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# CHIKYU HAKKEN

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EARTH DISCOVERY

# Fathom the Mysteries of the Deep Hot Biosphere

SPECIAL TOPIC 1 :

## Drilling the Okinawa Trough Hydrothermal Fields where Rich and Fertile Deep Hot Biosphere Spreads

SPECIAL TOPIC 2 :

## The Journey of Exploring for Life in the Deep Subseafloor has Just Begun


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10

CHIKYU HAKKEN Volume 10

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## Fathom the Mysteries of the Deep Hot Biosphere



# Fathom the Mysteries of the Deep Hot Biosphere

## Exploration of Subseafloor Biosphere by Deep Sea Drilling

Since the 1990s, it has been proved that a gigantic deep biosphere spreads inside the Earth.

One of the major scientific goals set by the IODP is to explore such biosphere by deep sea drilling and elucidate the relationship between the Earth's inner environment and biological phenomena. It is expected that the deep sea drilling vessel *CHIKYU* which Japan is proud of will shed light on the existence, activities and distribution of the deep biosphere we have not known yet.

# Drilling the Okinawa Trough Hydrothermal Fields where Rich and Fertile Deep Hot Biosphere Spreads

Interviewee:
**Dr. Ken Takai**
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)
Institute of Biogeosciences
Extremobiosphere Research Program

**To uncover subseafloor biosphere has been one of important scientific goal in the first phase of the Integrated Ocean Drilling Program (IODP). This time, after waiting until just the right moment, in the subseafloor of the Iheya-North Field in the Okinawa Trough, which is so rich and fertile in life as to be compared to 'the Amazon River', drilling is to be carried out in order to throw light on the river running through the subseafloor and the deep biosphere spreading in the flow.**

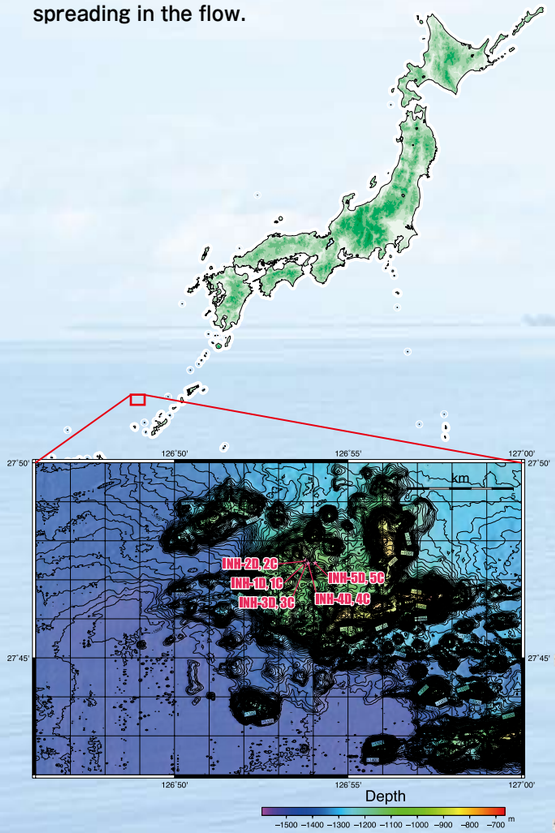



**Figure 1: Location of the Okinawa Trough**
It is in the Okinawa Trough, located to the northwest of the Okinawa Islands that subseafloor hydrothermal active fields were found for the first time in Japan. The Okinawa Islands form an island arc in the plates' subduction zone as the former Japanese Archipelago in the past. Geologically, the Okinawa Islands has the features of a miniaturized Japanese Archipelago brought into the modern era. The scheduled drilling sites are in the Iheya-North Field.

## Attention Given to Subseafloor Biosphere under Hydrothermal Vents

In 1977, seafloor hydrothermal vents and exotic organisms living around the seafloor were discovered at the Galapagos Rift off the western coast of South America. It was a great discovery that would overturn the conventional wisdom in biology and geochemistry.

With the discovery of the Galapagos Rift hydrothermal vent communities, it was considered that such communities might also exist near volcanoes in the areas where plate subduction would occur, like ocean around Japan, in addition to those communities most found along the Mid-Oceanic Ridge where plates would be born. About 10 years after the initial discovery, it was in the Okinawa Trough that the first hydrothermal vent communities were discovered in Japan. Triggered by the discovery, serious study of hydrothermal fields began in Japan, too.

After the 1990s, it is becoming clear that in the subseafloor the largest microbial biosphere on Earth exists. However, majority of the subseafloor microorganisms are minimally alive, for they are fed by dead creatures fallen to the seafloor, which subsist on organic compounds made by photosynthetic activities on the Earth's outer layers. The microorganisms have such low activities as to divide once during 3000 - 10,000 years. They barely exist between life and death. However, in 1993 Jody Deming and John Baross of University

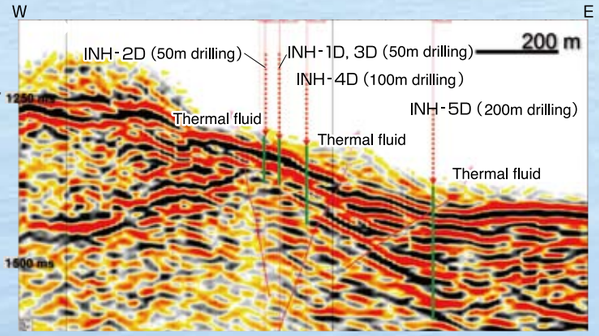



**Figure 2: Subseafloor structure of the scheduled drilling sites**
The subseafloor structure of the Iheya-North Field elucidated by the seismic survey. Based on the information, the drilling sites that would be the most expedient for the study were determined.

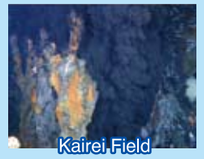
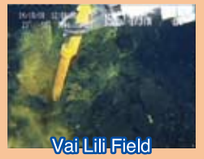
| | Great River of Sulfur | Great River of Hydrogen | Great River of Iron | Great River of Methane |
|---|---|---|---|---|
| |  TOTO Caldera Field |  Kairei Field |  Vai Lili Field |  Iheya-North Field |
| Primary driving force for hydrothermal circulation | Magma activity | Tectonics | Cooling of basaltic crust | Magma activity or tectonics |
| Predominant component in drainage basin | Magma | Ultramafic rock | Basaltic rock | Terrigenous sediment |
| Temperature | High-temperature | Middle-temperature ～ high-temperature | Low-temperature | Middle-temperature ～ high-temperature |
| Time scale | Several days ～ several hundred years? | Several thousand years ～ one million years? | One million years ～ several hundred million years? | Several hundred years ～ several thousand years? |
| Characteristic geochemical reaction | Magma degassing, high-temperature chemical equilibrium/ phase separation | Serpentinization and Fischer-Tropsch reaction | Weathering of basaltic rocks | Diagenesis caused by heat of organic matters and microorganisms |
| Dominant energy source in Great River (Potential) | Reduced inorganic sulfur compounds | Hydrogen | Fe (Ⅱ) | Organic matter, methane and ammonia |
| Primary producers supported by Great River (Potential) | Sulfur-oxidizing bacteria (oxygen, nitric acid), Hydrogen-oxidizing sulfur-reducing bacteria, sulfur-disproportionating bacteria, carbon monoxide-utilizing bacteria | Hydrogenovibrio marinus (oxygen, nitric acid, sulfuric acid, Fe (Ⅲ),sulfur), methanogen, formic acid -utilizing bacteria, anaerobic methane-oxidizing bacteria | Iron-oxidizing bacteria (oxygen, nitric acid), sulfur-oxidizing bacteria (oxygen, nitric acid), ammonia-oxidizing bacteria (oxygen) | Anaerobic & aerobic methane-oxidizing bacteria, Anaerobic & aerobic ammonia-oxidizing bacteria, methanogen, etc. |

**Figure 3: Classification of hydrothermal fluids fields into four types of 'Great Rivers"**
Hydrothermal fluids have very different chemical compositions depending on the conditions of their heat sources and chemical reactions with the host rocks. The Okinawa Trough corresponds to "Great River of Methane' in this Table.

of Washington, the United States announced a hypothesis that in the subseafloor of hydrothermal vents there should be a world of quickly dividing microorganisms with no dependence on photosynthesis. Following the hypothesis, more attention was drawn to the world of 'extremophiles' spreading in the subseafloor of hydrothermal vents, where it would be extremely difficult for normal creatures to inhabit.

In such circumstances, to uncover the true nature of the microorganisms in the subseafloor was set by the IODP as one of the main scientific goals. Also in Japan, a Lead country of the IODP, momentum was growing for submitting a proposal on the subseafloor microbial research that would meet the goal.

## First Proposal Submission and Challenge Standing in the Way

Those responsible for preparing the proposal in 2001 were the then members of the Deep-sea Microorganism Research Group of JAMSTEC. In those days, Dr. Ken Takai of Institute of Biogeosciences Extremobiosphere Research Program, who acts as Co-chief Scientist of the scheduled ocean drilling expedition from the standpoint of microbiology research, was carrying out research on different types of creatures and organisms inhabiting hydrothermal fluids, depending on their different chemical compositions, by comparing the Suiyo Seamount present in the seafloor of the Izu-Ogasawara Islands with the Okinawa Trough, deep sea waters to the northwest of the Ryukyu Islands. In hydrothermal fluids in the Suiyo Seamount, bacteria feeding on hydrogen sulfide live in symbiosis with bathymodiolid mussels, while in the Okinawa Trough, bacteria feeding on methane are in symbiosis with the mussels. The reason is that hydrothermal fluids from the Suiyo Seamount contain high concentrations of hydrogen sulfide, whereas those from the Okinawa Trough a lot of methane. Dr. Takai, together with researchers in geochemistry, went on exploring where such difference in composition of hydrothermal fluids would come from. As a result, they came to know the following: that there are sediment layers less permeable to water in the Okinawa Trough, therefore, the hydrothermal fluids running right above the layers do not flow toward the subseafloor but spread horizontally and expand their scale. And the hydrothermal fluids would contain a lot of methane by taking time to run above the layers and absorbing the components of the sediments, making such a prosperous world of microorganisms spread both in the seafloor and the subseafloor.

'In consultation with the geochemists who were researching together with me back then, a conclusion was made, "If we conduct drilling operations for highly active microorganisms in the subseafloor, the Okinawa Trough should be preferable where habitats for microorganisms are secured and energy sources are abundant". We thought the Iheya-North Field, which had been our research target, would be suitable, so I wrote a proposal of drilling for deep subseafloor biosphere in the Iheya-North Field'. (Dr. Takai)

Just writing an IODP proposal does not guarantee it will be easily accepted. A scientist who submits a proposal to the IODP must win out in the competition among numerous proposals coming in from scientists around the world. The IODP conducts a detailed review of the submitted proposal and requests its revision by pointing out problems to the proponent. The IODP adopts a system in which the proposal is nurtured through such a process until it is ready for its implementation. Initially, amid very severe opinions toward the submitted proposal, there was one remark, "The challenge toward the subseafloor biosphere has just begun. The positive sides must be found and nurtured. Now that this proposal presents a fascinating subject to study highly active microorganisms under hydrothermal fluids, efforts should be made by all means so that the proposal will meet the IODP's criteria'. This view saved me above all and made me go on to the next stage, said Dr. Takai.

| | the Amazon River | the Okinawa Trough hydrothermal system |
|---|---|---|
| Drainage basin | The largest in the world | The largest in the world? |
| Branch rivers | So many | So many |
| Characteristics of drainage basin | Rainforest<br>Jungle<br>Diverse creatures | Organic-rich sediments<br>Diverse subseafloor microorganisms<br>(subseafloor forest) |
| Flow | Extremely slow | Extremely slow |

**Figure 4: The Iheya-North hydrothermal system is 'the Amazon River**
As indicated in the Table, there are so many similarities between the Amazon River which nurtures prosperous biosphere, with the largest drainage basin among the great rivers flowing on the earth and the Iheya-North hydrothermal system, where the drilling is scheduled.

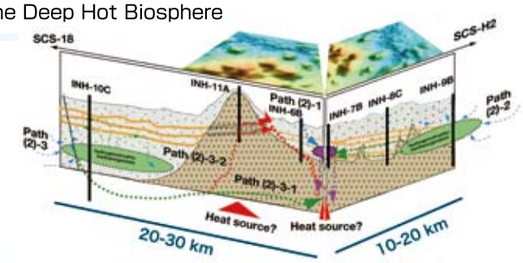



**Figure 5: Subseafloor drainage basin of the Iheya-North Field**
On the subseafloor of the Iheya-North Field, the heat sources beneath the seamount (magmas) create water circulation. With the seamount as its gateway, the water flows horizontally on the sediment layers less permeable to water and expands the scale of the hydrothermal fluids field. Overall, it is assumed that the water flow occurs as shown in the Figure.

## Clearing the Hurdle of Analyzing the Subseafloor Structure

The next problem was the preliminary study. One of its important issues was the analysis on geological structure of the subseafloor. Dr. Takai explained, 'The IODP pointed out at that time that the flow of the subseafloor hydrothermal fluids must be confirmed. With the accumulated experiences from seafloor researches, I could roughly expect the types of microorganisms in the subseafloor and I was also confident about my assumption. The problem was how to fit the flow of the hydrothermal fluids into the proposal. The IODP demands proper justifications of "the necessity of drilling at such sites down to such depth". For that reason, seismic survey had to be carried out to analyze geological structure of the subseafloor in order to create a story which would clarify that the drilling operations must be conducted at these sites, where highly active microorganisms were found to exist in the hydrothermal fluids flow'.

Those involved in this proposal were mainly scientists of microbiology or geochemistry. All of them had never analyzed geological structure of the subseafloor by conducting seismic survey. In those days, another proposal, drilling in the Nankai Trough, was submitted and under review. Every scientist, equipment and vessel that was capable of analyzing the geological structure of the subseafloor by seismic survey had been mobilized. The circumstances were such that nobody could be found that would analyze the geological structure of the subseafloor in the Okinawa Trough on behalf of Takai's group. With no other choice but to rely on his personal connections, Dr. Takai borrowed equipment from Ocean Research Institute, the University of Tokyo and placed the equipment on the research vessel *KAIYO* of Japan Agency for Marine-Earth Science and Technology (JAMSTEC) and managed to conduct a seismic survey called Multi-Channel.

Then, the survey data had to be interpreted to determine the geological structure of the subseafloor, but he did not know much about how to do that. 'The proposal deadline was approaching, so I asked various kinds of people all over Japan, "How should I interpret this?" When I submitted the data to the Site Survey Panel (SSP) of IODP, I was harshly criticized by such comments as, "This is insufficient" or "This interpretation is wrong", which gave me a real headache. At such time, one scientist, who had returned to a private oil company from JAMSTEC, kindly extended a helping hand to me. Out of genuine good will, he helped me to meet the requirements. I thought someone would never fail to help as long as the person is motivated'.

Thanks to his help, the geological structure of the subseafloor in the Okinawa Trough was determined, the evidence for the flow path of the hydrothermal fluids could be established and the drilling sites and depths were also fixed. While satisfying the IODP criteria, the proposal could provide the interpretation of the geological structure of the subseafloor in the form of additional information gained from the seafloor observations. The preparation of the proposal started in 2001 and it was adopted in 2007. It was worth the efforts; this proposal has been ranked at the 2nd by the IODP Science Planning Committee.

'But having said that, being in an upper-ranked tier does not necessarily mean the drilling is guaranteed. It took another long time for the proposal to be realized", added Dr. Takai. Even if the scientific significance of the drilling research is recognized, the drilling operations are realized only after various conditions are met, such as the survey area and the status of the vessel which actually conducts the operations.

## The Okinawa Trough, "the Amazon in the Subseafloor"

Under the seafloor, there is another space filled with water, such as hydrothermal fluids and cold spring water. Such water does not spread all over the subseafloor but flows where it is easy to flow, like subseafloor rivers. The water carries a variety of substances and energy is also supplied by magmas which may exist in the subseafloor. There should be a highly active microbial world.

The characteristic of the Okinawa Trough hydrothermal field, including Iheya-North, where the scheduled drilling sites exist, is that layers of pumice permeable to water like a sponge which originate from volcanic eruptions and layers of sediments containing a lot of organic matters flowing mainly from the Yangtze River alternately deposit, creating many layers just like mille-feuille cake.

The volcanic magmas in the Okinawa Trough region, with the scheduled drilling sites, contain lots of components which produce bubble as they become gas. When those magmas become rocks, they are like pumice, permeable to water. On the other hand, in this region, the water flow is complex: the sediments are watertight just like clay, so water running on the subseafloor flows on organic-rich sediments

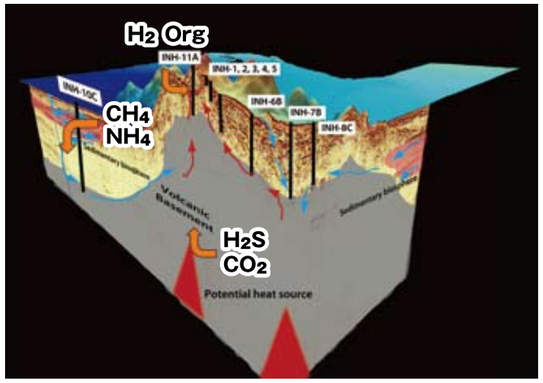



**Figure 6: Subseafloor structure and water flow**
The chart created by adding the subsurface structure obtained from the seismic survey to the water flow and the drilling sites shown in Figure 5 and changing the angle slightly. The red arrows indicate the rising hydrothermal fluids heated by the heat sources and the light blue arrows show the water circulation caused by the fluids. The orange arrows indicate the chemical components added to water from the areas. It is considered that hydrogen sulfide from the basalt host rocks, as well as methane and nitrogen compounds produced by numerous microorganisms in parts of layers of pumice and sediments are dissolved into the water. It is also expected that at the scheduled drilling sites, which correspond to 'the estuary of hydrothermal fluids', microorganisms right under the hydrothermal vents can be collected.

slowly but steadily in a lateral direction. Where faults exist, the water moves down and spreads on the sediments laterally again. Usually, in subseafloor hydrothermal fluids regions, water circulates vertically and the horizontal distance is said to be a few kilometers. However, in the hydrothermal fluids of the drilling sites, due to the sediments, the water also spreads laterally and expands the scale. It is just like the Amazon River with many branch rivers and wide drainage basin area.

The compositions of hydrothermal fluids vary depending on the geological conditions (the properties of the host rocks which the hydrothermal fluids flows through) of the area. Basalt-derived hydrothermal fluids found in Mid-Ocean Ridges contain large amounts of hydrogen sulfide. On the other hand, the characteristic of hydrothermal fluids in the Okinawa Trough is that they contain a lot of organic matters, such as methane, because of the layers of organic-rich sediments. There is a prosperous biosphere nurtured by the sediment layers in the drainage basin of 'the Great River of Subseafloor" in the Okinawa Trough. While hydrothermal fluids flow through the biosphere, they will contain plenty of methane. This characteristic also resembles that of the Amazon River which flows through rainforest soils and contain high levels of organic matters, Dr. Takai points out.

## Scheduled Drilling Sites and Subsequent Utilization Plan

The original proposal planned to conduct drilling operations at 11 locations in the Okinawa Trough in order to clarify the overall picture of 'the Amazon River of Subseafloor'. As for the 1st through 5th drilling sites (INH-1,2,3,4,5), water is heated by heat sources, flows through the seamount and finally spew out as hydrothermal fluids at the points. Actually, hydrothermal fluids may also come from the north, so drilling at the 6th through 9th sites (INH-6-9) should confirm the possibility. The 10th site (INH-10) is the area called , "Rainforest of Subseafloor", where water spreads horizontally. And the 11th site (INH-11) is the path of hydrothermal fluids that were found last. At the proposal stage, it was hoped that everything about these hydrothermal fluids would be clarified. But time is limited for the scheduled expedition, so our target is up to the 5th site. The site is the last part where hydrothermal fluids spew and the operations will be drilling at the estuary delta of the Amazon River, so to speak'.

Therefore, the main purpose of this drilling has become exactly microorganisms just under the hydrothermal vents. The hypothesis that there exists a world of highly active microorganisms has not been directly proved yet, even with indirect evidence by collecting hydrothermal fluids. This drilling should become its direct proof. 'For this purpose, the Okinawa Trough is the most suitable. In the case of the Indian Ocean, microorganisms live along the cracks of basalt rocks, so without digging through, microorganisms will be missed. In the Okinawa Trough, a lot of microorganisms inhabit the inside of pumice, which makes the encounter with them more likely. It is assumed that the scheduled drilling sites are not where abundant amounts of methane are produced. Still, by showing, 'Such amounts of methane should be impossible to be produced right under hydrothermal vents alone', the drilling can be linked to the next step', expects Dr. Takai.

Moreover, it is planned that pipes called casings, acting as inner frame, will be installed into some of the drilled holes in order to prevent them from crumbling and turn them into 'artificial hydrothermal vents' which can be used for at least the next 10 years. It is expected that the use of these vents as a kind of on-site incubators in a variety of microbial cultures will further advance the study of microorganisms on the subseafloor.

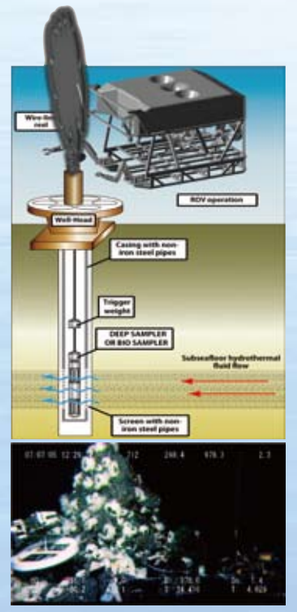



**Figure 7: Formation of artificial hydrothermal vents**
The drilling operations are scheduled at five locations right under the hydrothermal vents. Among the drilling sites, INH-1 (50m deep) corresponding to the hydrothermal mound, INH-4 (100m deep) covering the sediments from which low-temperature hydrothermal fluids seep and INH-5 (200m deep)、a Calyptogena colony covering sediments from which similarly low-temperature hydrothermal fluids seep will be maintained as the artificial hydrothermal fluids vents, by using the casings after the drilling. These vents will be utilized as on-site incubators in order to make continuous researches on subseafloor microorganisms.

# The Journey of Exploring for Life in the Deep Subseafloor has Just Begun

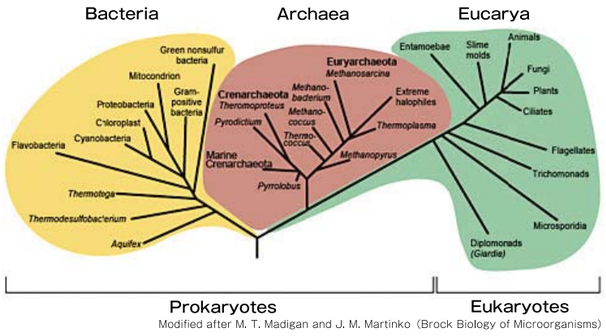
**For a long time, it was thought that no life existed within the deep-seafloor. In 1994, a theory was put forward which would overturn this assumption. The existence of huge amounts of microbial cells was revealed by the core sediment samples collected by the drilling operations of the Ocean Drilling Program (ODP), which reached 800 m below the seafloor. However, many questions remained: how deep do these creatures exist in the deep subseafloor, what types of creatures are they, and how do they live. Now, in search of the answers, IODP is about to begin a new series of investigations.**

Interviewee:
**Dr. Fumio Inagaki**
Geomicrobiology Group at the Kochi Institute for Core Sample Research

## The Achaea World in Marine Subsurface Sediments

Currently, the presence of microorganisms has been confirmed by analyses of sediment core samples from 1,626 m below the deep-sea seafloor, collected by the U.S. operated ocean-drilling vessel, *JOIDES Resolution*. It's estimated that in the deep biosphere of the entire Earth, the population of microorganisms is equivalent to $3.5\times10^{30}$ cells (more than 1 million cells in the space of one sugar cube), equal to about one-tenth of all life forms on the Earth, including all flora and fauna on land.

In 2008, Drs. Fumio Inagaki and Yuki Morono, Geomicrobiology Group of the Kochi Institute for Core Sample Research, in collaboration with the University of Bremen, Germany, analyzed marine subsurface sediment samples obtained from IODP drilling operations around the world. These included samples collected by *CHIKYU* to the east of the Shimokita Peninsula in Aomori Prefecture, Japan in 2006 and found microorganisms called *Archaea* in the subseafloor, in numbers far greater than had been expected.




Modified after M. T. Madigan and J. M. Martinko (Brock Biology of Microorganisms)

**Figure 1:** Life forms on the Earth can be divided into three groups: *Eucarya* (Eukaryotes)/*Bacteria*/*Archaea*. The *Eucarya* includes animals and plants, microorganisms such as *E. coli* and *Bactillus natto* as *Bacteria* and many of extremophiles, such as hyperthermophiles and halobacteria as *Archaea*. Studies have revealed that large amounts of *Archaea* inhabit subseafloor sediments. Eukaryotes refer to organisms whose genetic material (nucleus) is covered with films and prokaryotes organisms whose genetic material is not covered with films. Most prokaryotes are unicellular organisms whose physical structures are simpler than those of Eukaryotes. The outside of prokaryotes are covered with cell membranes and nutrients and other substances enter the cell through the cell membrane.

In general, microorganisms are phylogenetically classified into *Bacteria* and *Archaea* according to their DNA and cell membrane structure (see Figure 1). Dr. Inagaki and his colleagues paid attention to different cell membrane structures of both kinds of microbes and calculated the quantitative ratios of *Bacteria* and *Archaea*. *Archaea* accounted for about 87% of the sample sediments, showing that *Archaea* dominates the marine subsurface sediments. One reason why *Archaea* is so widespread throughout the subseafloor is that nutrients and oxidants are scarce in the closed subseafloor environment, which is cutoff from all sunlight, and the environmental adaptations reducing energy requirements of *Archaea* give them an advantage. The lipid structure of *Archaea* cell membranes, unlike that of *Bacteria*, makes it difficult for substances to move through the cell membrane. The more permeable *Bacteria* cell membrane lipid structure is considered more suitable for the world at the surface where sunlight is available. The *Archaea* in the subseafloor world indicates how microorganisms have expanded their habitat during the evolution of life on Earth.

However, "It's not that I'm satisfied with the research made so far. I don't think we have elucidated what types of microorganisms really inhabit the subseafloor", states Dr. Inagaki. "As for these (microorganisms), the result that *Archaea* was about 87% was obtained by focusing on the different structures of the cell membrane. However, according to the genetic analysis of DNA, *Archaea* accounted for 40-50% on average of all microorganisms within the sediment samples. The analytical methods made such difference. Maybe, there are other unknown phyletic groups of microorganisms, such as viruses, that we have not captured; that's what I think now. It should be necessary to realize that we may not have detected unknown microorganisms as life through conventional techniques. The extent of our understanding about the deep subseafloor is tiny. To recognize that we actually know nothing yet may be a point to make a new breakthrough", says Dr. Inagaki.

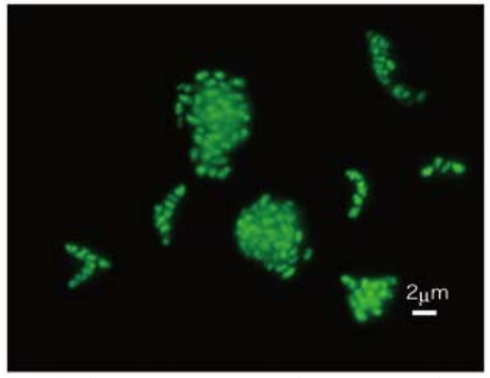



**Figure 2:** A fluorescence microscopy image of aggregate structure of active microorganisms confirmed in the drilling core sample obtained by *CHIKYU*, about 80 km, about 350 m below the seafloor to the east of Shimokita Peninsula in Aomori Prefecture.

## Challenge to the Unknown Biosphere

It's believed that the subseafloor biosphere exists at a slow metabolic rate over geological time scales and has long-term effects on the material circulation of the Earth's interior. However, the role of microorganisms in the circulation of matter in the Earth's interior is not well understood. To clarify the question is an important focus in future research.

For example, most research drilling in the deep biosphere has used seafloor samples of organic-rich sediments from along continental coasts, where the organisms observed in these areas are known to depend on organic matters produced in sea surface. What is happening in sea areas where such organic matter is rare or non-existent? *JOIDES Resolution* is scheduled to operate, IODP Expedition 329 in the South Pacific from October to December 2010. This region is known as an area where organic matter is extremely limited. Even in seafloor sediments here, microbial cells exist at a level of about one one-thousandth to one one-millionth of those in seafloor sediments along general continental coasts. However, "In such regions, a world different from the continental-organic driven biosphere may spread", expects Dr. Inagaki. "In continental coastal sediments, oxygen consumption occurs in surface layers on the seafloor, which results in anoxic deep sediments and reducing environments. In the South Pacific Gyre, however, oxygen consumption is very low in seafloor surface layers. Therefore, plenty of oxygen exists even in deep sediments, forming highly oxidative deep biosphere, where a lot of basalt rocks are found, rich in various elements such as iron and sulfur. There may be a biosphere within rocks that is created by microorganisms whose own nutrients are produced by the elements within the basalt rocks and oxygen contained in the seawater, a still unknown biosphere which is independent of organic matters produced by photosynthesis."

*CHIKYU*'s September 2010 IODP Expedition 331 in the Okinawa Trough hopes to find evidence of the existence of a biosphere independent of photosynthesis and even one different from the one Dr. Inagaki explains. "Furthermore, in order to study biospheres not depend on continental-organic matter, research methods directly accessing the interiors of rocks samples will be important in the future", predicts Dr. Inagaki. "Tools, such as water sampling equipment, will be placed to capture signals of life. From now on, the development of these tools will also be an important area", comments Dr. Inagaki.

Previous DNA studies have shown that many microorganisms inhabiting the deep biosphere are new to science. It is a major issue to establish a culture system for growing these microorganisms in a much shorter time, instead of a time scale of thousands of years. What is expected as the measure to overcome this issue is the area called single-cell biology. Dr. Inagaki explains that the age is approaching when almost all the genetic information can be read without cell cultivation by amplifying the DNA from a single cell.

In order to shed light on the deep biosphere, many issues remain. The exploration of life in the deep subseafloor has just begun. There is a great role to be played by *CHIKYU*, equipped with the facilities to process and preserve precious samples indispensable for research.

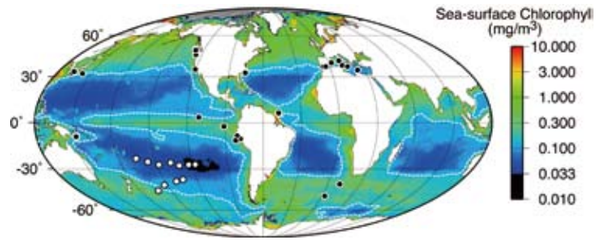



**Figure 3:** IODP Expedition 329 conducts research drilling operations in the South Pacific Gyre (white circles), where photosynthetic production of organic matters in sea water is the lowest on Earth. Similar sea areas cover approximately 40% or over of the Earth's surface (enclosed by dotted lines). It is a crucial expedition in investigating the extent and actual conditions of the deep biosphere on a global scale. The areas where information on the deep biosphere has been obtained so far are mostly along continental coasts with active production of organic matters (black spot), therefore a new unconventional discovery is expected.

World-class Research Base for Deep Subsurface Microorganisms

# The Kochi Institute for Core Sample Research, JAMSTEC

**In order to deal with deep biosphere research, which has become one of the important themes at the IODP, the Kochi Institute for Core Sample Research has established a system for storing core samples for more advanced researches on microorganisms and also put effort into developing advanced research technologies.**

Inside look at Microbiology Lab established within the Kochi Institute for Core Sample Research

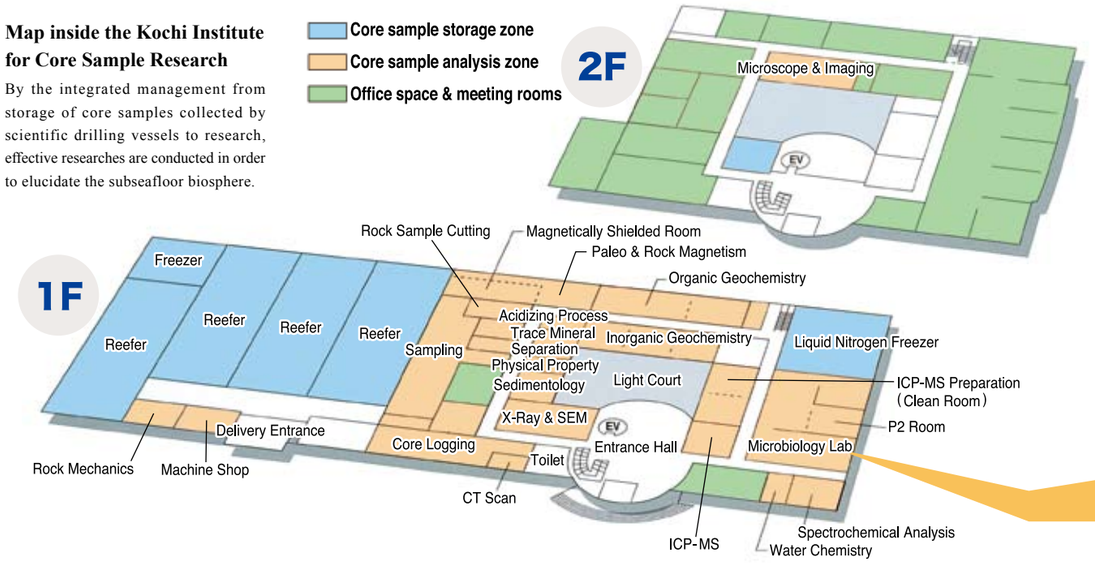
The Kochi Institute for Core Sample Research, as one of the research bases of Japan Agency Marine-Earth Science and Technology (JAMSTEC), manages the Kochi Core Center (KCC) in collaboration with Kochi University (the Center for Advanced Marine Core Research), while promoting researches using core samples obtained from IODP research expeditions. KCC is one of the IODP's three major core repository facilities bases in the world, along with University of Bremen, Germany and Texas A & M University, the United States. The KCC store and curate core samples collected by scientific drilling vessels, such as the deep sea drilling vessel *CHIKYU* , in the Western Pacific and the Indian Ocean.

One of the major characteristics of the Kochi Institute for Core Sample Research is that the core repositor facility and the research facility using the samples are managed in an integrated manner. The configuration and system of the facilities is constructed to ensure efficient operations in a line created from the core sample storage to the sample collection/research.

The Kochi Institute for Core Sample Research has internally established three research groups: Physical Property Research Group, Geochemical Research Group and Geomicrobiology Group. In recent years, the research system has been especially strengthened in the research area of the subseafloor biosphere. The area puts effort into technological development of advanced research as well as fundamental research using core samples. Under such circumstances, excellent results have been obtained, such as the new development of a technique to detect/quantify microorganisms cells from subseafloor samples by using difference in fluorescent colors (page 10, above). The core samples collected by *CHIKYU* for microbiological research, are first divided on board and immediately frozen and transported to KCC. The Center is equipped with −80℃ freezer at Microbiology Lab and −160℃ liquid nitrogen freezing system for the purpose of long-term storage of important samples containing organic matters and DNA in the most possible favorable conditions. Such storage enables long-term and effective utilization of precious core samples in microbiological research.

### Map inside the Kochi Institute for Core Sample Research

By the integrated management from storage of core samples collected by scientific drilling vessels to research, effective researches are conducted in order to elucidate the subseafloor biosphere.




**Core Storage**

In addition to vast Reefers for core samples whose room temperature is kept at about 2°C (4 rooms) and a − 20°C Freezer (1 room), Microbiology Lab is equipped with systems for storing the samples at −80°C and −160°C.

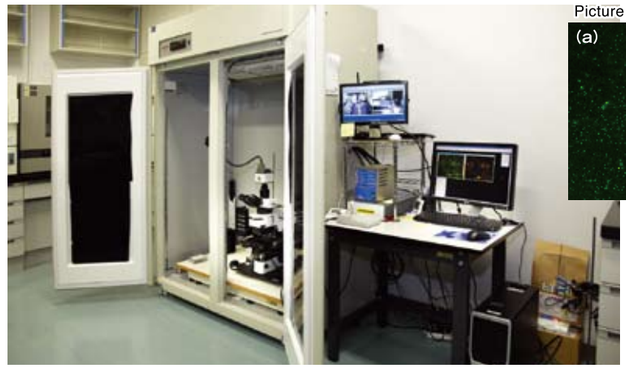
**Sampling**

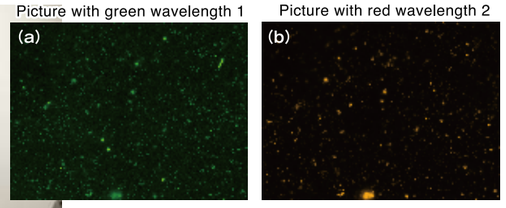
The Institute is equipped with the primary core sample analysis zone, adjacent to the Refers for the purpose of taking core samples and carrying out nondestructive physical property and paleomagnetic measurements.

**Analysis/Experiment**

The laboratories are located side by side equipped with various analytical instruments for geophysics, geochemistry and microbiology in order to conduct specialized researches of core samples.





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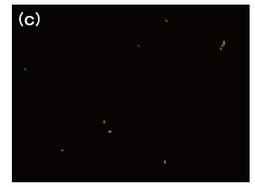



Microbial cells in subseafloor sediments arestained with DNA-specific chemical (SYBR Green I) which is known to emit fluorescence only when it binds to DNA. However, many inorganic particles are found to also emit fluorescence with SYBR Green I stain and make accurate measurement difficult. This SYBR-DiCE technique, which utilizes the difference in fluorescent color of the microbial cells and non-biological particles, has succeeded in retrieving only life-derived signals through taking pictures in twe wavelength region by optical filters differing pass-through light wavelength. The division of the green (a) with red (b) images generates image showing relative ratio of green/red in each position of the raw image. After deleting the regions where green/red ratio are low, life-derived signals are highlighted (c).

## ● SYBR-DiCE (Discriminative Cell Enumeration) Microscope System

This system is for discriminative detection and quantification of microbial cells in core samples based on the difference of fluorescent colors in automated manner, developed by the Kochi Institute for Core Sample Research. It has been difficult to precisely differentiate cells with non-biological grains in core samples in a visual inspection, however, this automated detection system contributes human error-free objective quantification of microbial biomass in a short time.

The microscope system is equipped with slide loader which automatically load sample slides in position and systematically take hundreds of pictures in two fluorescent colors in automated manner. Also, with an aid of computer macros, all steps for discriminative detection of microbial cells by image analysis and the quantification of signals are automatically done without eye sight inspection.

## ● Laser microdissection system

The system is for cutting the samples in micrometer scale under the observation with microscope by laser, for extracting DNA from single or several cells, for example.

## ● Core sample cut saw

Used for cutting frozen core samples into small ones without melting. Specially treated diamond particles are etched on the blade.

## ● Anaerobic glove box

The equipment used for aseptic processing of cores under anaerobic (oxygen-free), nitrogen atomosphere (also containing a little hydrogen).

## ● Liquid Nitrogen Freezer

The tanks in the photo are used for the long-term storage of small cut core samples at − 160°C. The microbial samples can be stored without exposure to oxygen. Another Freezer for −80°C is also installed.

Interviewee: Dr. Yuki Morono (The Kochi Institute for Core Sample Research, JAMSTEC)

# Efforts toward the Mantle Drilling

## with the Aim of Penetrating the Moho for the First Time in Human History

Interviewees:
**Dr. Shuichi Kodaira and Dr. Natsue Abe**
Institute for Research on Earth Evolution (IFREE),
Japan Agency for Marine-Earth Science and Technology (JAMSTEC)

The International Workshop held in Kanazawa in June 2010

**The project to reach the mantle by using *CHIKYU* to drill into the oceanic crust and penetrate the Mohorovičić discontinuity (the Moho), the boundary between the Earth's crust and the mantle, has steadily progressed towards its realization. Here is the outline of the project.**

### Long-time Dream for Ocean-Floor Scientific Drilling

Towards the realization of the drill into the mantle, an international workshop was held in Kanazawa, Ishikawa Prefecture on June 3-4, 2010, where narrowing down the candidate drill sites, future research projects and drilling/measuring technological development were discussed.

In 1909, a geophysicist in Croatian, Andrija Mohorovičić found the surface in the deep Earth on which the velocity of the seismic waves rapidly increases. This discontinuous surface forming the boundary between the Earth's crust and the mantle is called the Mohorovičić discontinuity (the Moho) after his name. The continental crust is 30 ～50km thick, while the oceanic crust is 5～8km, which is thinner. Therefore, 'Project Mohole' to reach the mantle through the ocean floor was attempted in the 1960s. However, its result fell far short of entering the mantle and even now, half a century later, the Project has not been realized. The drill into the mantle, which is a 'long-time dream for ocean- floor scientific drilling', has been taken up in the IODP Initial Science Plan (ISP) as its important theme and is likely to become one of the high-priority initiatives in the Second Phase of the Science Plan. The International Workshop in Kanazawa was also significant progress in realizing the drill into the mantle by using the deep sea drilling vessel *CHIKYU* which Japan is proud of.

### Towards Starting Preliminary Research

From the scientific aspect, there are two conditions on the drill site: ①above a fast-spreading oceanic lithosphere (the upper mantle) with average crustal thickness (<6km) and structure ②the presence of the clear oceanic Moho is indicated in seismic survey. Furthermore, technological requirements limit the site to be ①water depth < 4,500m, ②low Moho temperature < 250℃, ③Total drilling depth below rig floor <12,000m, ④closeness to operational base and ⑤stable sea current and weather conditions.

At the International Workshop, three sites on the Pacific Ocean were narrowed down as strong candidates: the Cocos Plate off Costa Rica (the region around IODP Site1256), the Pacific Plate off the Island of Hawaii (the eastern edge of the Hawaiian North Arch) and the Pacific Plate off Peninsula de Baja California, Mexico (Deep-Tow Site). And it was decided that preliminary research would be carried out off Mexico, where no data has been available so far. 'How to proceed with the preliminary research was also discussed at the Workshop. In 1997, Japan launched the research on the deep subseafloor structures using seismic survey in the Nankai Trough. Its results have been greatly useful in the current NanTroSEIZE Drilling. For this reason, there was also an opinion that the preliminary research should learn the experiences at NanTroSEIZE Drilling', says Dr. Shuichi Kodaira.

JAMSTEC has already proceeded with the preparations for implementing a long-term preliminary research (seismic reflection/refraction survey) using the deep-sea research vessel, *KAIREI* off Mexico. The concept is that under the agreement of the international community, Japan leads the efforts first in order to pave the way for realizing the drill into the mantle. 'The preliminary research is important not only for the purpose of determining the drill site but also for clarifying the scientific meaning of the site based on a solid understanding of large-scale tectonic field', comments Dr. Kodaira. 'In addition, an opportunity to actually validate what we seismologists have seen with imaging can be given by the drill into the mantle, so it is also important for the purpose of making the imaging more precise. We hope to make contributions to its realization in the preliminary research,' continues Dr. Kodaira.

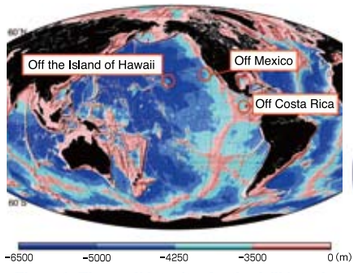



**Figure 1: The candidate sites for the drill into the mantle**

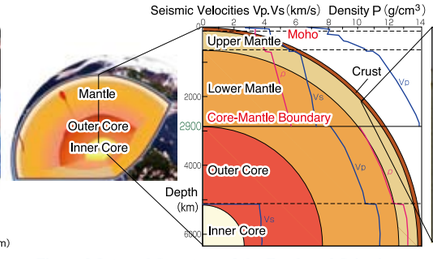



**Figure 2:Internal Structure of the Earth and Seismic Velocities/Density versus Depth**

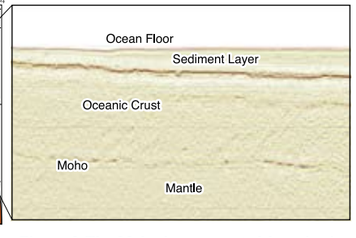



**Figure 3:The Moho image created by seismic structure survey**





Responsibilities & Roles of

# the *CHIKYU* Captain and Offshore Installation Manager (OIM)

Interviewees:
**Captain Yasushi Minoura**
**OIM Seizaburo Higuchi**

Captain Minoura standing on the bridge.

**The Captain is responsible for the expedition and navigation, and the OIM (Offshore Installation Manager) takes command of drilling operations on *CHIKYU* once at the drilling site. For safe and successful research expeditions, the Captain and the OIM, who lead their respective groups, shoulder a heavy responsibility for the on-site continual assessment of the situation.**

Responsibilities and roles are clearly defined aboard *CHIKYU*. Upon arrival at the drill site, the OIM takes charge of drilling operations and the Captain assumes command of other ship-handling matters. Both the Captain and the OIM work a 4-week-on, 4-week-off rotation, with their back-to-backs taking over.

'During drilling mode, we use special equipment called a dynamic positioning system (DPS) to hold the position of the vessel over a fixed point, and proceed to conduct drilling operations in cooperation with the OIM, with a close focus on safety and incident prevention. In the Nankai Trough, where the ocean current of the Kuroshio is very strong, special efforts must be made to hold the vessel in a fixed position while paying close attention to the atmospheric and oceanic conditions,' says one of *CHIKYU's* captains, Yasushi Minoura.

'The fundamental difference between *CHIKYU* and ordinary vessels is that one of the navigation modes of the vessel is to maintain station at a predetermined site, instead of moving. *CHIKYU* is loaded with the latest and highly reliable instrumentation. The vessel is, however, very susceptible to wind. There is also a unique difficulty, which is not present in normal vessels, in that the computer control must be correctly interpreted by human operators to maintain vessel control'.

OIM Higuchi (left) talking with a crew member in the Driller's House.

The Captain says that many crew members and scientists of different nationalities come aboard, so things are very different from those on general commercial vessels. And with the diversity, there is enjoyment in meeting people from different parts of the world, many of which he may not have known of. 'I have had more opportunities to talk with people involved in act ivies other than those maritime and have been inspired by their way of thinking and advanced technologies.'

One of the OIMs, Seizaburo Higuchi , is a veteran of more than 35 years' experience in offshore oil and gas exploration. He is also the first Japanese OIM of *CHIKYU*. 'In the past, all the work done on offshore drilling units was done by human power. So, I had a macho physique when I was young. *CHIKYU* is a 5th generation drilling unit, in which much of the drilling equipment has been automated. As a result, the probability of injury to the crew has been considerably decreased, compared with work that had to be primarily performed by human power. However, the number of personnel required has not changed, because it is the humans who make the final fine-tuning adjustments and the who make the judgments.'

During drilling operations, the most worrisome issue is the changing weather. 'We are dealing with nature, and we can't go against it.Typhoons in particular, are what we worry about. For riser drilling – although it also depends on the sea region and the water depth the vessel is operating in, – judgments must be made 5 days in advance in some cases in order to recover the riser and escape safely. If it is too late to avoid a typhoon, we must endure it'.

OIM Higuchi has another different sense of accomplishment with drilling operations on *CHIKYU* than those involving striking oil and natural gas. He says that he also feels happy when good quality core samples can be obtained. 'During IODP Expedition 322, when a precious sample, which would later be called "Miracle Core", was brought to surface I looked the core over carefully for the first time. The joy of the scientists is also our joy.'

The Captain and the OIM, are making every effort to ensure the safe conduct of expeditions and drilling operations, while hoping to contribute to research results.

# Teaching People about *CHIKYU* helping to Develop New Earth Science!

Scientific drilling carried out by *CHIKYU* is helping bring about a new era of earth science. The Center for Deep Earth Exploration (CDEX) has implemented many initiatives to help people better understand *CHIKYU*'s activities and scientific drilling.

High school students participating in the Summer Vacation program "Sand for Students".

## Special Page, (*CHIKYU* Expeditions), Follow the Activities of *CHIKYU*


The CDEX website established a special page for the Nankai Trough Seismogenic Zone Experiment (NanTroSEIZE), which includes: '*CHIKYU* TV', VIDEOs to convey the purpose of the research expedition and drilling operations, 'Daily Report' to introduce the activities on board during expeditions and also a Twitter feed. Please visit those pages.

Special page for NanTroSEIZE. Research expeditions have VIDEOs, photos and daily reports.
http://www.jamstec.go.jp/chikyu/nantroseize2010/e/index.html

A drill bit, which crewmembers check after use, used to drill through the subseafloor.

## Summer Vacation *CHIKYU* Classes

'Sand for Students (S4S)' field training and classes on scientific ocean drilling classes are held during summer vacation and attended by students from four schools, including the Super Science High School. CDEX implements S4S, led by Japan and the United States, with the participation of 24 countries from all over the world. S4S is an IODP outreach activity with the support of many earth scientists and high school teachers.

Participants examining riverbed stones during field training.

## TV Program Introduces Scientific Drilling Carried Out by *CHIKYU*

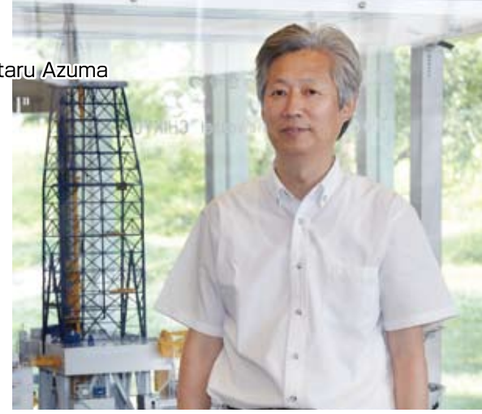
In July 2010, a special TV program in Japan, 'Toyota ECO Special: To the Miraculous Sea!' Great Expeditions of the Earth' introduced *CHIKYU* and deep subseafloor biosphere research.

It has been decided that another TV program will introduce the drilling operations and on-board research of IODP Expedition 331 'Deep Hot Biosphere' to be implemented during September – October 2010. Please stay tuned.





# Importance of Utilizing the Results from Scientific Drilling for Society



**What is aimed at in the IODP Expeditions of the Nankai Trough and the Okinawa Trough implemented in 2010? Wataru Azuma, the Director-General of CDEX, tells us.**

### New Subseafloor Biosphere Research Using *CHIKYU*

As introduced in the Special Topic of this issue, the full-scale research expedition using *CHIKYU* in the subseafloor biosphere is finally implemented in the Okinawa Trough.

The exploration of the subseafloor biosphere, also called the biggest biosphere on the Earth, has become a significant initiative also in the IODP Initial Science Plan and the research results are greatly expected. For us CDEX, which is in charge of the operations at *CHIKYU*, it is important to ensure the implementation of the total management, such as increasing the recovery rates of core samples as well as firmly controlling/storing these samples in order to prevent biological contamination caused by microorganisms in sea water and on land, improving a research environment, such as precise analyses and experiments on board and properly storing core samples at the Kochi Core Center to be provided for later research.

For the subsurface biosphere research, it is similarly important to collect and store the samples obtained by drilling while preserving the environment of the site, i.e. pressure and temperature. During the drilling off the Shimokita Peninsula to be subsequently carried out, a test is planned in order to ensure strengthened 'In-situ Research' where such environmental consideration is given. Another test is also scheduled on 'Mud Gas Lab' where gas components contained in collected drilling mud water are immediately analyzed. I think that it is also an important role for *CHIKYU* to develop a new era of the deep biosphere research by realizing such new technologies and techniques.

At the Reefer, the Kochi Core Center

As for developing new technologies and techniques, the same can be said about the drilling into the mantle introduced on Page 11 of this issue. I' m talking about still some time ahead, yet we have a number of technical problems to overcome, such as realizing deep-sea riser drilling technology for deep water (4500m) and subseafloor at 7000m and developing a logging tool which can be used under high temperature ( ≦ 250℃ or so) and high water pressure. In order to reach the mantle unexplored by humans, we must gather the wisdom through global cooperation to solve those problems.

### Aim to Contribute to Society by Drilling Whose Top Priority is Safety

In the subseafloor biosphere research, it is greatly expected that new biological resources will be found in the subseafloor. We hope that samples obtained by *CHIKYU* will be distributed to scientists as widely as possible so that new resources are not limited to satisfying the curiosity of scientists but such resources may be effectively utilized, in order to contribute to society and help with the Earth's future and the development of mankind.

In terms of contributing to society, great expectations have also been placed on NanTroSEIZE. The experiments of long-term borehole monitoring are conducted this year. Moreover, as early as possible in the future, measuring instruments for monitoring earthquake preparation process will be linked with DONET (Dense Oceanfloor Network System for Earthquakes and Tsunamis) to build three-dimensional, real-time seismograph network in the Kumano-nada region. Observation results obtained by the organic connection between the subseafloor seismograph network installed in DONET and the observation instruments installed within the subseafloor boreholes should contribute to the predictions of mega earthquakes and tsunamis as well as disaster mitigation measures.

Finally, let me talk about ensured safety of scientific drilling, which people, including those involved in fisheries have asked us about. In April 2010, in the Gulf of Mexico off the southern United States, an accident happened in which a lot of crude oil spilled out of an oil-drilling hole. Some people suspect that similar accidents can also happen in scientific drilling. However, in scientific drilling, we take long time of 4-5 years before the drilling, to conduct preliminary research, such as structural surveys of the deep-sea bottom, seafloor topography survey and seafloor soil sampling. The drillings take place by selecting safe sites where gases and high pressure fluids can gush out as little as possible. Also in the drilling operations, securing safety should come first before anything else. Please feel secure and we would appreciate your understanding and cooperation on scientific drilling.

CHIKYU HAKKEN
– EARTH DISCOVERY –
IODP Japan Implementing Organization Report
Volume 10 November, 2010
IODP INTEGRATED OCEAN DRILLING PROGRAM

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C L O S E U P

## Tweets on board the *CHIKYU*

Information on the research expeditions on board the *CHIKYU* is distributed on Twitter. During the expedition of NanTroSEIZE Stage 3, the occurrences on board and the situation inside the *CHIKYU* were reported real time, too.

The lines in the photo indicate the restricted areas on the drill floor, already introduced in the tweet.

Depending on the restriction levels, their colors are different: red, red/white and yellow/black.

Twitter account:
http://twitter.com/Chikyu_JAMSTEC/

CDEX
CENTER FOR DEEP EARTH EXPLORATION
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http://www.jamstec.go.jp/chikyu/