skipped the detailed description here but the important thing in preparation of the "standard sample" used for acquisition of the training data (database creation) is that "the standard sample is labelled (amino acid name, drink name, production region, origin, etc.)." It is easy to understand if one thinks of "blind sake testing," but there are no major restrictions on the object to be identified. Sake, wine, beer, soft drinks, etc., are items for which living creatures (human beings and *Escherichia coli*) can detect differences (the items have bioactivity), and if the brand is clear then they can be objects of identification. For example, water that has been used to wash rice, vegetable juice, etc., can also be selected as objects of identification. In the future, if these kinds of ingredient inferences and production region inferences, etc., of samples are achieved, applications to the safety of food and the quantification (visualization) of branding, etc., are expected.

In this article, we presented the fact that using living creatures as detection devices enables identification of chemical substances with bioactivity as the filter. It can be concluded that we are utilizing information that "has meaning for living creatures" such as the taste, aroma, toxicity, pharmaceutical benefits, etc., possessed by chemical substances. This can be concluded to be a basic technology for quantifying and transmitting "meaning and value" of chemical substances produced by interaction with living creatures, rather than composition or structure of chemical substances. In the future, it may be evolved to technology for communicating the "aroma" or storing the "taste" associated with memories. Our approach can be said to be an instance of utilizing biological features in ICT. I hope we can make a contribution from the starting point of developing the biomimetics field of information processing, which involves learning techniques of chemical substance information processing from living creatures.

Analysis of the Recognition Mechanism of Exogenous Materials in Living Cells

Toward an understanding, mimicking, and regulating the cellular ICT

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After completing a doctoral course in the graduate school, joined NICT in 2005 and has been engaged in researches on understanding cellular responses against invading artificial materials. Currently, a research manager (since 2018). Ph.D. (Engineering).

Our research project aims to create a new ICT paradigm using the information processing and communication capabilities of the cell, the smallest functional unit of life, by carrying out research and development into technology for measuring and controlling cell functions. This article focuses on what has been clarified for the first time regarding how cells obtain information on the outside world, and how they respond flexibly to it, by an original measuring technique using artificial beads.

Background

Living cells exchange information with the outside world through molecular communication. This concept of molecular communication applies not only to communication within individual cells, but for example, it also applies to, e.g., communication between cells that make up our immune system, as well as a variety of other phenomena (Figure 1). Molecular communication is biocompatible, and it is useful because it can even function in aqueous environments. Therefore, if it could be mimicked artificially and controlled, it could be used to complement existing telecommunications technology, e.g., optical telecommunication, or even surpass it depending on the environment in which it is used. It has the potential to become a new form of telecommunications technology.

It has only been 10 years since the concept of molecular communication was put forth, making it a new research field, and a lot of the research carried out so far has been theoretical. However, due to advances in bioimaging techniques, measuring techniques using microfluidic devices, etc., in recent years, the groundwork has been laid down for carrying out research involving demonstrations through biological experiments. This has resulted in increased attention to molecular communication research.

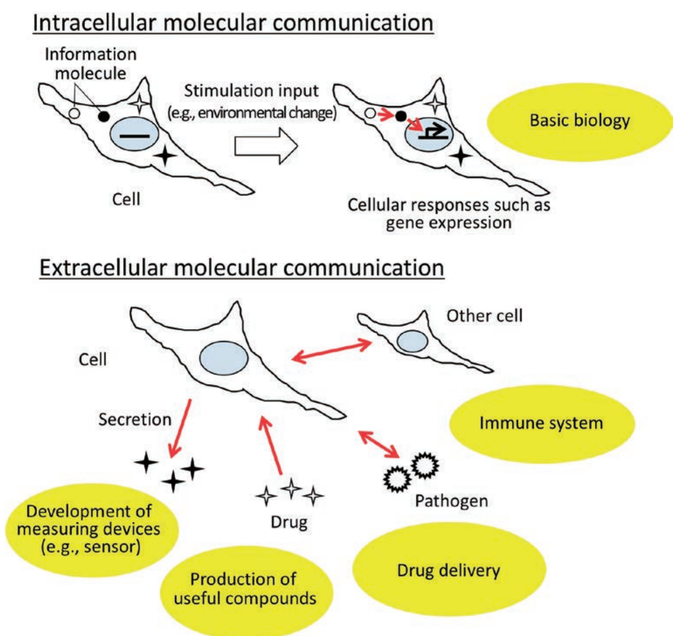

Figure 1　Molecular communication carried out by living cells, and extensive related fields

Introducing artificial beads into living cells and inducing autophagy

As a first step toward gaining a better understanding of how living cells carry out molecular communication, and controlling it, we focused on how cells obtain information on the outside world. Research on this issue had focused mainly on the signaling pathway via receptors, but due to, e.g., the small size of observation targets, little was known about the phenomena taking place immediately after drugs and other foreign substances entered cells.

This led us to develop a technique for measuring the cellular response (Figure 2a) that takes place in the vicinity of non-biodegradable, biomolecule-coated beads introduced into living cultured cells (HeLa cells). By using the non-biodegradable beads as markers in microscopy, this method enables detailed observation of the cellular response to the biomolecules attached to the surface of the beads. Moreover, the observations were carried out using fluorescence live cell imaging technology (Figure 2b) cultivated over many years by our group, and live CLEM imaging to examine the same area observed under a fluorescent microscope using an electron microscope.

So far, this method has been successfully used to induce autophagy (autophagy: a type of protein degradation mechanism in cells. Targeted substances are engulfed by a membrane structure, then broken down) in a limited area

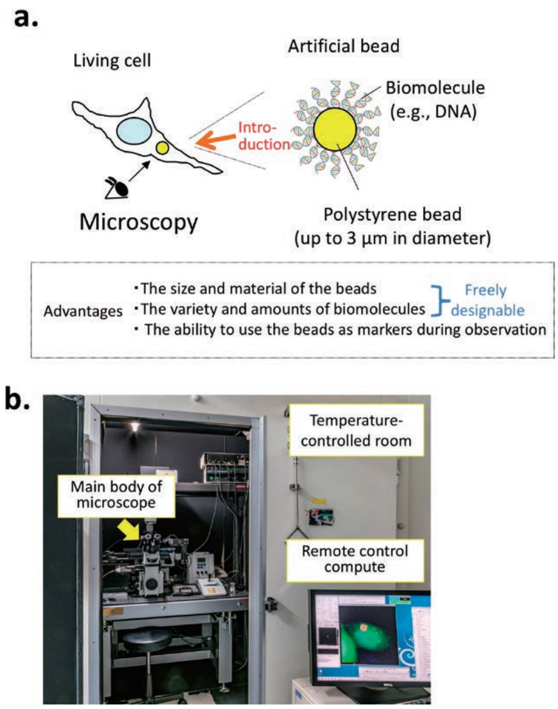

Figure 2 Technique for measuring the cellular response upon introducing an artificial bead into a living cell
a. Overview of experiment to introduce a bead into a living cell
b. A high-resolution fluorescence imaging system used for observing living cells. The microscope is set up inside a temperature-controlled room to maintain stable observation conditions.

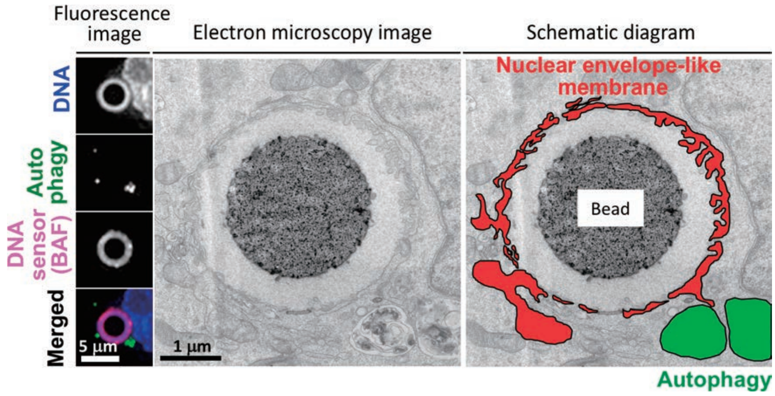

Figure 3 Image of DNA-coated bead 1 hour after introduction into a cell. The same bead seen in the fluorescence image was observed under an electron microscope. It can be seen that a membrane structure similar to a nuclear membrane has been formed around the bead (shown in red in the schematic diagram) to avoid autophagy (green).

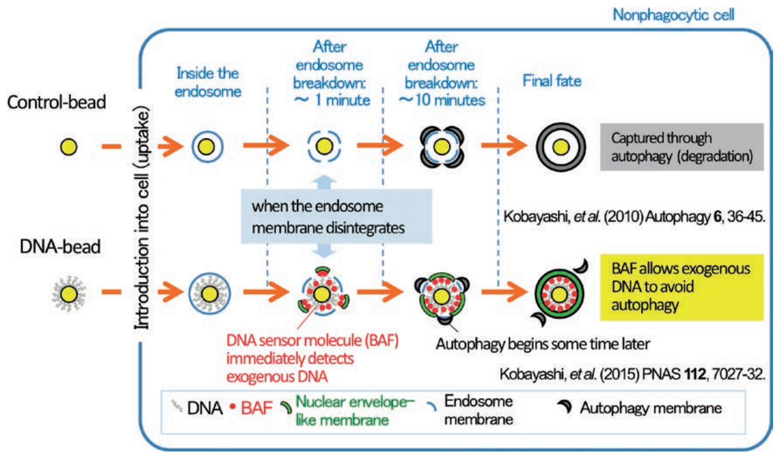

Figure 4 Mechanism by which exogenous DNA avoids degradation through autophagy

around the bead introduced into the cell, with all the details successfully monitored. This means that cells make use of the autophagy mechanism to deal with foreign substances that enter them.

The discovery of a mechanism for avoiding the autophagy of exogenous DNA

So, are all foreign substances captured and broken down through autophagy? Recent studies have revealed that at least DNA is treated differently.

The introduction of DNA into living cells is a technique that is indispensable to fluorescence live cell imaging, gene therapy, and a variety of other fields. Various kinds of techniques have so far been developed to introduce DNA into cells, but little was known about when and how the entry of DNA was actually being detected by cells. This prompted us to introduce beads coated with double-stranded DNA into cells, and analyze the cellular response. As a result, it was revealed that the DNA-binding protein, BAF (barrier-to-autointegration factor), immediately detects the exogenous DNA, and forms a membrane structure similar to a nuclear membrane around it within approximately 10 minutes. On the other hand, the membrane typical in autophagy was unable to engulf the bead and was finally observed only in areas far away from the bead (Figure 3).

In light of what we know so far, it seems that in autophagy, it is the invasion of exogenous material (the breakdown of the endosome* membrane) that is being detected rather than the foreign substance itself (upper part of Figure 4). This is a very good strategy to adopt, because it allows the handling of all foreign matter regardless of type. On the other hand, it is believed that the DNA sensor molecule, BAF, directly detects the introduced exogenous DNA, and forms a membrane structure around the bead before autophagy begins in order to protect it from degradation (lower part of Figure 4). If we could gain a better understanding of this flexible foreign substance recognition mechanism of cells, we believe it will lead to the development of a technique for introducing substances of our choice into living cells, and making it function reliably.

Future prospects

The experimental method of using artificial beads has enabled us to analyze what happens inside cells when foreign substances are introduced into them with high spatial and temporal resolution, which we knew little about until now. In the future, we hope to further improve this technique of measuring and controlling the functions of cells using artificial beads, so that we may control the timing of the entry of the artificial beads into the cells, create and use beads with multiple types of biomolecules attached to them, etc. We believe this will lead to the development of technology that would enable, by making use of the capabilities of cells, extraction and utilization of information associated with chemical substances and the like.

＊Endosome
A structure seen during the process of the cellular uptake of a substance from outside the cell (endocytosis), and it is formed by the invagination of the cell membrane as it engulfs the targeted substance. The foreign substance is released inside the cell when the endosome membrane disintegrates (= the invasion of a foreign substance).

Super-resolution Microscopy to Observe "Intellect in Nature"

Clarifying the nanostructures of organisms

Atsushi MATSUDA
Senior Researcher
Frontier Research Laboratory
Advanced ICT Research Institute

After receiving Ph.D., the author studied biology at Purdue University (USA), then changed subject to microscopy after having moved to University of California, San Francisco. In 2010, he became a researcher at NICT and began studying microscopy for biology.

The sophistication achieved by organisms far outperforms the technologies created by mankind. Mosquitoes that suck human blood, for example, are merely 2 mg in weight. The reason why this tiny little organism is capable of far outperforming today's robots is because it consists of microscopic molecular complexes with a variety of functions measuring only 10 – 150 nm (a nanometer is one-millionth of a millimeter), which are capable of functioning as molecular devices. Countless molecular devices like these work as high-sensitivity sensors, motors, memory devices and so on, forming cells and bionts with complex functions. Organisms harbor unknown principles and materials with the potential to revolutionize current robots and computers. High resolution measuring technologies for live organisms are indispensable to understand and make use of these principles. We are therefore currently developing new microscopy technologies using light.

A revolution in super-resolution microscopy using light

The cells that make up the bodies of organisms are so small that they are almost invisible, but it is believed that inside these tiny cells are particles and fibers that are one-thousandth or one-ten-thousandth the size of even these cells, undergoing drastic transformations to form structures that are elaborate overall. A characteristic of structures formed by organisms is that they are not static. In other words, they are constantly changing. These structures can be observed at extremely high resolution by using electron microscopes, but their dynamic features cannot be observed. Measuring the true nature of biological structures will require a high resolution imaging technique that uses light to monitor organisms as they are.

Fluorescence microscope imaging using visible light has resulted in a variety of breakthroughs in medical and life science research. One of the founding technologies was the dis-

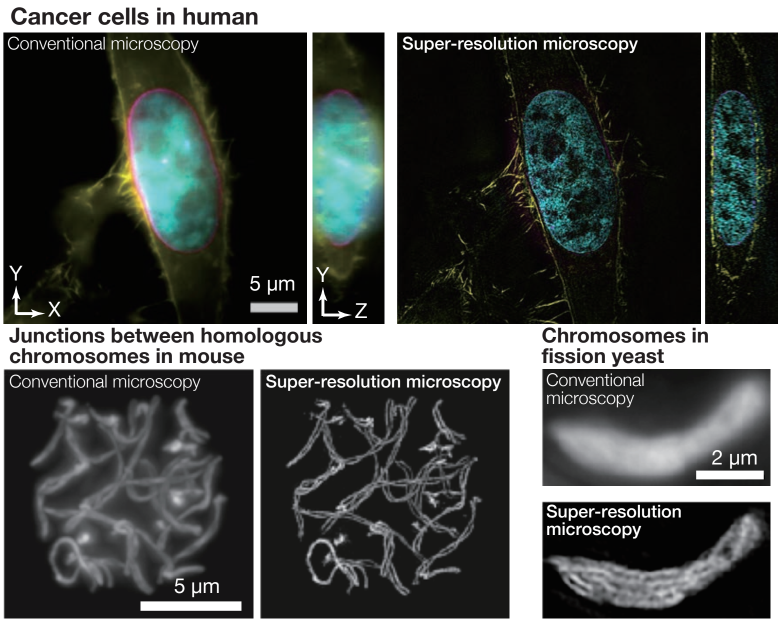

Figure 1　Comparison between conventional fluorescence microscopy and super-resolution microscopy. In the upper panel, different molecules within the cell have been labeled in three separate colors. To the right of the planar images are three-dimensional images seen from the side. In the lower panel are images of overlapping fibers that were indistinguishable from one another using conventional microscopes, but can now be seen clearly due to super-resolution microscopy (below left: modification of image taken from *Optical Alliance* (March, 2014), pp.31 – 35. Below right: modification of image taken from *Chromosoma* (2015) 125: pp.205 – 215).

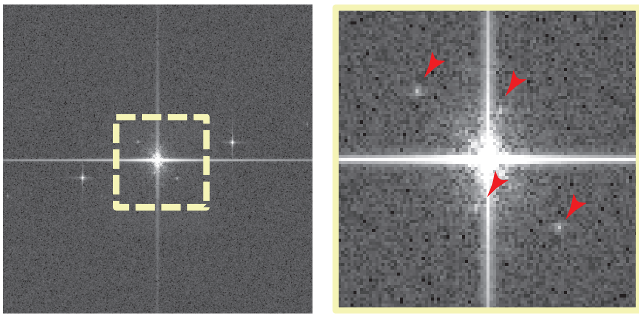

Figure 2 A super-resolution microscopy image seen in a frequency space. The image on the right is a magnification of the area in the image on the left enclosed in the yellow box. Bright spots due to stray light are indicated by red arrowheads (modification of image taken from *Nature Protocols* (2017), 12: pp.1011 – 1028).

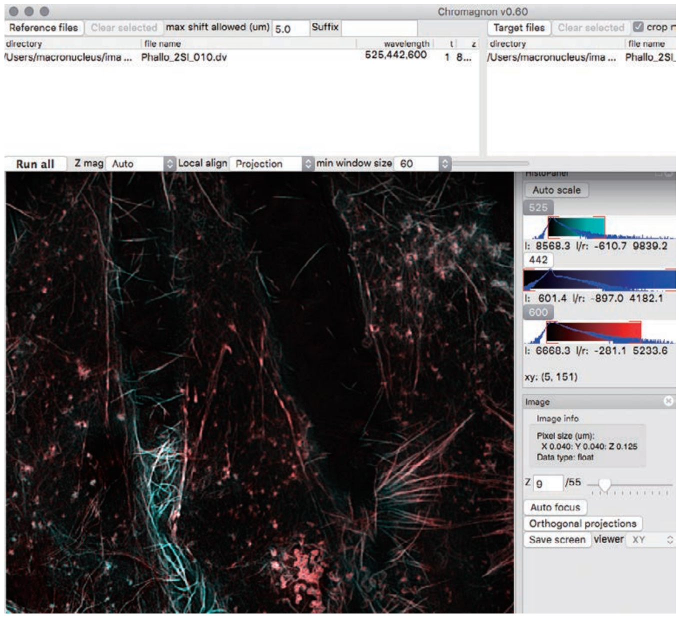

Figure 3 The newly developed chromatic aberration correcting software has been made available to microscope users throughout the world.

covery and application of a green fluorescent protein, which was awarded the Nobel Prize in Chemistry in 2008 (Osamu SHIMOMURA et al.). A protein synthesized through genetic recombination emits a fluorescent light, so that when an excitation light is illuminated on a living organism, only the targeted protein shines, enabling its observation against a dark background. Then the development of a super-resolution microscope, which was awarded the Nobel Prize in Chemistry in 2014 (Stefan Hell et al.), realized a method superseding the theoretical limit of optical resolution. The resolution of a fluorescence microscope is limited by the diffraction limit of light, which is around 250 nm. However, using super-resolution microscopy realizes a resolution of 15 – 100 nm, allowing things to be seen that have never been visible until now (Figure 1). Research and development in this area has greatly expanded the space-time of technology for measuring organisms, resulting in the possibility of continuous monitoring of smaller targets.

Technology for realizing extreme resolution

The principle behind super-resolution microscopy has been experimentally proven, but it is extremely difficult to take actual measurements using live organisms. It is difficult enough obtaining normal resolution using live organisms, so realizing an extreme resolution that is several times higher requires extreme care. We have therefore been developing the technology needed to use super-resolution microscopy on actual organisms. For example, in three-dimensional cells, fluorescent shot noise from out-of-focus planes increases in intensity, greatly reducing the resolution. In our super-resolution microscopy, we applied deconvolution to remove this fluorescent shot noise from out-of-focus planes, and image processing techniques such as denoising to reduce this noise, and succeeded in obtaining high resolution even in its presence. Moreover, images obtained through super-resolution microscopy faced the problem of interference patterns created by artifacts (errors in the data and warped signals generated during the processing of signals and images), but identifying their causes was difficult. In response to this problem, we proposed a solution for easily identifying problems in optical adjustments such as misalignments in the optical axis and presence of stray light, by carrying out a spatial frequency analysis of the obtained images (Figure 2).

Another thing that we have been working on is high-precision chromatic aberration correction. In fluorescence microscopy, multiple molecules can be targeted simultaneously for observation by labeling different molecules with different fluorescent colors (blue, green, red, etc.). However, the refractive index for light depends on its wavelength, resulting in minor chromatic shift in microscope images. With the enhancement of resolution through super-resolution microscopy, even the slightest chromatic shift makes the results difficult to interpret. The refractive index variation in organisms in particular is unpredictable, so it was extremely difficult until now to correct the chromatic shift in the observation of organisms. We developed a new method to acquire microscopic images and to measure the chromatic shift in the images, and achieved a correction accuracy of around 15 nm when observing three-dimensional samples (Figure 3). This level of accuracy will allow the correct interpretation of multi-colored super-resolution microscopy images. To contribute to the progress of biological research, we are providing this high resolution chromatic aberration correcting software that we developed for free.
https://github.com/macronucleus/Chromagnon

Future prospects

If we could elucidate the principles behind the impressive functions found in organisms, it would advance human innovation to a new level. Super-resolution microscopy has the potential to revolutionize the field of life sciences, but there are still numerous issues that need to be resolved, such as the toxic effects by exposure to strong excitation light to monitor living organisms over a long time. In addition to further enhancing resolution, we will continue our research into new directions to improve the method to observe living organisms, e.g., by mitigating its toxic effects.

Recent Initiatives by NICT for Realization of 5G
– From an NICT Press Release –

The fifth-generation mobile communication system (5G) has features that could not be provided by previous mobile communication systems, such as ultra-high speed, ultra-low latency, a large number of concurrent connections, etc. and is expected to connect all objects wirelessly and become the "infrastructure" of social life. NICT is advancing the implementation of demonstration tests toward the realization of 5G.

The NICT Wireless Networks Research Center, aiming to construct intelligent transport infrastructure utilizing the "ultra-low latency" of 5G, developed a system to ascertain the traffic conditions on the surrounding roads in real time (Figure 1). In this system, the sensors of an electronic roadside mirror (a standard roadside mirror with a stereo camera and laser range finder (LRF) installed) can carry out automatic identification, and then the information can be compressed and wirelessly send to a server and also integrate multiple pieces of diverse sensor information to construct a highly reliable and advanced map database (dynamic map).

Using this system, the Research Center constructed a test environment for an intersection simulated at the Yokosuka Research Park (YRP) and confirmed that it was able to accurately ascertain the positions of cars and pedestrians.

Furthermore, the Research Center used the wireless access method called the Grant Free method, the introduction of which is being considered for 5G, to confirm that "a large number of concurrent connections" of approximately 20,000 terminals were possible with regards to one base station. If this method is applied, even in a situation where congestion of communications occurs, such as in natural disasters, etc., information can be transmitted from many terminals within a limited time. This demonstration showed that these functions can be utilized effectively in the two use scenarios of a disaster prevention warehouse when there is a disaster, and a future smart office (Figure 2).*

At Wireless Technology Park 2018 held from May 23 (Wednesday) to May 25 (Friday), 2018 (at Tokyo Big Sight), demonstration exhibits and lectures related to 5G/IoT, including the above outcomes, were presented (Figure 3). The visitors showed interest in new concepts for wireless system utilization, and there were opportunities for discussions about technical issues, etc. with people from a variety of industries. Regarding 5G, a variety of issues can be considered based on the use scenarios, so the Research Center will continue to advance research and development while also advancing discussions with people from a variety of industries.

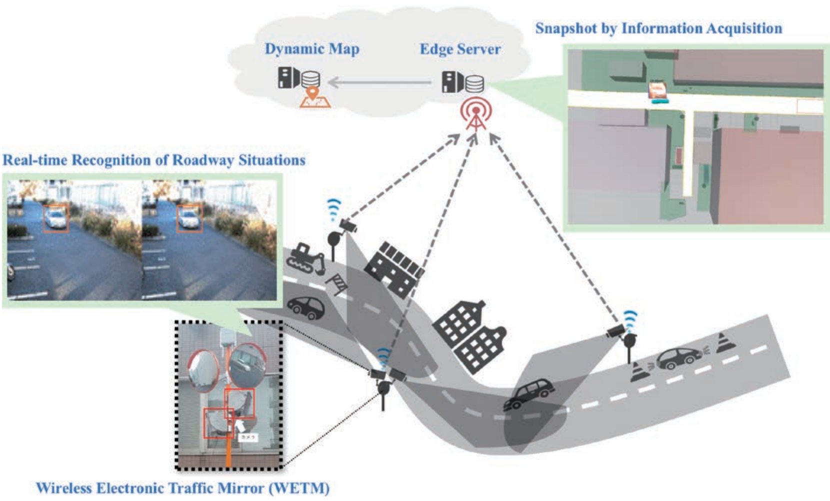

Figure 1 Configuration of the constructed intelligent transport infrastructure


Figure 2 Smart office environment utilizing a variety of 5G functions

Figure 3 The exhibits at WTP2018

＊Some of the research and development regarding electronic roadside mirrors is the outcome of an R&D of frequency-effective utilization technology from the Ministry of Internal Affairs and Communications. Furthermore, the demonstration of many simultaneous connections is the outcome of a contract for research and examination regarding the technical conditions, etc. for a fifth-generation mobile communication system from the Ministry of Internal Affairs and Communications, and was implemented through joint research with Itoki Corporation, ABIT Corporation, Sharp Corporation, and SoftBank Corp.

Related Press Releases

- **"Confirmation of Simultaneous Connection of 20,000 Terminals in the 5G Demonstration Test"** (Dated March 29, 2018)
  http://www.nict.go.jp/press/2018/03/29-1.html (only in Japanese)
- **"For Realizing an Intelligent Transport Infrastructure Utilizing 5G Ultra-low Latency"** (Dated June 7, 2018)
  http://www.nict.go.jp/en/press/2018/06/07-1.html

Awards

The Space Development and Utilization Grand Prize gives recognition to outstanding achievements in contributing greatly to the promotion of space development and utilization. The awards aim to contribute to the further promotion of space development and utilization, and cultivate greater awareness and understanding for it among people in Japan. The Maejima Award was established in commemoration of the achievements of Hisoka MAEJIMA, a founder of the telecommunications industry, and to pass on and build on his spirit. The awards are presented to people with outstanding achievements in the advancement of the information and communications (including postal services) or broadcasting industries, and two awards were won by people at NICT at the 63rd award-giving ceremony held this fiscal year.

Cabinet Office Minister for Internal Affairs and Communications Award 3rd Space Development and Utilization Grand Prize

National Institute of Information and Communications Technology*

*Awarded to the organization

Overview

●Description: Contribution to the social activities through the development and operation of space weather forecast system

We have been monitoring the ionosphere since 1958 to forecast and issue warnings regarding the transmission of radio waves. Since 1988, we have also been involved in ascertaining the status of space weather, which causes disturbances in the ionosphere, and providing information on forecasts for 365 days of the year, in addition to engaging in research and development aiming to improve the accuracy of forecasts, for which we were given the award. We won high appraisal, particularly for our major contribution to the development and utilization of space, including the issuing of warnings in the event of major solar flares to prompt the implementing of measures by satellite operators, airline companies, and so on.

●Date: March 20, 2018

Comment from the Recipient

It is honorable to recognize our continuous effort for space weather research and dissemination for a long period. We would like to contribute to social activities with improving and providing space weather information.

Mamoru ISHII
Director of Space Environment Laboratory, Applied Electromagnetic Research Institute

Tsushinbunka Association 63rd Maejima Award

Mizuhiko HOSOKAWA

Vice President

Overview

●Description: Research and development on time and frequency standards and standard time

As a leader of research at a time / frequency standards organization in Japan, he won high appraisal for leading research and development into high-precision time / frequency standards technology, a cutting-edge fundamental technology propping up the modern-day ICT society. In the development of the Cs primary frequency standard they created a type of equipment that qualified for participation in the development of International Atomic Time on which the world's standard time is based. As for the next generation of atomic clocks, the values measured by a Sr optical lattice clock they developed, said to be the most promising candidate for "redefining the second," has been adopted by an international committee.

●Date: April 10, 2018

Comment from the Recipient

I believe I was awarded this prize as a representative of all the people involved in R&D in the field of space-time standards at NICT.

We would like to continue contributing to achieving valuable results in this field. Thank you very much.

Daisuke INOUE

Director of Cybersecurity Laboratory, Cybersecurity Research Institute

Koei SUZUKI

Senior Technical Researcher, Cybersecurity Laboratory, Cybersecurity Research Institute

Yu TSUDA

Researcher, Cybersecurity Laboratory, Cybersecurity Research Institute

Yaichiro TAKAGI

Fixed Term Technical Researcher, Cybersecurity Laboratory, Cybersecurity Research Institute

At the party after the award ceremony. From left; Koei SUZUKI, Daisuke INOUE, Vice President Mizuhiko HOSOKAWA, Yu TSUDA, and Yaichiro TAKAGI

Overview

●Description: Research and development on an integrated analysis platform against cyberattack and its social application

The researchers won high appraisal for developing NIRVANA-KAI, the world's first "integrated analysis platform against cyberattack" capable of monitoring and analyzing traffic on the networks of organizations in real time to deal with targeted cyberattacks. The system enables the gathering and analyzing of huge volumes of alert information in almost real time, and three-dimensional visual displaying to allow the implementing of speedy measures, prioritization, and automatic protection measures.

The three-dimensional visualization and analysis technology of NIRVANA-KAI is being used at SECCON and other cybersecurity competitions as well.

●Date: April 10, 2018

Comment from the Recipients

NIRVANA-KAI is a platform that enables speedy responses to cyberattacks. The results of research and development, so far, have been used at universities, companies, government offices, and so on, throughout society. Moving forward, we plan to continue extensive promotion of our results.

We would like to take this opportunity to express our gratitude to all the people who have contributed to R&D and the spread of NIRVANA-KAI throughout society.

NICT NEWS 2018 No.4 Vol. 470

Published by **Public Relations Department, National Institute of Information and Communications Technology**

Issue date: July. 2018 (bimonthly)

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ISSN 2187-4050 (Online)