Workshop on

Subseafloor Ocean Biosphere and Borehole Observatory Science (SOBBOS)

Research Coordination Network: Dark Energy Biosphere Institute

Kona Coast, Hawaii
October 18-21, 2009

Steering Committee

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Executive Summary

An international workshop on the "**Subseafloor Ocean Biosphere and Borehole Observatory Science"** was held at the Fairmount Orchid Resort on the Kona Coast of Hawaii, over October 19-21, 2009. It was the first of five theme-based workshops planned by the NSF-funded Research Coordinated Network (RCN) for 'Dark Energy Biosphere Institute' (DEBI). The theme of the Kona workshop was subseafloor (CORK) observatories and the exploration of the deep biosphere.

The workshop was comprised of a series of keynote speakers, open microphone time, poster sessions, breakout discussion sessions and hands-on training/demonstration sessions. The keynote speakers covered aspects of CORK subseafloor observatory history and design, technical specifications and options for future modifications, drilling and other operational considerations, monitoring and contamination concerns, key results from earlier subseafloor experiments using observatories, and future directions for subseafloor biosphere science involving observatories. Participants were also provided time and encouraged to make short presentations ('open microphone' time) during the first day's plenary session to draw attention to key issues, ideas or recent results related to studies of the subseafloor biosphere. In addition to oral presentations, all participants were asked to present during a series of poster sessions highlighting work completed or in progress involving subseafloor observatories and/or the deep biosphere, or conceptual proposals describing how to move the science forward through novel application of observatory technology for passive (monitoring) or active experiments; 12 posters were presented. The deep subseafloor biosphere research area is both technologically challenging and extremely new to science, especially microbiology. Both realities were emphasized in the plenary presentations and reflected in the composition and discussion of the participants. A large proportion of the 43 workshop participants were new to this field; early career scientists (10 graduate students and 6 postdoctoral associates) accounted for 37 percent of the group. The intent of the workshop was to mix lecture and poster/discussion formats so as to encourage engagement and development of collaborative opportunities between individuals and groups that have not previously worked together or have had difficulty understanding each other's science. On the basis of observations, comments and workshop evaluations, the workshop was successful in achieving these major objectives.

The training component at this meeting focused on access and use of CORK observatories for deep biosphere studies and the crossing over between microbiological sampling and monitoring and marine hydrogeology. Three hands-on demonstrations were featured during the second day of the workshop: i) *in situ* incubation experiments using CORK observatories; ii) application of voltammetric electrochemistry to biogeochemical studies of subseafloor fluids and iii) practical introduction to hydrogeological calculations for fluid flow, solute fate and transport.

The three breakout group discussion sessions were far-ranging. The initial breakout discussions (Breakout Groups I) generated science questions, ideas and concerns, and technological wish-lists and some solutions, that were a dynamic mix of material familiar to old-DEBI hands and new innovative looks at this environment and its many challenges. There was a strong confirmation of the importance of, and still very much unresolved, questions related to the limits of life, potential physiological-metabolic diversity and novelty, and the ecological connectivity of deep subseafloor environments. Each of the discussion groups offered up long lists of compelling scientific questions and objectives, both overarching and specific, many of which will lead to exciting proposals and studies. Technology discussions (Breakout Groups III) provided an opportunity for newcomers to further learn about the attributes as well as limitations of CORK observatories and associated instrumentation. These tech discussions also unleashed a treasure trove of anticipated technologies needed to support routine and innovative research approaches in the deep biosphere, from improved contamination avoidance to a greatly enlarged quiver of *in situ* sensors and samplers to new drilling technologies (e.g., improved rock-core recoveries) and improved power supply and communication (e.g., cables and beyond). The groups acknowledged that while some of the required technological advances may need to be developed de novo from within the DEBI community, there are many advantages to partnering with other technologically savvy organizations (i.e., NASA, oil and gas industries). The 'Scenario Planning' group discussions (Breakout Group IV) considered what fundamental deep subseafloor biosphere science questions cannot be adequately addressed by the existing observatory facilities (including upcoming North Pond deployments). It is understood that the deep subseafloor basement and sediment environments are globally heterogeneous and that a full appreciation of the ecology and biogeochemistry of the deep subseafloor biosphere will require serious investigation of a much broader selection of subseafloor type-environments than represented by the Juan de Fuca Ridge flanks, North Pond, and the South Pacific Gyre. Breakout Group IV considered many important alternate type environments, including new (spreading ridges) and old (>80 My) hydrothermal systems, peridotite/serpentinite formations, coastal regions and many others. All of the discussion results are presented in the body of the report.

Finally, as noted above, this was the first of 5 planned RCN-DEBI workshops. The RCN is committed to use these community meetings as effective means of informing and recruiting scientists of diverse backgrounds and experiences to the DEBI community and to strengthen and enable the DEBI research community. Consequently, a sincere effort was made to solicit participant evaluations of key aspects of the Kona workshop. A complete summary of the 'Workshop Evaluation' survey is appended to this report (Appendix E). Participants appreciated the lectures (some even wishing there were more) and breakout discussion times, though some suggested that meeting flow would have been improved by slightly less breakout session time and mixing some of the lectures in between breakout sessions. The setting of the meeting was strongly appreciated as was the atmosphere with respect to its conduciveness to open discussion and casual chats; unfortunately, most folks felt that the venue was a bit too expensive after accounting for travel and accommodations and funding. As evidenced by many positive comments during the workshop and in the subsequent evaluation survey, the hands-on 'training sessions' were one of the highlights of the meetings; about half of the respondents urged more, longer training sessions for future RCN-DEBI workshops.

Scientific Motivation

In a 1992 essay, Thomas Gold postulated the existence of a "deep, hot biosphere", fueled by geological energy sources (Gold, 1992). A few years later, Whitman and colleagues (Whitman et al., 1998) elaborated on this concept by integrating available data on aquatic, soil, and subsurface prokaryotes, and concluded that the microbial subsurface biomass may exceed that of all the plants, animals, and microorganisms at the surface. The ramifications of a 'hidden' biosphere are numerous, leading to paradigm shifts in the biosciences and geosciences. The potential contribution to the global budget and cycling of carbon, and the potential source of novel organisms employing novel metabolisms and enzymes are just two of many examples. To date, deep biosphere studies have focused, often by necessity, on enumerating cells in relatively shallow marine sediments or in mine, well, and drill-core samples. However, these data do not permit a true global census of subsurface life. Note also that the largest potential subsurface biome—the volcanic ocean crust —is the least accessible.

In response to increasing interest in the subsurface marine biosphere, the first IODP-sponsored workshop on 'Exploring Subseafloor Life with the Integrated Ocean Drilling Program' was held in Vancouver, British Columbia, in October 2006. A follow-up workshop was held in 2007 at the University of Southern California (USC) Wrigley Institute on Catalina Island. Whereas the 2006 meeting was large and focused predominantly on drilling and sampling techniques as a means to explore the deep biosphere, the 2007 meeting focused more narrowly on emerging topics in subseafloor microbiology, and establishing a collaborative infrastructure that might facilitate research. Catalina workshop participants agreed to establish the virtual Dark Energy Biosphere Institute (DEBI) to foster research interactions and help coordinate financial, human, technological, and organizational resources. A major component of DEBI is a series of thematic meetings—part scientific conference, part educational workshop, intended to help disparate scientific communities to learn to talk about their work, important problems that should be addressed, and the use of sophisticated monitoring, sampling, and analytical tools in achieving joint objectives. The first of these DEBI meetings is to focus on the use of subseafloor borehole observatories (CORKs) for exploration of the subseafloor biosphere in volcanic ocean crust.

Background and rationale. The last decade has seen a substantial increase in deep biosphere studies, proposals, publications, working groups, and field opportunities (e.g., (Amend and Teske, 2005; Biddle et al., 2006; Chivian et al., 2008; Cowen et al., 2003; D'Hondt et al., 2004; Inagaki et al., 2006; Kormas et al., 2003; Roussel et al., 2008; Teske and Sorensen, 2008). This relatively new field now is starting to mature from predominantly opportunistic investigations (often piggy-backing on other science) to targeted research. However, marine subsurface biosphere investigations are logistically complex, requiring close coordination and detailed planning. They must generally rely on dedicated research cruises, drilling platforms, specialized instrumentation, submersible vehicles, highly-trained technical staff, long-term observatories, and substantial financial commitments. In this sense, they are akin to flight missions in the space sciences.

There also are similarities to continental deep biosphere operations, including issues of contamination during drilling, sample preservation, and ownership and distribution strategies. However, there are notable differences between marine and continental studies of the deep biosphere. The marine studies are generally in remote locations, and they often depend on scientific ocean drilling for establishing a presence, monitoring, and sampling the subseafloor environment. The high costs and difficulty in getting scheduled for Integrated Ocean Drilling Program (IODP) expeditions, the need for international cooperation, and the exceedingly long planning cycle can be daunting for even the most well-integrated of scientific groups. While the deep biosphere and the subseafloor ocean was identified as one of three primary themes in the Initial Science Plan for the first phase of IODP (2003-2013), there have been relatively few IODP proposals built around microbiological objectives, and only a couple of these have been forwarded for scheduling. Microbiological and drilling communities need closer coordination, and a better understanding of their mutual scientific goals and technological needs if successful IODP programs focusing on microbiology are to be completed in coming years. The primary purposes of the Workshop on The Subseafloor Ocean Biosphere and Borehole Observatory Science (SOBBOS) were to enhance scientific and technical coordination, and engage researchers in discussion at the interface between ocean drilling and microbiological disciplines.

Three examples highlight recent integration of microbiology and scientific ocean drilling. ODP Leg 201 targeted the microbial communities in deeply buried sediments of the eastern equatorial Pacific and Peru Margin. The second example is the Juan de Fuca Ridge microbial observatory, which, is exploiting the sampling and monitoring opportunities provided by the first and second generation of long-term borehole observatories in young, ridge-flank ocean crust. This project also interfaces extensively with the complementary hydrological CORK Tracer Transport project. The third example is the “North Pond” project, which will focus on sampling sediments, basement rocks, and cool crustal fluids on the flank of the Mid-Atlantic Ridge. In each case, microbiologists have teamed up with others experienced in drilling, sampling, and/or observatory techniques.

General Introduction to Deep Biosphere RCN Themed Meetings

The marine subsurface may be the largest biome on Earth, but it is also the least accessible. The last decade has seen a tremendous growth in deep biosphere research activities, focusing on global-scale biogeochemical cycling of carbon and energy and on a census of subsurface life and metabolic activities. To advance our understanding of microbial processes in the deep subsurface, a coordinated effort of theoretical, field, and laboratory investigations is required. A Deep (subseafloor) Biosphere Research Coordination Network (RCN) was recently funded by NSF to assist the coordination and promotion of this rapidly evolving and complex field of research. A key component of this RCN is annual, theme-based meetings to address the research and coordination efforts of this growing community. These meetings are intended to enable scientists and students to exchange ideas in deep biosphere studies, to learn about practical developments and study opportunities in the field, and to coordinate research and education activities. Each meeting will be part *scientific conference* and part *training workshop,* both parts focused on a specific theme within deep biosphere research. The conference portion provides a forum to introduce concepts and to present research activities and findings to a broadly trained, but scientifically focused audience. The training component should serve as a means to further educate scientists and students in the key research techniques and methods commonly employed, to discuss the advantages and disadvantages for specific applications, to produce consensus recommendations, and to make available detailed lab and field protocols. Each of these workshops is intended to combine scientific conference and education workshop components and designed to achieve the following objectives:

- Develop an interactive *community* of deep-biosphere researchers;
- Facilitate *coordination* of science *between* deep-biosphere drilling projects;
- Stimulate *interaction* and *education* among and between the disparate disciplines involved in deep biosphere research; and
- Enable *synthesis* and *integration* of data and technology advances generated from deep biosphere projects
- Support *interlaboratory* experiences of students in deep biosphere research;
- Align scientific, technical, education, and outreach project components for maximum benefit.

These objectives stem from assessment of the most pressing questions in deep biosphere research. At any specific location or in any particular sample, 'who is there?', 'who is active?', and 'what are they doing?'. The investigations then transition, via biogeography, to global concerns. What are the nature and extent of life on Earth? What are the physico-chemical limits of life on Earth? How metabolically active is the deep biosphere, and what are the most important redox processes? What are the dispersal mechanisms for life in the deep biosphere? How does life evolve in deeply buried geological deposits that can occur more than a km beneath the ocean floor? These questions are very diverse, and the underlying research approaches are highly interdisciplinary. As evident from these sample questions, there are several overarching themes:

- The limits (extent) of life in the deep biosphere – considering the extremes and norms of carbon, energy, nutrient, temperature, pressure, and pH regimes;
- The activity of life in the deep biosphere – considering function and rates as they relate to global biogeochemical processes;
- Biogeography and dispersal/transport of life – how does life get in the deep biosphere and the degree to which there is a distinct deep subsurface biome;
- Evolution and survival of life in the deep biosphere – adaptation, enrichment, selection, repair.

In addition, DEBI and the RCN feature a series of five annual marine deep-subsurface biosphere meetings, each at a different location, with a different overarching theme. The first of these (SOBBOS) was a stand-alone meeting, focused on using borehole observatory technology to achieve subseafloor microbiological objectives. However, it also should be viewed in context of the larger, longer-term goals; in that sense, SOBBOS sets the stage for the subsequent meetings to be held at the University of

North Carolina in 2010 (theme: *microbiology in marine sediments*), the University of Bremen, Germany in 2011 (*drill cores and the biogeochemistry of marine sediments*), Woods Hole Oceanographic Institute in 2012 (*microbiology and biosignatures in marine basement*), and Washington University in St. Louis in 2013 (*bioenergetics*). Each meeting will be part scientific conference, part education workshop. The series of meetings will establish a strong, cross-disciplinary, international community of scientists and engineers interested in the marine deep-subsurface biosphere, and it will identify the most exciting research opportunities and IODP target sites.

Introduction to Kona, Hawaii Workshop, October 19-21, 2009

The **1nd Workshop on Dark Energy Biosphere Institute** was held at the Fairmount Orchid Resort on the Kona Coast of Hawaii over October 19-21, 2009. It was hosted by the University of Hawaii. Organizers included James Cowen (U. of Hawaii; Chair), Brian Glazer (U. of Hawaii), Jan Amend (Washington U.) and Andrew Fisher (U. of California, Santa Cruz).

The Kona workshop, reported upon below, was the first of 5 planned RCN theme-based meetings. The theme of the Kona workshop was subseafloor (CORK) observatories and the exploration of the deep biosphere. The workshop was comprised of a series of keynote speakers, open microphone time, poster sessions, breakout discussion sessions and hands-on training/demonstration sessions.

The keynote speakers covered aspects of CORK subseafloor observatory history and design, technical specifications and options for future modifications, drilling and other operational considerations, monitoring and contamination concerns, key results from earlier subseafloor experiments using observatories, and future directions for subseafloor biosphere science involving observatories. Participants were also provided time and encouraged to make short presentations (open microphone' time) during the first day's plenary session to draw attention to key issues, ideas or recent results related to studies of the subseafloor biosphere. Some 15 people took advantage of the 'open podium' time during the Day 1 plenary session to present one or two slides emphasizing a new idea or a particular issue or concern, spawning rigorous discussion.

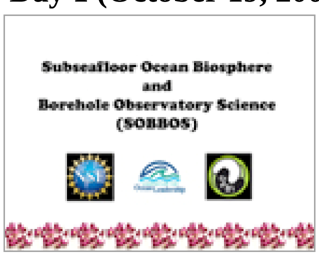
In addition to oral presentations, all participants were asked to present during a poster session (also viewed throughout the day in association with coffee and meal breaks) highlighting work completed or in progress involving subseafloor observatories and/or the deep biosphere, or conceptual proposals describing how to move the science forward through novel application of observatory technology for passive (monitoring) or active experiments. Our intent was to mix lecture and poster/discussion formats so as to encourage engagement and development of collaborative opportunities between individuals and groups that have not previously worked together or have had difficulty understanding each other's science. Twelve posters were mounted in the 'poster room' for the entire workshop (see Appendix C for poster abstracts). A focused poster session was held during the late afternoon/evening of Day 1 leading into a dinner served just outside of and adjacent to the meeting rooms. The poster session was well attended, with discussions continuing through the dinner hours. Poster viewing and discussions were encouraged throughout the workshop, at breaks and after hours.

Training component. The training component at this meeting focused on access and use of CORK observatories for deep biosphere studies and the crossing over between microbiological sampling and monitoring and marine hydrogeology. The primary goal was to develop a basic level of understanding among two disparate groups: those involved in development and use of CORK systems for marine hydrogeological studies, and microbiologists working in the deep biosphere or in other settings. It is important to note that this is a technologically challenging area of research and one that is extremely new to science and in particular microbiology. In order to succeed, this workshop moved beyond the standard introductory lectures that attempt to survey an entire discipline in 45 minutes. Instead, the workshop endeavored to link the lectures, discussions and hands-on demonstrations. Three hands-on demonstrations were featured during the second day of the workshop: i) *in situ* incubation experiments using CORK observatories; ii) application of voltammetric electrochemistry to biogeochemical studies of subseafloor fluids; and iii) practical introduction to hydrogeological calculations for fluid flow, solute fate and transport. It was not possible to provide a comprehensive curriculum in any of these topics given the short time available. Instead, demonstration leaders developed a series of presentations and exercises that provided a mechanism for non-specialists to gain a basic grasp of one or more key concepts.

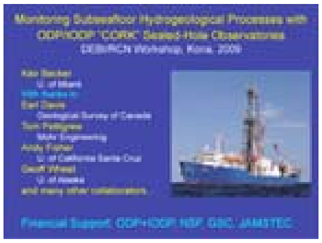
Plenary Presentations

Following an introduction to the concept and intent of our DEBI-RCN by Jan Amend, Keir Becker reviewed the history of CORK observatory designs, especially from a hydrogeological perspective. Geoff Wheat addressed general CORK technology and downhole samplers and sensors. Jim Cowen followed with a discussion of deep biosphere studies at Juan de Fuca Ridge flanks with an emphasis on seafloor sampling and sensor technologies. Katrina Edwards presented a look forward into future deep biosphere studies using the upcoming North Pond CORK Observatory system and its associated opportunities. Andy Fisher gave a final plenary talk on the final day of the workshop, providing an experienced view of the workings of IODP and the proposal-to-expedition process. (See meeting website for PDF versions of presentations, http://www.darkenergybiosphere.org/meetings/2009meeting/2009agenda.html)

Day 1 (October 19, 2009)



Welcome and RCN introduction
Jan Amend, Washington University



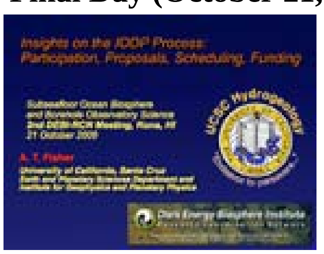
CORK design: Foundation in Hydrogeology
Keir Becker, University of Miami

CORK technology: Geochem/Biosphere Observatories
Geoff Wheat, University of Alaska

Juan de Fuca case study and subsurface biosphere applications
Jim Cowen, University of Hawaii

North Pond future directions
Katrina Edwards, University of Southern California

Final Day (October 21, 2009)



Insight on the IODP process
Andy Fisher, University of California Santa Cruz

Breakout Group Reports

Workshop participants were divided into 2 or 3 subgroups for each of the Breakout Sessions. Each group had an assigned discussion leader and a scribe. Each group leader summarized their group's discussion in a plenary session that followed each Breakout Session. Each of the Breakout Session (I, III and IV) reports below integrates the 2 or 3 group reports for that session.

Session I. The Big Science Questions

This breakout session had two parts. Each of the two breakout groups was asked to first consider questions listed under 'Session IA', then, following a short break, to consider the related questions listed under 'Session IB'.

Session IA: No holds-barred discussion of the important outstanding science questions that need to be asked with regard to the deep subseafloor biosphere. Do not be concerned with technological limitations at this point (that will come in Session IB).

1. What are the "big science questions" with respect to the deep subseafloor biosphere? Distinguish between science questions that are specific to sediments, basements (basaltic crust), or either.

Session IB: Revisit the results of the "big science questions" discussions from a technological perspective. Emphasize the CORK observatories: how can they help, where can they be improved, and what are their perceived limitations?

1. Which of the "big science questions" are (or may be) addressable using CORK observatories?
2. Which questions may not be addressable using CORK observatories? Why? What other approaches are needed?
3. Which questions seem to be presently technology limited? At what level (e.g., CORK infrastructure, sampling systems, sensors)?

Discussion Group Leaders: Wolfgang Bach (University of Bremen)
Rick Colwell (Oregon State University)
Discussion Group Scribes: Alberto Robador (University of Hawaii)
Mark Lever (Aarhus University)

Session 1A Group Report (working group reports are integrated)**:**

Questions raised are roughly distributed below as points generally relevant to subseafloor biosphere, or as more or less specifically to basement versus deep sedimentary environments.

(i) General subseafloor

- Can we identify new forms of life, i.e., life as we don't know it? If yes, how?
    - We need to be mindful of the method bias that interferes with this effort
    - Use of tracer/isotopically labeled compounds and study of their uptake or fractionation
- What are the limits of life? Where would we expect life? Where would we expect that it would not occur? What habitat, energetic, physical and other conditions are required? Requires consideration of the 'biological fringe' and just beyond. The search would inform strategies for where and how to look for life beyond Earth.
- What microbial physiologies are needed to survive in the seafloor? How do organisms deal with energy limitation, extreme pressure, etc.? What are physiological adaptations? What pathways are present and utilized?

- Can we determine the age of deeply buried communities, the role of microbial transport in different geologic systems, the in-place evolution of subsurface biological communities? Have microbes evolved adaptations in place, or were microbes with these adaptations always present and then able to become dominant over time? How adaptive are subsurface communities with very long generation times? What are the adaptive potentials of subsurface communities? Is evolution in the subsurface fundamentally different than at the surface (e.g., consider the roles of horizontal gene transfer and phage-mediated transfer of DNA)?
- Are microbial communities as old as the habitat in which they occur, or have microbes colonized their habitat after its formation/deposition? What is the connectivity of subsurface ecosystems, and how does this affect dispersal, transport and distribution of microbial communities/activities? How important is fluid flow?
- How does microbial activity affect global biogeochemical budgets, C and redox flux, crustal composition? How active are subsurface communities? How significant are subsurface autotrophic metabolisms in global elemental budgets, and how important is chemoautotrophy compared to photoautotrophy? Integrate the knowledge gained regarding the subsurface with the existing view of surface biogeochemical budgets.
- What is the spatial and temporal heterogeneity of subsurface microbial metabolic diversity and activity? What is the importance of hydrogeology, heat flux, or other geophysical parameters?
- Do biogeographic provinces exist in the subsurface through comparative studies? (Do boundaries correlate with overlying ocean? Defined by plate boundaries?)
- Are microbial communities/microbially-driven reactions in steady-state or not? Where are such conditions met? Do microbial communities “recycle” in the subsurface?
- How can we identify past microbial communities?
- What dictates the similarities and differences between subsurface environments in terrestrial vs. seafloor settings? Are certain microbes prone to survival in the subsurface regardless whether the setting is presently covered by water?
- What are the age relations between rocks/sediments, fluids, and microorganisms? How and when are sediments inoculated with microbes? What role does fluid flow within the underlying basement play? Specifically, what processes at the sediment-basement interface mediate inoculation?
- What “type” localities need to be examined to complete our conceptual model of the subsurface. What are ‘typical’ vs. ‘atypical’ locations? Which major zones of the oceans have not been studied and need to be targeted? What are big-science questions that justify going to places that have been neglected by (microbiological) sampling? Which present-day locations provide “windows” into Earth’s past or will be drilled in the near future or offer exceptional opportunities for microbiological investigation?

- oxygen minimum zones, anoxic basins, Guaymas Basin
    - Juan de Fuca Ridge (Flank)
    - system on Cocos Plate
    - North Pond
    - Black Sea?
    - Okinawa Trough
    - serpentine seamount with "hole" off of Guam
    - transects across land-sea interfaces
    - ophiolites
    - compare geologically active to inactive settings
    - hydrothermal vents/black smokers in anoxic basins (Mediterranean mud volcanoes, northern Gulf of California?) to examine origins of metal ore deposits; possible manipulation experiments?
    - locations where carbon sequestration experiments may occur
    - Locations where large flux gradients occur (may dictate surface-like strategies) vs. locations where low flux gradients occur (may dictate starvation/survival)
- Shall microbiologists be "added" on all/more non-microbiology-focused cruises (and take samples for archives)? Need for quality control (e.g., proper storage, contamination monitoring) whenever samples are collected for microbiological investigations.

(ii) Basement or Crystalline Crust (e.g., volcanic, gabbroic, mantle rocks)

Activity and extent of life in hard rock

- How does textural evidence for microbial activity (cumulated) relate to present-day activity within rocks (instantaneous)?
- What molecular techniques are best suited in tracing microbiological activity?
- What are the biogenicity criteria for tracing microbial activity within ancient rocks?
- What are the controls of lithology, permeability, fluid flux, etc. on microbial activity and physiology?
- Vise versa: what are the controls of microbial activity on crustal weathering rates. How are different materials (e.g., olivine, basaltic glass) affected by it?
- Does rock mineralogy control microbial community composition?
- What is the relation between the free-living microbial communities in basaltic fluids and particle-attached organisms?
- What is the importance of a rock-hosted deep biosphere for biogeochemical and geochemical cycles? What is the standing stock? How does it compare to the sediment-hosted deep biosphere, both in size and physiology? How does microbial activity affect the release of Ca and Si. (The ocean crust is a prominent source of those biogeochemically relevant solutes.)
- The ultimate goal is global 3-D image of the presence and activity of subsurface life

Evolution of microbial life in aging plate

- What are the relations between basement age and colonization age?
  - Terrestrial studies highlight the importance of fluid age dating
  - Transects - in age and, more importantly, in parameter space - will be crucial in examining critical functional relationships
- What are the dominant microbial colonization processes (fluid advection versus seafloor inoculation followed by burial)?
- What is the relationship between microbial biogeography and global hydrological cycle?
- Vice versa: Can the distribution of microbes/biomarkers be as a proxy for geological sources (e.g., hydrothermal) and/or events (e.g., LIP formations)?

(iii) Deep Sediments

- What is the effect of metabolic activity on paleo proxies (isotopic and molecular) and how does it modify Earth history sedimentary archives?
- How has the deep biosphere changed in Earth history, e.g., within a Wilson cycle with changing sediment accumulation and seawater composition?
- How do microbes move in geochemical gradients, in particular in low-energy environments?
- How does life cope with severe energy limitation in some deep-sea sedimentary settings?

Session IB Group Report (working group reports are integrated)**:**

Here we asked participants to change gears, to revisit the results of their 'big science questions' discussions (Session 1A) from a technological perspective, to emphasize the CORK observatories. How can CORKs help, where can they be improved, what are their perceived limitations?

Advantages of CORKs:*

- CORKs allow *in situ* experimentation and time series studies
- Allow study of *in situ* activity in the deep biosphere
- Can study effect of perturbation on *in situ* communities
- Can create artificial hydrothermal vents
- Can be used to address most scientific questions related to the subseafloor biosphere
- Best tool we have to study subseafloor life under (near) *in situ* conditions
- Geochemical profiles do not reflect cryptic microbial processes that can be investigated experimentally within CORKs

** CORK studies must be linked to coring and surveys*

Limitations of CORKs:

- Can experiments really be representative of *in situ* processes?
- Cannot provide numbers on microbial biomass within basalt

- If the duration of an experiment is too long for the CORK-lifetime then a CORK may not work. But this was questioned.
- High financial cost
- Logistically challenging, often do not work as intended (but better than a purely laboratory approach)

General CORK-related issues/questions?

- What future tools will be important?
- Need to introduce "best practices" or recommendations for CORKs?
- Can CORKs (always?) be designed so that they evaluate the validity of the data acquired?
- Can data from CORKs be cross-compared?

1. *Which of the 'big science questions' are (or may be) addressable using CORK observatories?*
   - New opportunities for assessing the *activity of deep biosphere*
   - Microbial observatory and active experimentation leads to critical insights into microbial identity, physiology, activity, physiological response to perturbation. This approach should be used for basement and sedimentary settings. Also useful in examining effects on paleo-archives

2. *Which questions may not be addressable using CORK observatories? Why? What other approaches are needed?*
   - How do we distinguish between the activities and physiologies of planktonic microbes versus biofilms versus endolithic microbes?
   - Active experimentation (perturbation-response) - and comparison with information obtained from fluid samples - provides useful but limited insights
   - Improved core recovery is needed to be able to investigate rock-hosted microbial communities!

3. *Which questions seem to be presently technology limited? At what level (e.g., CORK infrastructure, sampling systems, sensors)?*
   <u>Sample recovery and quality</u>
      - improved core recovery needed for providing minimally disturbed samples
      - new drilling tools (sidewall drilling)

   <u>Borehole installations</u>
      - *in situ* measurements (i.e., *in situ* mass spectrometer, electrochemical sensors)
      - using osmo sampling for capturing (fixing) short-lived metabolites

   <u>Sensing string packages (logging)</u>
      - video camera provides powerful continuous visual information, should be used routinely
      - sensors detecting organics (cells), e.g., UV fluorescence

Session III. Technology Innovations and Limitations

The assignment for this session will get down to the nuts and bolts of the technologies needed to effectively interface the opportunities afforded by CORK observatories to deep biosphere studies.

1. What are the useful existing technologies?
2. What new technologies are needed? Which of these might be adapted from technologies already developed for other environments? Which technologies require complete ground-up development?
3. What are hot areas of technological innovation that might have relevance to the deep biosphere?
4. Which of these technologies are likely to be adaptable to CORK observatories? What modifications to CORK infrastructure are needed to accommodate these technologies? Downhole vs. seafloor?
5. How can CORKs be improved in the context of this technology discussion?

Discussion Group Leaders: Fabien Kenig (University of Illinois)
Matt Schrenk (East Carolina University)
Wiebke Ziebis (University of Southern California)

Discussion Group Scribes: Peter Canovas (Arizona State University)
Tina Lin (University of Hawaii)
Sean Jungbluth (University of Hawaii)

Session III Group Reports (working group reports are integrated)**:**

Overarching Goals:

1. More sensitive analyses
2. To develop *in situ* devices compatible with being sent downhole
3. Accommodation of extended time scale related to sample preservation: *in situ* vs. ship board analysis. Temporal resolution of sampling (both long and short term)
4. Direct downhole observations: able to examine the rock and identify where microbes are
5. Overall, more data
6. Extend infrastructural capabilities (e.g., Deep: deeper drilling capability; Harsh environments: no sediment covered crust)
7. Communication and knowledge transfer between subseafloor science community and other communities (e.g., oil/gas industry; engineering; NASA)

Experimental ideas:

1. Induce microbial blooms
2. Inject microbes/organics into the subsurface in a contained manner

1. *What are the useful existing technologies? Make available (i.e., DEBI web site) list of available logging technology and other field instrument resources available.*

Ready for Seafloor Instrumentation

- Fluid Deliver Lines (and other features) of advanced CORK observatories
- OSMO samplers
- incubation devices (e.g., FLOCS)
- GeoMICROBE instrument sleds
- Mobile Pump Unit (MPU)
- Large Volume Bag Sampler (LVBS)
- *In situ* mass spectrometers
- UV nitrate sensors
- Wet chemistry nutrient (NO_3^-, NO_2^-, PO_4^-, silicate, NH_4^+) analyzer (commercially available)
- Wet chemistry ATP & LAL measurement. (ref. NASA)
- Miniaturized NASA technologies (e.g., GC; combustion mass spectrometer)
- Available Preservation technology:
    - Single cell genome and transcript studies. *In situ*, shipboard or lab?
    - *In situ* preservation, e.g., pre-charged (fixative) filtration system.
- Cable observatories
- Power supply
- Communication/data transmission

Ready for downhole deployment

- still/video camera monitoring
- Ultra sonic borehole tele-viewer: fractures, textures.
- Diamond core barrel provides better rock core recovery.
- different polymers for materials (fiberglass casing)
- side-wall coring
- communication/data transmission
- packers
- Existing technologies for drilling that need to be communicated from the oil industry (e.g., calibrations; logging sensors; camera and microscope for down hole observations/movies; Borehole side sampler [cleaner sampling than initial coring] has already been developed [for legacy holes, done after hole recovery])

2. *What new (or improved) technologies are needed?*
    - Ability to sample at specific intervals within the borehole (available now to limited extent: 1-3 discreet intervals via fluid delivery lines, or placement of downhole instruments)
    - Temperature & corrosion-resistant materials (significant improvements under development for JdFR and North Pond: e.g., fiberglass, PVDF, Teflon, Titanium and Stainless steel; costs are real issue)
    - Remote data downloading (beyond AUV/ROV/HOV and cable systems)

- Automated release of data recorders and/or experiments
- Communication between borehole sites
- Continued Sensor development
- “Asceptic” drilling for better sampling (reduction of the number of bottom sea water cells by 1 to 3 orders of magnitude???)
- Minimize impact of drilling water that is pumped down hole for drilling or find way to filter
- Use of redox-controlled drilling fluid (adapted to hypothesized environment)
- “Inert” casings for drilling and minimization of contamination (i.e., possible remaining problems with fiberglass resin, etc. (e.g., different linings/coatings for different applications, at different depth Teflon, or titanium, fiberglass)
- Downhole power for *in situ* sensors
- Larger diameter pipe, side rock coring device.
- Rock abrasion tool (RAT): to help install incubation device into side - - Rocks; spectral of dust. (NASA)
- *In situ* mineralogy
- *In situ* Microscopy*;* Micro-came: able to deployed both seafloor and downhole.
- *In situ* fluorescence; UV spectrometry (very specific to certain types of proteins)
- *In situ* Raman spectrometry (Wash U; MBARI; UW)
- *In situ* hybridization MBARI-MESP, Q-PCR
- Cell-counting, sorting, and characterization by flow cytometry
- Long term electrochemistry deployments
- *In situ* sample preservation (e.g., freezing?—high power, require cable system? ; chemical poison/preservative—low cost options)
- Extraction: Low concentration organic or inorganic compounds can be pre-concentrated on extraction columns (e.g., resin exchange)

Which of these might be adapted from technologies already developed for other environments?

- Flexible Liner Underground Technology (FLUT)— from Terrestrial technology
- Triggered/initiation of sampling/analyses (e.g., PCR etc.) due to change in chemical or physical (e.g., pressure, temperature) signal (UCLA, MBARI, JPL)
- NASA (space mission) microsensors (including rock surface scanners for biologics)
- *In situ* PCR and hybridization exp. for characterizing microbial activity (e.g., geochip? for mini PCR)
- Isolate single cells for genomic study: laser tweezers, spatial NanoSIMS.
- Apply electrochemistry for microbe activity study: Microbe must be on the electrode to measure the redox activities. Colonize the electrode surface

- Measure *in situ* metabolic activity: e.g., C_{14}-thymidine; incubation system required

Which technologies require complete ground-up development?
- Dry drilling (logistically impossible?)
- Different water sources for drilling
  - pre-treated water sources (sterilization)
  - ship-generated fresh water
- Monitoring contamination of drilling water as it exists the pipe
- Assessment of the entire drilling process from the perspective of microbiological contamination
- Encapsulated coring techniques
- Robotic coring systems
- Extended core barrel for basement recovery of short intervals (1-2 meters)
- Motor-driven core barrel
- Heave, rotation, and geometry improvements to drillbits
- Placing CORKs into bare rock (e.g., hammered casing; expandable casing)
- Enhanced communication between scientists and oil/gas industry
- Use surface & down-hole robots for subseafloor study. (ref: Sukane, Ubi Nitra.) Robots can be built very small.
- Bio-sensors; for low concentrations of metabolites; integrated with GFP.
- Novel biosignatures (e.g., alteration of magnetic properties of basaltic rock by bio-activities)

3. *What are hot areas of technological innovation that might have relevance to the deep biosphere?*
   - Making electronics work at high temperatures (problems are connections)
   - Autonomous power supplies
     - equipment efficiency
   - Nanotechnology
   - Sensor longevity (materials)
   - Sensor development for dissolved gases
     - downhole mass spec
   - Telemetry
   - Internal CORK profilers
   - Containment of instruments within the casing that have the ability to leave and profile bare basement
   - Instrumentation to monitor internal seismic structure

4. *Which of these technologies are likely to be adaptable to CORK observatories? What modifications to CORK infrastructure are needed to accommodate these technologies? Downhole vs. seafloor?*
   - All responses listed in #3 above.

5. *How can CORKs be improved in the context of this technology discussion?**
   - Making the boreholes wider

- Movable sampler/sensors within the CORK
- Moveable packers
- Increasing the number of packers per CORK (i.e., more sample intervals)
- Sampling at multiple intervals within the perforated casing
- Materials (casing, umbilicals, packers) employed according to specific requirements of project; can vary by depth [make list of alternative materials and their costs: place on DEBI website]
- Easier, more frequent data and experiment recovery (e.g., automated or acoustic release)
- Tidal pumping
  - Make holes produce
- Sealing techniques
  - Improved methods for testing the seals
- Osmo-sampler membrane technology
- Retrieval of large volumes of borehole water (capable of collecting 100 liters now using CORK fluid delivery lines and seafloor sampling equipment-UH)
- Adapt CORKs to function in less permeable regions (sandy locations)
  - requires screening at input
  - Casing/umbilical screen materials adapted to sandy environments
- Pre-cruise and pre-drilling communication to maximize opportunities
- Mechanical components of CORKs (connections)
  - Aeroquip connectors
- Welding materials vs assembly using lubes
- Drilling deeper into basement, past biotic fringe into abiotic zone (i.e., requires high temp [>100°C] drilling capability—lessons from Iceland drilling?)
- Retrieval of pressurized samples (entire osmo string or experiment)
- "Intelligent" (or automated event responsiveness) CORK (e.g., sensor/software directed changes to sampling regime based on physical or chemical signal; power management for long-term deployments)
  - Continuous, renewable power supply for CORK experiments (e.g., use of mooring outfitted for generating electricity—wind, wave, tide etc.)

** Attract engineers from other disciplines; create informational lists on DEBI website (CORK designs, available equipment/instrumentation; standard materials;*

Session IV. CORK Scenario Planning

The community currently has 3 successful (drilled or scheduled for drilling) deep biosphere programs (JdFR flanks, North Pond, and the Pacific Gyre). Where do we go from here?

1. What environmental, geological, biological criteria should be considered for identifying important target sites for new deep biosphere studies, in particular for installation of new CORK Observatories?
2. What technological constraints exist for any of these target sites?
3. What are top 3 future new priority CORK projects?

Discussion Group Leaders: Julie Huber (Marine Biological Laboratory)
Tom McCollom (University of Colorado)
Craig Moyer (Western Washington University)

Discussion Group Scribes: In Chieh Chen (University of Hawaii)
Sarah Bennett (University of Southern California)
Jason Sylvan (University of Southern California)

Session IV Group Reports (working group reports are integrated)**:**

1. *What environmental, geological, biological criteria should be considered for identifying important target sites for new deep biosphere studies, in particular for installation of new CORK Observatories?*
   - Trough/Trenches at Active Margins (e.g., Costa Rica, Nankai, Barbados, Cascadia)
   - High-temp hydrothermal system (e.g., Manus, Middle Valley)
   - Exposed basalt seamounts with lots of water coming out (e.g., Dorado, Cocos Plate)
   - Hydrothermal system in an anoxic basin: Analogue for early Earth (e.g., Northern Gulf of Mexico)
   - Old hydrologically-active crust (e.g., West of the Mid-Atlantic, 80 MY [Embley et al]; Madeira Abyssal Plain, 106 My, has been drilled for sediment, but is in 4700m of water)
   - Comparison of sites with high versus low fluid circulation
   - Sub-aerial and Sub-marine: fresh water filling in on the land part, seawater filling in on the marine part (e.g., Helena slope in Hawaii)
   - Peridotite/serpentinite formation (e.g.**,** Atlantis Massif drilled in 2005)
   - Comparison between areas of high and low productivity in the overlying ocean (Pacific gyre)
   - Transect away from MOR to evaluate impact of age on evolution of biological community (CORKs on JdFR Flanks afford good start; issues with more than one variable)
   - Sites with different crustal composition to examine impact of biological community, chemistry (ultramafic, andesitic, dacitic)
   - Compare microbial communities across an ocean-continental crust transition
   - Coastal regions – fresh water to salt water
   - Off-axis recharge zones – oxidized fluids circulating into crust should stimulate biological activity
   - Flanks of hot-spot seamount (e.g., <u>FeMO deep</u>, the flank of the Loihi Seamount—large mats, low temp ecosystem: a type hydrological system for seamounts)
   - Transform fault: we have only two of three plate boundaries (ridges, subduction zones) drilled—BTSZ, seismically active plate boundary not well understood, some hydrothermal activity, lithologic heterogeneity, serpentinization (maybe- serpentinites present), gabbros.

- Fault zones that have generated massive earthquakes—what is effect on subsurface biosphere and how does it recover?
- Fore-arc systems
- Serpentinizing systems
- Anaerobic water overlying subsurface (northern Gulf of California?)
- Mediterranean Sea with its multiple shallow water hydrothermal systems at 10s of meters depth (e.g., local seismic activity accompanied by changes in system gas chemistry and presumably by changes in microbiology)
- High latitudes with shallow, gassy sediments; good climate change outreach implications (e.g., gas hydrates, for example there is already a CORK at Hydrate Ridge, installed 1992)
- Areas of known petroleum generation (GOM)—microbiology near oil reservoirs poorly understood (possible problems with rentals of quadrants of the oceans here).
- Biotic-abiotic transitions: Limits of life (e.g., Super deep—joint effort with Moho efforts; high temperature sites; back-arc basins with extreme alterations over short distances)
- Coastal Margin: sediment and continental crust (e.g., New Jersey, Great Barrier Reef)
- River delta environments—implications for carbon transport, links between continental and oceanic crusts? Coupling between light and dark ecosystems?
- CO_2 sequestration in basalts experimental CORK observatory—possible small scale injection studies (like Fe addition experiments in HNLC areas). Should have liaisons with DOE, etc. to address concerns about this issue - this is a big DEBI question, let's address it!
- Cold seeps - accretionary prisms, others?

2. *What technological constraints exist for any of these target sites?*
   - Dorado- Hammer in technology
   - Peridotite- Hammer in technology
   - Continental Crust- thickness of sediment
   - Bare Rock Hydrothermal- Hammer in technology, Temperature and electronics
   - Hydrothermal System in Anoxic Basin- Temperature and electronics
   - concern that important sites w/o current site survey data will be passed over in favor of sites w/ current site survey data
   - Depth limitations, ~8km total (hole and water) (e.g., North Pond is deepest CORK so far at 4400 m)
   - For shallow sites, must figure out MSPs - not necessarily a technological constraint, but may be expensive to rent rigs

- Serpentinzation systems tough? doesn't seem like there is a constraint on how much sediment is needed to install a CORK, but one must adapt the CORK to specific sites if shallow sediment cover
- Fractured, fresh basalt (no sediments) could be a problem? However, we've only tried concentric arm bits, but ring bits may work. Site 921 lessons- there are still subsurface features you may not be aware of that can "get you." The Hammer as a solution to this type of system? Hammer drilling has worked in other types of basalt.
- Horizontal CORKS (multiple replicates of a single depth interval); horizontal drilling possible, but is a CORK possible? Best way to get replicates of the same stratigraphic material/horizon.
- Temperature is a constraint, about 150°C is currently hottest possible.
- Pre-deployment testing at a land site a good idea to be sure we can get measurements we want- proof of concept
- Other constraints: High sulfide; low pH; water depth – accessibility by sub/ROV

3. *What are the top 3 future new priority CORK projects?*

The intention of asking the working groups to attempt an "interim" prioritization was to further focus discussions of #1 and 2 above on most serious candidates and to stimulate interest and enthusiasm for initiating new CORK observatory projects.

One of the working groups actually tallied a 'strawman' vote for their group's top candidate for future CORK project sites:

- 4 votes: Dorado
- 4 votes: Peridotite
- 3 votes: Continental Crust
- 1 vote: Bare Rock Hydrothermal
- 1 vote: Hydrothermal System in Anoxic Basin

Much more work to be done in areas that are already characterized

However, all three subgroups for this session also emphasized the importance of continuing to consider a number of more general issues in their discussion summaries:

- Science driven choice of future sites: Essential to consider important science questions and hypotheses and see what sites best address those.
- Diversity of geological context/substrate is important—for example, all sites so far/planned are basalt-hosted, but basalt is not representative of all sites. For example, other rocks untested by CORK observatory deep biosphere studies include:
    - Ultra-mafic rock– trio of sites for comparison? (e.g., Marianas fore arc already corked, pH 12, microbial methane, hydrogen, potential abiotic organic compounds)

    - Felsic rock
- Allocthanous vs autocthanous? sites that are evolutionary interesting? How alive are the sediments?
- Biotic fringe sites- too hot, too low energy- transect concept would address this
- Array of diverse habitats: how LARGE/variable/complex is subsurface microbial standing crop? Is there new production? How tight is the linkage between biotic communities and hydrogeology?
- Old crust end-member – (801b?)
- Back arc basin (Lau basin, Okinawa trough [currently scheduled for drilling, but CORK?])
- Terrestrial – continental shelf transition

Training Session Synopses ('Breakout Group Session II')

A significant portion of Day 2's morning was devoted to three 'Training Sessions', each of which was rerun over three half-hour periods. The participants were divided into three groups. Each group was then directed to a separate room to participate in one of the training sessions. At the conclusion of each training session period, the groups rotated to the next training session room, allowing all participants to experience each of the three training sessions. According to participant workshop evaluations, these training sessions were very well received. A brief overview of each session follows.

Borehole Microbial Observatory Construction (Beth Orcutt, University of Southern California)

Flow-through Osmo Colonization System (FLOCS) design* was demonstrated with example cassettes and chambers. Discussion included variations in packing materials such as colonization substrates, including glass wool, polished rock chips and glass beads, and deployment modes such as at the seafloor, on a CORK platform, and within a CORK borehole. (*See website for design schematics for FLOCS, http://www.darkenergybiosphere.org/meetings/2009meeting/files/Orcutt_DEBI-RCN_FLOCSdesign.pdf).

Further details can be found in: Orcutt, B., Wheat, G., and Edwards, K. 2010. Subseafloor ocean crust microbial observatories: Development of FLOCS (FLow-through Osmo Colonization System) and evaluation of borehole construction materials. Geomicrobiology Journal 27:2, 143-157. http://dx.doi.org/10.1080/01490450903456772

Electrochemistry Measurement and Application (Brian Glazer group, University of Hawaii)

Environmental electrochemistry in the Glazer lab is investigated using solid-state mercury-gold amalgam sensors which can detect O_2, H_2O_2, HS^-/H_2S, S_2O_32-, S_4O_62-, S_x^{2-}, S^0, Fe^{2+}, Mn^{2+}, Fe^{3+}_{aq}, FeS_{aq}, I^-, Cu^{2+}, Pb^{2+}, Cd^{2+}, Zn^{2+}. Electrochemical techniques can be used for identifying and quantifying organic and inorganic compounds, determining adsorption processes on surfaces, measuring kinetic rates, establishing electron transfer and reaction mechanisms, specifying thermodynamic properties, and discovering complexation and coordination values. Glazer's lab group demonstrated and encouraged workshop attendees to participate in hands-on construction, calibration, and deployment of sensors for *in situ* voltammetry.

Jenny Murphy (University of Hawaii graduate student) instructs workshop participants Katrina Edwards (University of Southern California), David Fike (Washington University) and Matt Schrenk (East Carolina University) on polishing of gold electrodes prior to amalgamation for making voltammetric measurements.

Hydrogeology Modeling and Computation (Andy Fisher, University of California Santa Cruz)

Hydrogeologic properties of the ocean crust were calculated using cross-hole testing. The Microsoft Excel "Solver" tool was used to explore different options for determining formation properties. [The Solver is provided as part of standard Excel software, but is not always installed automatically. Installing the Solver differs somewhat on different platforms and versions. Google "How to install solver" and your Excel version and platform for instructions specific to your hardware/software configuration.]

The material was introduced in a Powerpoint presentation*, then participants were taken through the calculations in the Excel spreadsheet* step by step. A background file* contains some mathematical and hydrologic background on aquifer testing. (*See website for PDF and Excel presentation files, http://www.darkenergybiosphere.org/meetings/2009meeting/2009training.html)

Further details can be found in: Fisher, A. T., Davis, E. E., and Becker, K. 2008. Borehole-to-borehole hydrologic response across 2.4 km in the upper oceanic crust: Implications for crustal-scale properties. Journal of Geophysical Research 113, B07106. http://dx.doi.org/10.1029/2007JB005447

Appendices