

NanoSIMS ion imaging analyses for biological samples: Applications to seafloor life.

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The CAMECA NanoSIMS 50L ion microprobe represents the *in-situ* microanalysis by secondary ion mass spectrometry, combining unprecedented spatial resolution (minimum spot size of ~50 nm for Cs⁺ or ~150 nm for O⁻) with ultra-high sensitivity. Up to 7 elemental and/or isotopic images can be acquired simultaneously by 7 electron multipliers with sensitivity in the ppm. The capability for maps of multiple elements and isotopes within a sample with permil precision and accuracy and nm scale spatial resolution is unique to the NanoSIMS and provides a new approach to study of the isotope and trace element distributions within the sample, i.e., extraterrestrial, terrestrial and biology samples, including meteorites, Earth rocks and microbial cells in deep and ancient seafloor sediments by the Integrated Ocean Drilling Program (IODP).

In last decade conventional SIMS technique has been used to microbiology to match chemotaxonomic and phylogenetic signature of microbes. Recently NanoSIMS ion imaging introduced to a stable isotope probing study (i.e., ¹³C, ¹⁵N labeling) for a single cell to understand microbial metabolic activities, and metal-probed *in-situ* hybridization for phylogenetic identification.

Subseafloor sediments of the South Pacific Gyre (SPG) obtained by IODP Expedition 329 represent a large proportion of organic-poor, oxidized sediments in the open sea. The sediment is characterized as rich in oxygen but poor in energy sources. In an energy-limited sedimentary environment, a small size of microbial community perseveres functions for life with extraordinary low oxygen-consumption rate. However, the nature of deep sedimentary microbial life in the SPG remains still unknown. In this study, we will investigate metabolic activity of the SPG sedimentary cells with a NanoSIMS ion imaging.

Isotope labeled SPG sedimentary cells (incorporation of substrates after 1.5- years incubation) were analyzed by a raster ion imaging in a NanoSIMS 50L ion microprobe at the JAMSTEC Kochi Institute for Core Sample Research. A focused primary Cs⁺ beam of ~0.8 pA was rastered over 20 x 20 to 28 x 28 micrometer areas on samples. Negative secondary ions of ¹²C, ¹³C, ¹²C¹⁴N, ¹²C¹⁵N and ³²S were measured using 5 electron multipliers in multidetection mode at a high mass resolution of about 9,000 that is sufficient to separate all relevant isobaric interferences (i.e., ¹³C on ¹²C¹H). Each run was started after stabilization of the secondary ion beam intensity following presputtering of approximately 5 to 10 min with strong primary ion beam current. Each image run repeatedly scanned (30 to 40 times) the same area, with individual images consisting of 256 x 256 or 512 x 512 pixels, depending on the region-of-interest, having a dwell time of 2,000 to 3,000 microsecond. We prepared E.coli cells as the standard samples, which have different carbon isotopic ratios of 0, 5, 10, 15 and 20 % enriched in ¹³C (relative to the ¹²C) or ¹⁵N (relative to the ¹⁴N) to evaluate an instrumental mass fractionation for C and N isotopes as well as to find target mass peaks (¹²C, ¹³C, ¹²C¹⁴N, ¹²C¹⁵N and ³²S). This presentation will highlight results to illustrate critical analytical issues affecting precision and accuracy including sample preparation and data processing.

Keywords: NanoSIMS, seafloor microbes, isotope imaging, stable isotope labeling

Development of Gold-ISH for sensitive detection of microbial phylogeny with a NanoSIMS ion microprobe

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As yet most of prokaryotes in subseafloor are not culturable, linking prokaryotic phylogeny and metabolic activities at single cell resolution by culture-independent techniques is one of big concerns to understand biogeochemical nutrient cycles. Isotopic or radioisotopic labeling of microorganisms and subsequent phylogenetic identification by in situ hybridization of rRNA-targeted probes can directly link metabolic activity and phylogeny at single cell resolution. After the recent development of a NanoSIMS ion microprobe with high spatial resolution of ~50 nm, isotope probing studies at single cell resolution are nowadays more popular to understand microbial metabolic activities related to carbon, nitrogen and sulfur metabolisms. Methods for simultaneous isotopic measurements and phylogenetic identification of single microbial cells were reported in 2008 from three different laboratories, and they all used halogen elements due to their high ionization yields and relatively low abundances in biomass. However, halogen-based these techniques still have drawbacks when apply to subseafloor samples, especially with halogen rich samples.

Gold is the one without exception for a SIMS analysis and it shows comparable ionization yield to halogen elements. In addition, gold is also relatively low natural abundance in biomass: it can make lower background signals. Furthermore, gold signals can be enhanced by gold enhancement to achieve high sensitivity. In this study, we focused on undecagold, which is consisted of 11 Au atoms with the diameter of only 0.8 nm. Here we present applicability of undecagold-labeled probes for the identification of single cells by an ion imaging analysis using the JAMSTEC NanoSIMS 50L.

For probe generation, mono-maleimide functionalized undecagold was successfully conjugated with thiol-linked oligonucleotide. After PAGE, more than two bands were observed. The band expected to be undecagolds labeled with single oligonucleotide was cut, purified, and used for in situ hybridization. Oligonucleotide probes were also labeled with Cy3, allowing verification of specific hybridization signals by epifluorescent microscopy before NanoSIMS analysis.

For the proof-of-concept, purely cultivated and ¹³C enriched *E. coli* cells and non-enriched *M. maripaludis* cells were mixed and used for FISH experiment with the EUB338 probe. Specific fluorescent signals were obtained only from *E. coli* cells and the undecagold-derived Au signals detected by nanoSIMS were identical to ¹³C signals, indicating only *E. coli* cells were successfully detected by the undecagold-labeled probe and the method has sufficient sensitivity for NanoSIMS analysis.

Further experiment was conducted using a granular sludge sample. The granular sludge was incubated with ¹³C-labeled lactate and sulfate to label lactate-utilizer under a sulfate-reducing environment. After hybridizing with the *Desulfovibrionales*-targeting SRB385 probe, specific FISH signals were obtained from rod-shaped cells. Undecagold-derived Au signals were identical to ¹³C signals by NanoSIMS analysis, indicating *Desulfovibrionales* is the main lactate-utilizer in the environment. The signals obtained from undecagold-labeled probes had high signal-to-noise ratio (approximately 10), enabling clear discrimination from background signals. These results indicated that undecagold is stable under the parameters used in this study and can be used for in situ hybridization study with NanoSIMS for ecological understanding in microbial ecology. Gold-ISH may open the door to decipher biogeochemical processes by linking uncultured microbial metabolisms with microbial phylogeny in complex microbial communities.

Keywords: Gold-ISH, NanoSIMS ion microprobe, Undecagold

Detection of coenzyme F430 in deep sea sediments: A key molecule for biological methanogenesis

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We report the presence of coenzyme factor 430 (F430), a prosthetic group of methyl coenzyme M reductase for archaeal methanogenesis, in the deep sub-seafloor biosphere. At 106.7 m depth in sediment collected off Shimokita Peninsula, northwestern Pacific, its concentration was estimated to be at least 40 fmol g sediment⁻¹ (i.e. 36 pg g⁻¹ wet sediment). This is about three orders of magnitude lower than typical concentrations of archaeal intact polar lipids in similar sub-seafloor sediments. On the basis of the concentration of F430 in methanogens and conversion to biomass composed of typical sub-seafloor microbial cells, we estimated that ca. 2×10^6 cells g⁻¹ could be methanogens in the deeply buried marine sediment.

To our knowledge, this is the first study reporting F430 in a methanogenic environment of deep sub-seafloor biosphere. Further studies on the spatial and vertical distributions of F430 in the sedimentary column could potentially provide crucial information on sub-seafloor biological methanogenic processes.

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Reference:

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Genetic Signatures from the Aging Oceanic Crust: Evidence for Ancient Subvent Biosphere?

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Several lines of evidence strongly suggest that microbial communities exist within young ocean crust on the flank of mid-ocean ridge systems where fluid circulation is vigorous. Aging of ocean crust is accompanied with significant decreases in porosity due to secondary mineral formation and oxidative alteration within the first 10-15 million years. After thermally driven fluid circulation ceases at ~65 Ma, basalt weathering appears to be a sole energy source in the aging ocean crust, the habitability of which remains to be largely unknown. Basaltic cores obtained during Expedition 329 (U1365: >100 Ma; U1367: ~33.5 Ma; U1368: ~13.5 Ma) provide a systematic opportunity to determine age variations in habitability and microbial community within the basalt basement thinly covered with oxic and organic-poor sediment. In this study, flame sterilization of the core exterior (Lever et al., 2006) and a newly developed technique for DNA extraction (Kouduka et al. 2012) were applied to minimize contamination and to maximize DNA recovery from low biomass habitat, respectively.

Although the numbers of microbial cells were below a minimum detection limit of ~10⁵ cells/cm³, 16S rRNA gene sequences were successfully obtained from all core samples associated with fracture-filling assemblages of oxidized or reduced secondary minerals. Contamination sources including drilling mud and surface and bottom seawater were thoroughly inspected, and it was found microbial communities in the basalt cores were clearly distinct from those from contaminant ones (<97% similarity). Phylum- or class-level distributions of microbial communities also suggested that neither formation age, depth nor chemical alteration is clearly correlated with the community structure. Many phylotypes belonging to Deltaproteobacteria and Fimicutes were closely related to strictly anaerobic metal and/or sulfate reducers (>95% similarity), despite the oxygenated bottom sediment. As overall community structures resemble previously reported ones from seafloor and subseafloor basalt with young age (< 1Ma) (Lysnes et al., 2004; Santelli et al., 2008) and low-temperature vent fluids (Huber et al., 2009), there is the possibility that genetic material originated from the subvent biosphere has been preserved owing to extremely low habitability and/or irreversible binding of DNA to rock matrix in the aged oceanic crust.

Radon measurement during IODP Exp.337

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D/V Chikyu is capable of riser-drilling using circulation mud. The circulation mud repeatedly comes back on the deck of D/V Chikyu from the deep bottom of the borehole. Thus, it is regarded to be a carrier of formation gases and other information, which could be utilized for real-time monitoring on formation gases and fluids. On D/V Chikyu, a degasser is placed on its mud circulation line, we could retrieve the dissolved gases and supply into various monitoring and sampling apparatus. As such monitoring apparatus, Radon (Rn) monitor is also available as a third party tool connecting to one of the auxiliary ports of scientific gas line on D/V Chikyu through fine-mesh dust filter.

Radon (Rn) is an inert radioactive gas of the daughter nuclei of Uranium and Thorium with rather short half-lives. Among Rn isotopes we focus ²²²Rn having longest half-life of 3.82dy. Although ²²⁰Rn shows second longest half-life of 55.3s, most of ²²⁰Rn decayed during transportation to the surface. Due to such short half lives and volatility, Rn has widely been monitored to detect micro-cracking prior to the seismic activities (e.g. Igarashi et al., 1995). The concentration of Rn dissolved into the circulation mud is anticipated to reflect the lithological units of the formation; its parental elements, Uranium and Thorium, are generally rich in the terrigenous sediments and felsic rocks. In addition, some coal bed contain abundant Uranium up to 200 ppm (Takeda, 1981), which may enables sensitive detection of coal bed or lithological change during the drilling with continuous profile.

The measurement was performed by an AlphaGUARD PQ2000 Pro apparatus (Saphymo GmbH, Germany) based on an ionization chamber method. The extracted gases dissolving in circulation mud certainly contained formation gases from deep borehole although careful calibrations are required. The newly obtained time sequential Rn data will be presented. The parameter of the measurement are as follows; effective chamber volume is 650mL with 10 min integration, the flow-rate was controlled as 65ml/min.

References:

Igarashi G. et al. (1995) *Science*, 269, 60-70.

Takeda E. (1981) *Bull. Geol. Surv. Japan*, 31(11), 583-682 (in Japanese).

Keywords: IODP Exp.337, Gas monitoring, Radon measurement

Thermal properties of the sedimentary rocks at Site C0020, IODP Expedition 337 in Sanriku-oki basin

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Thermal properties of thick sedimentary basins are important parameters to evaluate the thermal structure at depth and the maturation process for organic materials. Therefore, thermal conductivity measurement is listed as routine measurements for IODP program on board. However, to understand non-steady state processes for heat flow and diffusion, thermal diffusivity and specific heat are necessary to be evaluated as well. Therefore, in this study, a series of thermal property measurements were conducted on the sediment cores at site C0020 from IODP expedition 337 in Sanriku-oki basin. Thermal conductivity and thermal diffusivity were measured by Hot Disk method (Hot Disk AB Ltd., TPS 1500). Hot disk method (or the transient plane source technique) enables us to measure both thermal conductivity and thermal diffusivity at the same time within few minutes. We used half round core samples with 4 cm length in all measurements. The flat sensor was placed between the surfaces of a half round core sample and a heat insulating material (expanded polyethylene, thermal conductivity = 0.034W/mK) during measurements. Before measurements, half round core samples were saturated by NaCl solutions with 35 per mil, and we loaded 4.9 N during measurement at room temperature.

Thermal conductivity of sample ranged from 0.4 to 2.9 W/mK. Thermal conductivity of sandstone and siltstone increased with depth from 1.4 (1,278 mbsf) to 1.9 W/mK (2,466 mbsf). Lignite showed very low thermal conductivity and the largest thermal conductivity values were observed in carbonate cemented sedimentary rocks. A half-space line source method using TK04 (TeKa Ltd.) was applied to measure the half round core samples on D/V CHIKYU during IODP expedition 337, and the thermal conductivity and the lithological variation of the thermal conductivity measured on board were similar with our data. Thermal diffusivity was decreased with depth from 0.5 mm²/s to 0.9 mm²/s as well. Thermal conductivity was scattered at depth from 1,900 to 2,000 mbsf, and this scattering reflects the lithological variation of core samples. Thermal diffusivity of lignite (or coal) was 0.16 mm²/s, and the largest value of 1.9 mm²/s was observed on unconsolidated coarse sand. The increase in the thermal conductivity and thermal diffusivity is consistent with reduction in porosity with depth. On the other hand, thermal conductivities of lignite and cemented rocks will depend more on the pore structure and chemical compositions. Thermal diffusivities for most samples are exponentially increased with increasing thermal conductivity, though, the thermal conductivity of unconsolidated coarse sand is deviated from the exponential curve. We measured thermal properties under atmospheric condition; therefore, our data might underestimate in-situ values. Therefore, to investigate the relationship between thermal property, porosity, and pore structure at depth, we can estimate the in-situ thermal property indirectly.

Keywords: IODP expedition 337, thermal conductivity, thermal diffusivity, Sanriku-oki basin

Compaction and dewatering process in New Jersey Shallow Shelf inferred from IODP Exp.313 core samples

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IODP Exp.313 was conducted on the New Jersey shallow shelf in May to July 2009. The L/B Kayd chartered for a mission-specific platform recovered 612 cores at three sites. This study focuses on compaction and dewatering processes associated with the architecture of the sedimentary sequences under frequent sea-level changes (based on core-log integration). Particularly, a high sedimentation rate often induces underconsolidation of sediments with overpressured pore fluids which complicates consolidation behavior. Understanding the compaction process and its effect on architecture of the sedimentary sequences reveals probable geometry of sedimentary structure before deformation, and provides important information to estimate frequency and amplitude of eustatic changes. In this study, we aim to discuss initial in situ diagenesis and dewatering processes based on physical properties measured or estimated from down-hole logging, the Multi Sensor Core Logger System (MSCL), and discrete core samples. Correlation of the three Expedition 313 sites and the seismic profile will provide better estimation of the spatial distribution of dewatering paths.

Onboard and offshore MSCL measurements provided physical properties of whole-round cores including porosity, density, electric resistivity, P-wave velocity, magnetic susceptibility and natural gamma ray. Bulk density derived from individual core samples show good correlation with gamma-ray density from MSCL, and the other physical properties from discrete core samples also correspond to WL-logging and core logging data. Porosity-depth curve measured on MSCL exhibits slightly lower porosity than discrete samples in the glauconitic-sand interval which shows relatively higher density. In M0027A, comprehensive trend of porosity-depth curve in each lithology parallels standard curve in North Sea (e.g. Sondergeld et al., 2005). Therefore these data indicates normal compaction process in the study area. On the other hand, on-board geochemical analysis indicates distribution of abnormal fresh water at M0027A (Mountain et al., 2010). Though estimated pore pressure using density profiles do not show confining layers, porosity fluctuations measured by MSCL and discrete samples suggest that those water lenses are possibly sealed by impermeable layers. This presentation will discuss estimated pore pressure and permeability from discrete core samples.

This research used data provided by the Integrated Ocean Drilling Program and is supported by Japan Drilling Earth Science Consortium (J-DESC).

Lithology and paleoenvironments at drilled Site C0020 off the Shimokita Peninsula, IODP Exp. 337

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Marine subsurface hydrocarbon reservoirs and the associated microbial life in continental margin sediments are among the least characterized Earth systems that can be accessed by scientific ocean drilling. We penetrated a 2,466 m-deep sediment sequence with a series of coal layers around 2 km below the seafloor. Here, we report the lithology of sediments and paleoenvironments from drilling Site C0020, IODP Expedition 337. Based on macroscopic and microscopic description of cutting and core samples during this expedition, which was supported by XRD and XRF data as well as all available data (e.g. logging, micropaleontology and physical properties data) we defined four different lithologic units present in Site C0020. The succession of lithofacies at Hole C0020A also provides insight into the evolution of depositional environments in the site region.

Keywords: IODP Exp.337, Marine subsurface hydrocarbon reservoirs, microbial life, depositional environments

X-ray CT images of drilled cores and CT value data from IODP Expedition 337; Deep coalbed biosphere off Shimokita

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X-ray computed tomography (CT) on board 'Chikyu' is a revolutionary analysis suited to scientific ocean drilling. It is a quick and nondestructive method to produce geological image of cores and to quantify the porosity and permeability in sediment and rock of cores using CT value.

Here, we will introduce some examples of CT image of cores and CT value data from IODP Expedition 337; Deep coalbed biosphere off Shimokita, Japan, northwestern Pacific Ocean.

Keywords: X-ray CT, Ocean drilling, IODP Expedition 337

Mud logging: its importance and potential for IODP future riser expeditions

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New IODP structure will start from October 2013 and D/V Chikyu operated by CDEX/JAMSTEC will continue to act important role as a riser drilling vessel for deeper scientific drilling (to ~6500+ mbsf) in IODP.

Mud logging is a conventional technique in petroleum industry to investigate well site geology, to search for oil/gas reservoirs and to carry out safety control. Three IODP riser expeditions were carried out with the Chikyu in 2009 and 2012, and mud logging operations were also included in the expeditions. Meanwhile mud logging as a new scientific measurement technique has been attempted on the Chikyu, continuous coring has also been a basic approach in shallower riserless scientific drilling (~2000 mbsf) by not only D/V JOIDES Resolution (JR) but also the Chikyu in IODP. However, since continuous coring generally takes great amount of operation time with high risks of hole instability; the deeper a hole becomes, the more it will be difficult to accomplish. Therefore, mud logging has been recognized as one of important scientific measurement techniques to investigate lithological, petrophysical and geochemical features of deep crust and upper mantle.

The Chikyu conducted two riser operations in JFY 2012 (Exps 337 and 338). Mud logging in these expeditions was carried out both for operational safety control (operation mud logging) and scientific cuttings/mud gas monitoring. Operation mud logging is mainly composed of lithological description of cuttings and real-time mud gas monitoring. Cuttings for lithological description are ordinarily sampled at every 5 m in depth. In mud gas monitoring, concentrations of hydrocarbons (C1 to C5), CO₂ and H₂S gases are monitored in real-time.

In this presentation, results of operation mud logging in Exps 337 and 338 will be shown. In addition, potential of mud logging as a method of scientific measurement and issues for future expeditions will be discussed.

Keywords: Scientific drilling, IODP, Riser drilling, Mud logging, Core, Real-time gas monitoring

Diatom and nannofossil biostratigraphy of DSDP Holes 366 and 369A, offshore north-west African margin

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We re-investigated the standard coccolith and diatom biostratigraphy zonations from Deep Sea Drilling Project (DSDP) Holes 366 and 369A in the eastern equatorial Atlantic Ocean in order to determine the relative ages for the low-latitude diatom zonation. Applying the ages of nannofossil zonations to diatom ones, the ages of several diatom bioevents (first common occurrence, FCO and first occurrence, FO) which determined biozones of DSDP Holes 366 and 369A are evaluated as followed:

- FCO of *Baxteriopsis brunii* (ca. 38.4 Ma)
- FO of *Coscinodiscus excavatus* (ca. 33.4 Ma)
- FO of *Cestodiscus reticulatus* (ca. 32.8 Ma)
- FO of *Rocella vigilans* (ca. 30.5 Ma)
- FO of *Rossiella symmetrica* (ca. 29.5 Ma)
- FO of *Bogorovia veniamini* (ca. 25.2 Ma).

These ages may applicable for not only these holes but also others and more biostratigraphical studies in other holes which contain Paleogene diatoms are needed in order to compare with each other.

Keywords: diatoms, nannofossils, biostratigraphy, Paleogene, DSDP 366, DSDP 369A

Refined diatom biostratigraphy of the IODP Expedition 320/321 Sites U1334 and U1338

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Two hundred sixty slides from IODP Expedition 320/321 Holes U1334A, U1338A and U1338B were investigated to define the diatom biostratigraphic datums and acquire the paleoceanographic changes in the eastern equatorial Pacific Ocean from the middle Eocene to middle Pleistocene. As a result, the datums of twenty-four diatom zones were defined as follows:

Fragilariopsis doliolus (NTD 17; 0-0.69 Ma), *Nitzschia reinholdii* (NTD 16; 0.69-1.05 Ma),
Rhizosolenia praebergonii (NTD 15; 1.05-3.06 Ma), *Nitzschia jouseae* (NTD 14; 3.06-4.57 Ma),
Thalassiosira convexa (NTD 13; 4.57-5.40 Ma), *Nitzschia miocenica* (NTD 12; 5.40-7.36 Ma),
Nitzschia porteri (NTD 11; 7.36-8.23 Ma), *Thalassiosira yabei* (NTD 10; 8.23-8.88 Ma),
Actinocyclus moronensis (NTD 9; 8.88-10.18 Ma), *Craspedodiscus coscinodiscus* (NTD 8; 10.18-11.09 Ma),
Coscinodiscus gigas var. *diorama* (NTD 7; 11.09-11.70 Ma), *Araniscus lewisianus* (NTD 6; 11.70-12.41 Ma),
Cestodiscus peplum (NTD 5; 12.41-14.36 Ma), *Crucidenticula nicobarica* (NTD 4; 14.36-15.58? Ma),
Triceratium pileus (NTD 3; 15.58?-20.00 Ma), *Craspedodiscus elegans* (NTD 2; 20.00-22.18 Ma),
Rossiella fennerae (22.18-23.37 Ma), *Rocella gelida* (23.37-25.96 Ma),
Bogorovia veniamini (25.96-27.56 Ma), *Rocella vigilans* (27.56-29.98 Ma),
Cestodiscus trochus (29.98-30.95 Ma), *Coscinodiscus excavatus* (30.95-33.71 Ma),
Baxteriopsis brunii (33.71- ? Ma), *Asterolampra marylandica* (?- ? Ma) Zones.

Keywords: IODP Expedition 320/321, diatoms, biostratigraphy, Site U1334, Site U1338

The Neogene records of the fossil diatoms from the core ODP Leg 145 Site 887, North-eastern Pacific

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Gulf of Alaska located in high latitude region of Subpolar Gyre in the northeastern Pacific. In this region, Alaskan Gyre is formed in eastern part of Subpolar Gyre as the subarctic current goes north and flows as Alaskan stream. Additionally, ice sheet expansion in Pleistocene contributed the drop of the global mean surface temperature with the large-scale cooling in the Northern Hemisphere (Broccoli, 2000). However, detail process of the cooling from the middle Miocene to present has been under discussion.

Moreover, the North Pacific Diatom (NPD) Zones established by Yanagisawa & Akiba (1998) have been used to define the geological/sedimentary ages in this region. However, there have been little discussions on the age gaps of diatom bioevents, including the first/last appearances of each taxon which define the NPD zones, between northwestern and northeastern Pacific because of little analyses using the same core to compare the diatom biostratigraphy with paleomagnetism around these regions.

There is two previous works for establish of northeastern Pacific diatom biostratigraphy with paleomagnetism data acquired from single core such as Barron & Gladenkov (1995) and Watanabe & Yanagisawa (2005). The former showed important result for the establishment of the Neogene diatom biostratigraphy in northeastern Pacific using with several Ocean Drilling Program (ODP) cores including Site 887, however the sampling intervals were lower to decide the detail ages of diatom bioevents because they mostly used only core catcher samples. Moreover, the ages of each diatom event used diatom biostratigraphy have been settled based on the magnetostratigraphic ages, but the paleomagnetic time scale has renewed by Gradstein et al. (2012). Therefore, each paleomagnetic event corresponding to biostratigraphy must be needed to recalculate. On the other hand, Watanabe & Akiba (2005) achieved higher-resolution analysis, but they focused on short interval from the early to middle Miocene.

Accordingly, to establish the detail northwestern diatom biostratigraphy with recalculate ages with Gradstein et al. (2012), this study used the core samples of ODP Leg 145 Site 887 drilled in Patton-Murray Seamounts, southern Gulf of Alaska, as well as Integrated Ocean Drilling Program (IODP) Expedition 341 planned to drill on May to July, 2013 in northern part of Gulf of Alaska. The objectives of this study are 1) to establish high-resolution diatom biostratigraphy from the Miocene to Present in Northeastern Pacific with comparing to paleomagnetism of Gradstein et al. (2012) and 2) to reveal paleoenvironmental changes in this region.

Reference: Barron, J.A. & Gladenkov, A.Y. 1995. Proc. ODP. Sci. Rslt , 145 , 3-19; Broccoli, A.J. 2000. Journal of Climate , 13 , 951-976; Gradstein, F.M., et al. 2012. A Geologic Time Scale 2012. Cambridge (Cambridge Univ. Press); Yanagisawa, Y. & Akiba, F. 1998. Jour. Geol. Soc. Japan. 104(6). 395-414; Watanabe, M. & Yanagisawa, Y. 2005. The Island Arc , 14, 91-101.

Keywords: Gulf of Alaska, Northeastern Pacific, IODP, diatoms, biostratigraphy, paleoenvironment

Refined diatom biostratigraphy and paleoceanography in the ODP Leg 145 Hole 884B

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In this study, fossil diatom assemblages from the Ocean Drilling Program (ODP) Leg 145 Hole 884B were investigated to refine the diatom biostratigraphy in the northwestern Pacific Ocean. As the results, twenty-one diatom zones with the Neogene North Pacific Diatom zone codes (NPD) were defined and their high resolution datum ages (~10 kyr on average) were recalculated based on the geomagnetic polarity time scale in Hilgen et al. (2012) from the early Miocene to the Pleistocene. The refined diatom biostratigraphy is intended for practical use to determine the precise datums and correlation with other regions in the north Pacific. The analysis of paleoenvironmental indicators for an interval of 0-5 Ma was also carried out in this study. The cold-water indicators, which showed high abundances throughout the interval, suggest the cold environmental conditions throughout from the Pliocene to the Pleistocene. Relatively higher abundance of temperate-water species at ca. 2.8 Ma, appearance of sea-ice related species at ca. 2.7 Ma and a slight increase of neritic species observed at ca. 1.9 Ma may reflect a series of Northern Hemisphere Glaciation (NHG) events. The peak of cold-water species, like *Neodenticula seminae*, at ca. 0.9 Ma might be affected by the global cooling of the Middle Pleistocene Transition (MPT), which is characterized by a severe increase of glaciations which started at 1.25 Ma and completed at 0.7 Ma. The first occurrence of *N. seminae* (ca. 2.6 Ma) and the last ones of *N. kamtschatica* (ca. 2.6 Ma) and *N. koizumii* (ca. 0.9 Ma) also seem to correspond to the NHG or MPT events.

Keywords: diatom, biostratigraphy, North Pacific, Neogene, paleoceanography

Exp 906: The Kumano Mud-Volcano Drilling II and Hybrid Pressure Coring System

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D/V Chikyu implemented pressure coring by using the newly developed Hybrid Pressure Coring System (Hybrid PCS) for the first time in Expedition 906 in June 25-28, 2012. The expedition was carried out at the Kumano Mud Volcano #5 (1900 m water depth), and penetrated to 203 mbsf by riserless drilling. Pressure coring of 3 m advance was attempted five times at selected horizons. Pressure core was analyzed onboard by using Pressure Core Analysis and Transfer System (PCATS) of Geotek Limited. In addition normal HPCS/ESCS coring was also carried out by turns.

Drilling Site: Mud volcano, typically formed at convergent margin, works as a natural pipeline that conveys material from deep source to the surface. In the Nankai Trough area, tectonic activity of subducting plate created mud volcanoes, among which Kumano Mud Volcano #5 is one of the most active site in terms of fluid and gas seepage. Surface cores to 20 mbsf were collected in previous Chikyu Exp 903 in 2009.

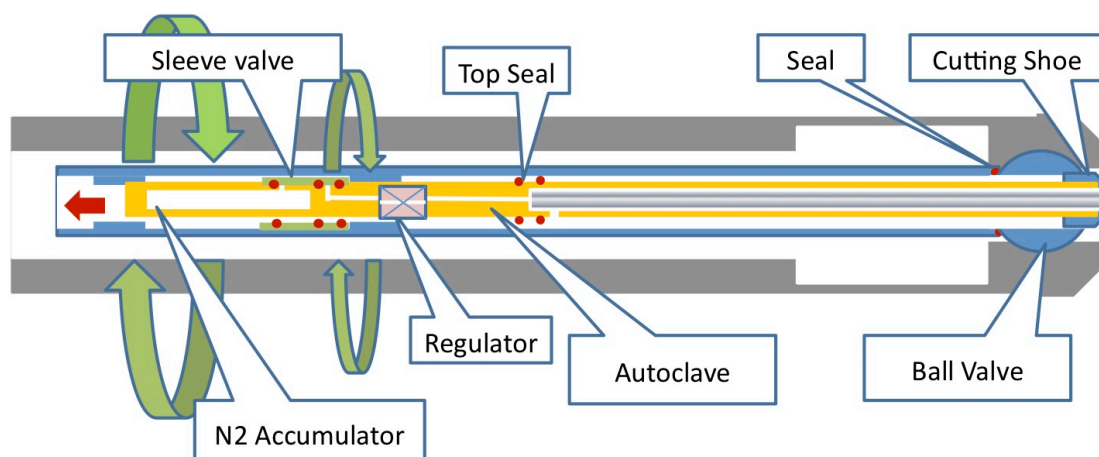
Scientific Purpose: Pressurized core samples will provide methane hydrates and in-situ volatile gas components such as H₂ and CO. With accurate evaluation of geochemical, geophysical and microbiological characteristics of deep material in mud volcano, we can infer that the mud-volcano in the Nankai Trough forearc basin is the window to the deep seismogenic zone, in which geochemical and biological characteristics and behaviors may be highly sensitive to the seismogenic fault activity.

Hybrid PCS: Hybrid PCS was designed to capture a pressurized core sample with 51 mm in diameter, 3 m in length, and up to 5,000 psi in pressure. The system is compatible with Chikyu's ESCS wireline coring system, which runs through 5 and 5-1/2 inch drill pipes. The core liner closure mechanism includes three valves; lower ball valve, top seal for mud return port, and pressure control valve connected to the accumulator. These valves work by running / retrieving core wire line.

PCATS: PCATS provides non-destructive analysis of X-ray CT, P-wave velocity and gamma density of core samples in pressure chamber. Sampling and analysis of gas derived through controlled depressurization process can also be carried out. Core samples are transferred to storage chamber for transportation for further analysis in an onshore laboratory.

In Exp 906, core recovery was limited due to sticky formation in mud volcano, but 0.9 m of pressurized core material was recovered at the last attempt. This presentation, an introduction to the newly developed Hybrid PCS and a summary of Exp 906 will be presented.

Keywords: Pressure coring, Chikyu, Mud volcano, Hybrid PCS, PCATS



Sea-level changes around the Last Glacial Maximum based on large benthic foraminiferal assemblages: IODP Exp.325

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The shelf edge of the Great Barrier Reef was cored during the Integrated Ocean Drilling Program (IODP) Expedition 325 Great Barrier Reef Environmental Changes. Lower parts of cores drilled on deeper shelf slopes, consisting mainly of unconsolidated carbonate sediments, may record sea-level changes around the Last Glacial Maximum (LGM). The purpose of this study was to reconstruct sea-level changes around the LGM, based on large benthic foraminiferal (LBF) assemblages.

Grain-size and foraminiferal analyses were conducted for 64 unconsolidated sediment samples from the lower parts of cores drilled at HoleM0040 and M0041 on the HYD_02A transect. Paleo-water depths were estimated by comparisons of fossil LBF assemblages with modern LBF assemblages. LBF assemblages in these two cores were dominated by *Operculina* sp. and *Amphistegina* spp. Relative sea-level changes based on the paleodepth estimations were generally consistent with reported sea-level changes around the LGM.

Geology of the subduction boundaries and suggestion for the future work - How to avoid ultra-mega-earthquakes -

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The Philippine Sea plate is surrounded by several subduction boundaries, which are subducted and subducting boundaries in the southern & eastern margins and northern & western margins, respectively. I have been working to establish the geological cross section of the Southern Mariana trench inner wall area, and mentioned that mantle peridotites exposed along the trench inner wall. I hope that those basic data from the southern end can contribute to consider the physical plate model in the northern end of the plate at Nankai trough. If subducting slave contacts with the mantle peridotite of the subducted slave like in the Southern Mariana, slippery serpentine mud layer can be deposited easily under hydrous environment at subduction boundaries. On the other hand, those geological evidences give us some ideas about how to avoid ultra-mega-earthquakes in the Japanese Islands. Injection of serpentine mud into the asperity zone may be a possible answer.

Keywords: Philippine Sea plate, subduction boundary, ultra-mega-earthquake, serpentine mud, scientific ocean drilling, mantle peridotite

Partial melting and assimilation at the basal part of sheeted dike complex in Hole 1256D, ultra-fast spread oceanic crust

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Hole 1256D is located on 15 Ma oceanic crust formed at the superfast spreading East Pacific Rise (220 mm/yr full spreading rate). This hole is the first successful drilling that penetrated the entire upper oceanic crust from 250 m thick sediments, 811 m thick extrusives (including the Transition Zone) and a thin (346 m) sheeted dike complex and drilled 105 m into the upper gabbro (Teagle et al., 2006) and important as the reference of oceanic crust from fast spreading ridges. The gabbro appears as two distinct units between the metamorphosed dike complexes. These were defined as gabbro 1 (52 m thick) and gabbro 2 (12 m thick) from above (Teagle et al., 2006). The sheeted dike complex just above the gabbro 1 is highly metamorphosed and called as granoblastic dikes. The Similar metamorphosed dike complex appears between the gabbro 1 and 2, and also the deepest part of the hole below the gabbro 2, and is called as dike screen 1 and 2, respectively.

Exp. 335 was operated as the fourth expedition at the hole 1256D. This expedition drilled 14.5 m of metamorphosed dike complex. Because of numerous fractures and very hard lithofacies, recovery was very low. Instead, a large amount of rock samples were collected from junk baskets. Some of the samples from the junk baskets were larger than core samples and showed the lithofacies that have not reported before at this hole.

The samples collected from Exp. 335 are mainly composed granoblastic dikes that are characterized by granular clinopyroxene and orthopyroxene with minor dioritic to tonalitic veins and patches. These granoblastic dikes correspond to the lithofacies of the dike screen 2 in the hole 1256D. We report petrological and geochemical features of granoblastic dikes from Exp. 335. On the basis of petrographic and petrological facts, we present evidence suggesting partial melting in the deepest part of the granoblastic dikes, which may be responsible to the generation of silicic melts.

Keywords: oceanic crust, magma, partial melting, IODP, superfast spreading ridge, Hole 1256D

Emplacement and solidification of a large off-ridge lava flow from ODP-Hole 1256C, Guatemala Basin

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Site 1256 is located at 91deg56.1' W in the 3650-m deep Guatemala Basin on Cocos plate formed at ~15 Ma on the eastern flank of the East Pacific Rise. A large off-ridge lava flow 75-100 m in thickness and ca. 10 cubic kilometers in volume drilled at site 1256 provides an unprecedented opportunity to understand the internal structures and solidification and emplacement processes of large lava flows. Hole C penetrated a 35-m thick lava from 280 mbsf to 315 mbsf. We present mineral compositions and grain size analyses of the core samples from Hole 1256C, and discuss the crystallization and emplacement processes of the large off-ridge lava flow. Phenocrysts are olivine, clinopyroxene and plagioclase, and the groundmass minerals are clinopyroxene, plagioclase and magnetite with mesostatic quartz and biotite. The core samples show variolitic, subophitic and poikilitic textures and mesostatic intergrowth of plagioclase and quartz. The lava flow consists mostly of NMORB with high-K²O EMORB intervened at a depth interval of 290-300 mbsf (Wilson et al.,2003). High-K²O EMORB has high-Mg# (60~70) clinopyroxenes and is interpreted to have intruded into the solidifying lava body at the final stage of the lava emplacement. Given the interval of EMORB was intruded after the upper and lower lavas solidified, olivine phenocryst size and number density distributions prior to the intrusion of EMORB are similar to those formed by crystal precipitation by Rowland and Walker (1988). Mapping analyses of augite and pigeonite demonstrated domains different in Al²O₃, CaO and MgO concentrations within a single grain. Three types of augite (Augite-N1, Augite-N2 and Augite-N3) and two types of pigeonite (Pigeonite-N1 and Pigeonite-N2) are identified for the domains in pyroxene crystals from NMORB lava intervals. The EMORB lava interval has three type augite (Augite-E1, Augite-E2 and Augite-E3) and two types of pigeonite domains. Two pairs of Pigeonite-N1 & Augite-N3 and Pigeonite-E1 & Augite-E1 are in equilibrium with each other in terms of Fe-Mg partitioning. The crystallization order is Augite-N3 & Pigeonite-N1, Pigeonite-N2, Augite-N1, Augite-N2(rim), followed by the intrusion of EMORB, and then Augite-E1 and Pigeonite-E1, Pigeonite-E2, Augite-E2 and Augite-E3 (rim).

Keywords: Ocean Drilling, Hole 1256C, Off-ridge volcano, Mid-ocean ridge, Large lava flow, Oceanic crust

Southern Ocean drilling proposal: Outline and future plan of Antarctic Cryosphere evolution project (AnCEP)

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The Southern Ocean has played a very important role in the global climate system through geologic history to the present day. To understand the processes that change atmospheric CO₂ concentrations, it is important to understand the sub-systems and processes of the Antarctic cryosphere, such as changes to the Antarctic Circumpolar Current (ACC), the Weddell Gyre, the biological pump, sea surface temperature, the polar front location, the distribution of sea-ice, and the Antarctic ice sheet on modern and geologic time scales. The evolution of the Antarctic cryosphere for major climate shifts during the Cenozoic is still poorly understood. The key motivation for the IODP scientific drilling in the Southern Ocean stems from a lack of knowledge of the complex role the Antarctic cryosphere plays in the global climate system and water circulation. Understanding the history of variations of ice volume and associated cryospheric changes during the Cenozoic is of great importance because changes of ice volume and distribution change global sea levels, affect albedo, control the latitudinal temperature gradient of the Southern Hemisphere and thus heat transport via atmospheric and oceanic circulation, and influence the distribution of ice shelves and seasonal sea ice, which are commonly considered to cause the cold bottom waters that drive global ocean circulation.

We will propose the drill sites along a north to south transect in the Indian sector of the Southern Ocean. The proposed sites are on sediment drift deposits on the Conrad Rise, and on the Del Cano Rise. The arrangement of those sites is crossing the oceanic fronts, the subtropical front, subantarctic front, Antarctic polar front, and winter sea ice limit. These sites contain continuous sedimentary sequences exhibiting moderate to high sedimentation rates. Age control will be provided by oxygen isotope stratigraphy, relative geomagnetic paleointensity with conventional geomagnetic polarity stratigraphy, and high-resolution biostratigraphy. The data from this drilling will greatly advance our understanding of the relationship between Antarctic climate change and Southern Ocean paleoceanographic variability.

The proposed sites are strategically located to reveal the evolution of the Antarctic cryosphere and to investigate the role of the ACC and Weddell Gyre system in that evolution. The Indian sector of the Southern Ocean is a most suitable high-latitude ocean to elucidate the evolution of the Antarctic cryosphere. Our main objectives are:

1. Evolution of the ACC and Weddell Gyre system: To test the hypothesis that considerable expansion of the ACC and Weddell Gyre system caused global cooling and a large decrease in the thickness of the EAIS during the mid-Pleistocene transition (MPT).
2. Variability of the Antarctic climate and sea-ice distribution during the Quaternary: To reconstruct high-resolution records of ACC migration, sea ice coverage, surface-water stratification, the biological pump, acidification of ocean waters, and eolian dust inputs in the Southern Ocean.

Keywords: Southern Ocean, Antarctic Circumpolar Current, Antarctic ice sheet, IODP, sea ice, paleoceanography

Neogene history of Mediterranean hydrology based on sedimentary osmium isotopic records

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The Mediterranean Sea has experienced an extreme event called Messinian Salinity Crisis (MSC) that represents a formation of gigantic evaporite deposits in deep basins. In this study we report an osmium (Os) isotopic record of marine sediment cores from four deep-sea drilling (DSDP) sites in the Mediterranean; DSDP Site 372 in the western Mediterranean, DSDP Site 374 in the central Mediterranean, and DSDP Sites 375 and 376 in the eastern Mediterranean. The Os isotopic ratios of the pre-MSC sediments (Burdigalian to Serravallian) in the western Mediterranean are identical to that of the coeval global seawater. In contrast, the pre-MSC sediments (Langhian to early Messinian) in the eastern Mediterranean have significantly low $^{187}\text{Os}/^{188}\text{Os}$ values than those of the global Middle-Late Miocene seawater. Our data suggest that Os in the eastern Mediterranean was not fully mixed with that of other seas such as western Mediterranean and North Atlantic, and that the basin isolation has already started before the MSC, probably as early as Middle Miocene. The unradiogenic Os would have been supplied to the eastern Mediterranean by selective weathering of ultramafic rocks in the surrounding ophiolite bodies, which contains high amount of non-radiogenic Os. The isotopic compositions of Os in gypsum samples from all sites are significantly lower than the end-Miocene ocean water values, suggesting isolation of all Messinian basins. Sediments from the Pliocene show Os isotopic ratios more radiogenic, and close to the global seawater values of the Pliocene, indicating that Os started mixing with global seawater again.

Keywords: osmium isotopes, Messinian, Mediterranean, evaporite