To Be Submitted to *Applied Spectroscopy*

Spectroscopic Successes and Challenges:

Raman Spectroscopy at 3.6 Km Depth on the Ocean Floor

INTRODUCTION

The deep ocean is an “extreme aqueous environment”, permeated by fine particles of degraded organic matter and characterized by high pressures (about 360 atmospheres at 3.6 km depth), low temperatures (~ 2° C), and a high concentration (3.5 wt. %) of potentially corrosive salts. Such conditions make it difficult to analyze interesting geologic materials and dynamic processes on the ocean floor, such as the fluids and solids that issue from hydrothermal vents, the rocks that cool from undersea lava eruptions, the skeletons and shells of reef-building animals such as corals and clams, and ice-like clathrates that form when natural gas seeps upward through the ocean-floor sediments and into the water column above. Moreover, because many of the phases of interest are not stable once they are brought to the surface and exposed to ambient pressure and temperature, only an *in situ* analytical technique can enable detailed investigation of the ocean environment.

Raman spectroscopy is well suited to meet the challenges of analysis on the ocean floor: the technique is very amenable to materials that reside in an aqueous environment; analysis is possible on solids, liquids, gases, and dissolved species; and modern Raman instrumentation has fiber-optically-coupled components that can be encapsulated in pressure-resistant housings for operation underwater.

In the present paper we report on the development, modification, calibration, deployment, and first successful applications of a Deep Ocean Raman System (DORS), which is based on a laboratory-model laser Raman spectroscopic system from Kaiser Optical Systems, Inc. (KOSI). This paper documents the analytical successes to date and explores some of the challenges that we are still coming to terms with.

SCIENTIFIC INTERESTS IN THE SEA FLOOR AMENABLE TO RAMAN SPECTROSCOPY

The selection of the components of the spectroscopic instrument that was the starting basis for the deep ocean Raman system was determined by the anticipated analytical requirements and challenges posed by our scientific interests. Those interests include the mineralogy of the sea floor and the chemistry of pore water, gas seeps, and sea floor vents. We would like to monitor dissolved gases such as CH_4, CO_2, CO, O_2, H_2S, H_2, N_2 and to distinguish speciations that are sensitive to oxygen concentration (e.g., CH_4 vs. CO_2, sulfate vs. sulfite) and pH (e.g., HSO_4^- vs. SO_4^{2-}). We also want to make time- and spatially-resolved measurements of the ocean chemistry, for instance, investigating gradients in dissolved gases, such as CO_2, and dissolved aqueous complexes, such as sulfate and carbonate. The concentrations of such species typically are homogeneous and unchanging in the deep ocean. When there are compositional variations and concentration gradients, however, they often are transient and thus need to be recorded quickly, by a technique that does not disturb the compositional distribution.

Other interests include biologically (including microbially) precipitated solids such as the polymorphs of elemental sulfur produced by bacteria such as *Thioploca* and *Beggiatoa* (Pasteris, et al., 2001), the distinction between the biologically produced $CaCO_3$ polymorphs aragonite and calcite, and the investigation of phosphate minerals and deposits on the seafloor. Other solids of interest on the seafloor include the silicates quartz and feldspar, the iron oxides

magnetite and hematite, and clathrate hydrate phases, which incorporate methane and/or carbon dioxide into their structure.

For the past several years, researchers at the Monterey Bay Aquarium Research Institute (MBARI) in Moss Landing, California, have been investigating the possibility of sequestering carbon dioxide on the deep ocean floor. This research addresses the attempt to develop large-scale strategies to limit the amount of greenhouse gas in the atmosphere by placing it in geologically stable environments (REFS??). It is well known that at sufficient pressure (i.e., depth, in the ocean) and at temperatures above (but approaching) the freezing point of water, natural gas (methane) interacts with seawater to form clathrate hydrate, which is an ice-like phase in which gas molecules are trapped in crystalline cages formed by the H_2O molecules (Sloan, 1998; Buffet, 2000; REFS??).

Carbon dioxide, i.e., the most strongly implicated greenhouse gas, also can form clathrate hydrates. Therefore, for the past several years, researchers at MBARI have been using remotely operated vehicles (ROVs) to release liter-scale aliquots of liquid carbon dioxide into the deep ocean, where they photographically have monitored its dissolution, downward percolation into sediments, and formation of clathrate hydrates (Peter B. your favorite REFS??). In such experiments, the newly developed Deep Ocean Raman System (DORS) will be used to make specific geochemical measurements on the liquid CO_2, the seawater surrounding the CO_2, the nature of clathrate hydrates, and the sediments that potentially interact with the CO_2.

THE CHALLENGES

How to Deploy the Deep Ocean Raman System (DORS) at 3.6 km Depth.

The encapsulation of a Raman spectrometer in a pressure-resistant, water-tight housing and its successful deployment by ROV on the ocean floor are very challenging tasks. Scientists and engineers at MBARI work together to routinely send ROVs to the ocean floor with various

payloads (i.e., several types of instruments and monitors) to carry out biological, geological, and chemical experiments. An ROV is an integrated, unmanned submersible that is connected via electrical wiring and fiber-optic cables to the research ship on the ocean surface ("mother ship"). An ROV can be lowered through the water column (at a speed of ?? m/minute) to a depth as great as 4 km, and then placed on the sediments of the deep ocean floor **(Fig. 1: Box Sketch of Ship, ROV, Drawer, Raman probe head, with dimensions sketched in)**. The ROV is well equipped with lighting and video cameras, and its position can be controlled to +/- 0.1 m (??) from the mother ship via GPS. MBARI operates two research ships (*Western Flyer* and *Point Lobos*), which are equipped with two different ROVs (*Tiburon* and *Ventana*, respectively). The ROV *Tiburon*, for instance, measures ?? X ?? X ?? ft (.... m) and has a maximum weight of 7400 lbs. (3356.6 kg), including a payload of 1100 lbs. (499 kg).

Part of an ROV consists of modular tool sleds ("drawers") that carry mission-specific pressure-tight and water-tight payloads and instrumentation, such as the Deep Ocean Raman System (DORS), which is the subject of the present paper. An ROV has robotically operated claws (i.e.,"robot arms") that are manipulated by specially trained pilots from the control room in the mother ship. These claws, which can be extended as far as ?? m from the ROV, can be used to move mission-specific equipment (e.g., animal cages) or an instrument (e.g., the probe head of the DORS) around on the sea floor or to lift it from the sea floor. The position of the DORS probe head can be manipulated with an accuracy of +/- 1 mm (??) via the ROV's robot arm and the skills of the human pilot in the mother ship. Video imaging of the flood-lighted underwater scene enables selection of the sample surface of interest and the proper positioning of the probe head near the sample. The video cameras are mounted on the ROV, and images are monitored live by both ROV pilots and ocean scientists in the ship's control room. The dynamic display of the Raman spectrum also can be observed live on a dedicated computer in the ship's control room, which helps guide the optimum positioning of the Raman probe head

with respect to the target sample. The number of spectral acquisitions and the collection times can be controlled in real time by a scientist in the ship's control room. Raman spectra can be acquired not only while the instrument is stationary on the sea floor, but also during descent and ascent of the ROV, while the probe head is sitting in the ROV's tool sled.

In April 2002, the DORS was deployed to the deep ocean for the first time, in the tool sled of the ROV *Tiburon*. Since then, both of MBARI's ROVs have been involved in a total of five additional successful deep-ocean deployments, all in Monterey Bay off the coast of California.

Specification and Selection of the Core Instrument.

The Deep Ocean Raman System that we deployed on the ocean floor is based on a laboratory-model, laser Raman spectroscopic system from Kaiser Optical Systems, Inc. (KOSI), which we call the "core instrument". The need for the DORS to be placed repeatedly at different sites on the sea floor demanded that the core instrument have as few moving parts as possible, and that the laser give a stable output under the thermal conditions pertinent on the sea floor.

A frequency-doubled Nd:YAG laser operating at 532 nm was chosen (model DPSS532 by Coherent) because of its stability and its ability to be cooled simply by thermal conduction through the high-pressure housing and through its contained air. In addition, the 532-nm excitation wavelength was selected for its relatively efficient propagation through sea water.

The analysis of the wide range of materials and processes of scientific interest on the sea floor requires a core Raman spectrometer that is physically robust, measures the full spectral range of 100 to 4000 Δcm^{-1}, and has a resolution on the order of 2-3 cm^{-1}. The wide spectral coverage is required so that data can be collected in the low-wavenumber range (on sulfur and typical minerals, which incorporate inorganic complexes), mid-range (on volatiles such as CO_2, O_2, N_2), and high range (for organic compounds and for the OH-groups of

clathrate hydrates and hydrated minerals such as zeolites and clays). Appropriate resolution is required to distinguish mixtures of phases with similar band positions, such as minerals undergoing phase changes and experimentally introduced materials with contrasting isotopic shifts (e.g., ^{12}C and ^{13}C).

Our requirements were met by the following core instrument: Kaiser's HoloSpec f/1.8i spectrometer with a transmissive grating; a front-illuminated CCD camera with 2048 x 512 pixels, by Andor Technology; and Kaiser's Mark II holographic filtered probe head with two interchangeable optical elements: a "dry" optic (i.e., an ~10X objective lens with a focal length in air of ~ 6.4 cm and ~ ??cm (Sheri??) when projected through a fish-eye lense into seawater; these are also the approximate working distances) and an immersible optic that consists of an approximately ??X lens integrated into the tip of a 25.4-cm--long metal cylinder that can be immersed into pressurized liquids over a wide range of temperatures, including the waters of the open ocean.

Making the Core Instrument "Seaworthy".

The core Raman instrument was re-configured to permit its deployment in the deep ocean (up to 4000 m depth) by either of MBARI's two remotely operated vehicles. The as-received laboratory spectrometer, plus a single-board computer, were re-packaged into three pressure-tight housings **(Fig 2.:Cut-away diagrams of the 3 pressure-cannisters with the Raman components. Request MBARI to make these illustrations in black and white)**: the electronics housing (fiberglass cylinder 101.6 cm long, 25.4 cm in diameter), the spectrometer housing (aluminum cylinder 76.2 cm long, 32.4 cm in diameter), and the probe head housing (titanium cylinder 30.5 cm long, 14 cm in diameter). The electronics housing holds the Nd-YAG laser, a power inverter (48 VDC to 110 VAC), and a single-board computer with the CCD controller card and flash memory. The spectrometer housing holds the spectrometer, the CCD

detector, and the associated electronics. Because the CCD detector is mounted perpendicular to the spectrometer, an 20.3-cm--long, 17.8-cm-diameter cylinder protrudes from the body of the housing to contain the detector. The probe head is alone in its housing, which has a handle that allows the ROV's robot arm to grab the probe head (in its housing) and move it to bring the laser to focus on interesting targets. When the dry optic is deployed, it needs to be mounted in the probe head. The latter is terminated by a pressure-resistant, ~1.3-cm-thick glass dome ("fish-eye lens") through which the exciting laser emerges. For deployment of the DORS to depths as great as ??m, the immersion optic can used. In this case, the dome window is replaced by a flat titanium endcap with a Conax Buffalo Technologies packing gland, which seals the endcap against the 1.3-cm-diameter tube of the immersion optic. The tip of the immersion optic projects directly into the water.

An electrical cable connects the electronics housing to the ROV to provide power to the DORS and to communicate through the ROV to the surface via Ethernet protocol. For our application, Kaiser and MBARI software engineers developed a remote protocol for controlling the instrument on the sea floor via a computer in the mother ship's control room. Two additional electrical cables connect the electronics housing to the spectrometer housing: one connecting the CCD detector to its controller card on the single-board computer, the second providing power and other communications. The probe head is connected to both the laser (in the electronics housing) and the spectrometer via fiber-optic cables. The excitation fiber (connecting the laser to the probe head) is 62.5 μm in diameter, and the collection fiber (connecting the probe head to the spectrometer) is 100 μm in diameter. The electrical cables use standard Seacon Mini-Con™ underwater connectors. Penetrators that were designed and built at MBARI permit the pressure-jacketed (to prevent losses associated with fiber optic connectors) fiber-optic cables to enter the pressure housings.

The electronics housing and the spectrometer housing remain in the ROV's tool sled

during the entire dive. The probe-head housing stays in the tool sled during descent and ascent of the ROV (each trip takes about 1 hour per kilometer??), but once the vehicle is on the sea floor, the probe-head housing can be picked up, tilted, rotated, and translated by the ROV's robot arms. The translation distance (about ?? m) is limited by the "arm's reach" and the lengths of the fiber optic cables that connect the probe head to the DORS spectrometer and electronics housings **(Proposed cover figure, showing configuration of all major parts of the system)**.

The dangers of cold temperatures and a corrosive seawater environment also pose challenges that need to be addressed. Temperature, humidity, and water sensors are installed in the electronics housing and the spectrometer housing to alert the user to leaks before serious water damage can occur. Knowledge of the instrument's temperature and other possible instrumental effects is very important for proper interpretation of the spectral data.

Seagoing instruments are often subjected to significant jostling and vibrations, unlike in a laboratory environment. In addition, between ocean dives, the DORS typically is removed from its pressure housings and taken apart for inspection, repair, and cleaning. For these reasons, a few components in the original core instrument were removed and replaced with more robust components. These parts included the laser injector and the slit optimization mechanism. A motorized single-axis stage recently was installed such that the slit position can be optimized via remote control while the spectrometer is enclosed in its pressure-tight housing.

EXPERIMENTAL PROTOCOLS

Laboratory Simulations.

Many comparison measurements were made with a duplicate KOSI Raman instrument (not enclosed in pressure housings, but with the same laser, gratings, and CCD detector as the MBARI instrument) in our laboratory at Washington University in St. Louis. Simulation experiments were done in a 38-liter aquarium tank using probe-head optics identical to the two

types deployed on the seafloor (dry optic and immersion probe optic), except for the lack of a "fish-eye lens" in front of the dry optic. We analyzed aqueous salt solutions at temperatures between 2 and 25 °C, and we analyzed mineral samples submersed in water. Even though we were able to simulate the low temperatures appropriate to the ocean floor, however, we were not able to simulate in our laboratory the high pressure that is encountered on the deep ocean floor (e.g., ~360 atm at 3.6 km).

Calibration Routines.

The Kaiser Raman system, like many modern Raman systems, can be purchased with spectral calibration standards (He-Ne lamp and white light for wavelength and intensity calibrations, respectively) and appropriate software to allow automatic correction of each spectral acquisition for both wavelength and intensity. Such a system works very well under laboratory conditions: the two calibration lamps are operated and then a known spectral standard (such as cyclohexane or isopropanol) is analyzed as a so-called "Raman-shift standard" to complete the calibration protocol. The unknown samples are run at the same conditions, and the calibration factors automatically are applied to the resultant spectra.

The situation in deep-ocean spectroscopy, however, is much different. Although it is still possible to carry out a calibration shipboard immediately before the DORS is lowered into the ocean (procedure we have been following), the calibration is "lost" as the instrument moves downward: The decreasing water temperature cools and differentially contracts the components of the laser, spectrometer, and CCD detector. Moreover, the pressure-tight housings flex slightly, thereby affecting the physical alignment of the components. Our results suggest that the above factors shift the recorded band positions by several wavenumbers and probably affect the recorded band intensities differently across the spectral window.

In six dives during the year 2002, the DORS was calibrated for wavelength only; an

intensity calibration has not yet been attempted. Absolute wavelength calibration with a Ne lamp standard was performed immediately before each dive on shipboard. This was followed by a "Raman shift calibration" (i.e., relative wavenumber calibration) on shipboard using both a liquid standard (isopropanol in a glass vial) and a mineral standard (1-mm-diameter diamond wafer glued on the outside of a glass vial). Both Raman shift standards were used for shipboard calibration immediately before and immediately after each dive. In addition, the standards also were taken down on specific dives, and were measured about once an hour at ambient *in situ* sea floor conditions. The purpose was to evaluate dive-induced changes in the instrument's response, so that these extraneous effects could be corrected for.

The isopropanol standard was contained in a glass vial connected to a deformable, plastic reservoir, which injected additional isopropanol into the vial as the plastic reservoir became compressed at increasing depth. **(Fig. 3: Isopropanol on deck vs. isopropanol at depth vs. isopropanol in laboratory; Fig. 4: isopropanol and diamond on deck vs. at depth vs. in lab).** Depth-induced shifts in the band positions were recorded not only for isopropanol and diamond, but also for seawater as a function of its pressure and temperature, which control any fluid's density. The spectra for seawater as a function of depth were obtained by directing the laser (in its housing in the ROV's tool sled) into ambient seawater as the ROV descended and ascended.

In some of our later dives, we placed a small, polished diamond chip within the probe head, but considerably off-focus (REF ?? John??). Nevertheless, the extremely strong Raman-scattering efficiency of diamond assured that this arrangement provided continuous monitoring of the diamond's band position as a Raman shift standard; the 1332 Δcm^{-1} band was weakly superimposed on each of the spectra. Because the diamond was within the pressure-tight housing, it was shielded from pressure and only underwent cooling. Thus, no pressure-induced shifts could have occurred in the band positions (spectra not shown). Because we previously

had established in the laboratory the band shift of diamond as a function of temperature, the continuous monitoring of the diamond band during a dive provided information on the dive's effect on the spectrometer. Based on several types of information, we estimated that we needed to correct the spectra obtained in the deep ocean by a shift of about +3.2 cm^{-1}; the exact value was slightly different for each dive. The spectra shown in the present paper, with the exception of those in Fig. 6, are our best attempts to correct the observed band positions for dive-induced instrumental shifts. The large uncertainties, of ± 3 cm^{-1} in accuracy and ± 0.4 cm^{-1} in precision, reflect the problems outlined above. As mentioned above, the spectra shown are not corrected for relative intensity across the spectral window.

Typical Analytical Conditions.

laser power?? No spectral slit used on the first ?? cruises. With slit, spectral resolution is about ?? Thus, early dives had lower resolution than this.

During the full duration of the each ROV dive (i.e., both during descent and ascent as well while the ROV is residing on the sea floor), the laser remained on. It could be seen (as captured live by the ROV's video cameras and watched by the scientists in the ship's control room) as a focused green light beam exiting the probe head.

how many spectra co-added??

EARLY SUCCESSES OF THE DEEP OCEAN RAMAN SYSTEM

Fluids.

Among the first spectra obtained with the DORS were those of seawater. An early concern that did not materialize into a problem was the possibility that laser irradiation of the organic debris ("marine snow") that constantly rains down through the ocean water column would create a high fluorescence background in Raman spectra taken in the deep ocean. Fortunately, the spectra

we have taken of seawater at depth (100's to 1000's of meters) have low backgrounds. **[Fig. 5. Spectrum of just seawater at 3600 m depth compare to that of pure water]** The Raman spectrum of ocean water is characterized by the OH-bend and -stretch, centered at ?? and ?? Δcm^{-1}, together with the v_1 sulfate band (?? Δcm^{-1}), as has been reported for *ex situ* analysis of natural and synthetic seawater (REFS??). The covalently bonded anionic complex, SO_4^{2-}, which has an average concentration of 28 mM in seawater, is readily recorded. Although the purely ionic species such as Na^+ and Cl^- do not yield explicit Raman bands, the nature of and the concentration of dissolved salts have a measurable effect on the band-structure of water. This effect is illustrated by the difference in the OH bands between the spectra of pure water and seawater, as shown in Fig. 5 (REFS Mernagh and Wilde, 1989; Furic, et al., 2000 ??).

In the past, we have done Raman analyses in the laboratory (Seitz, et al., 1996) on pure CO_2 enclosed in a glass capillary under controlled conditions of temperature (23°C) and pressure (up to 700 bars). In April, 2002, we made comparable measurements, using the DORS, on several hundred (how many ml??) ml (measured in liquid state) of essentially pure CO_2, which were injected underwater into an inverted glass vessel. The glass beaker resided within the tool sled of the ROV, which has numerous openings that permit the passage of seawater through it. The open-ended, inverted beaker permitted the CO_2 to equilibrate with both the pressure and temperature of the surrounding seawater as the ROV descended to 664 m depth at ?? °C and then returned to the surface. The beaker underwent video monitoring during the entire experiment. During the initial descent, CO_2 was introduced in several aliquots (beginning at 200 m depth), as the increase in pressure caused its compression. At about 400 m depth, we visually and spectrally recorded the CO_2 phase change from vapor to liquid. At depths shallower than this, two interfaces could be observed in the beaker, i.e., 1) a higher interface between air containing small concentrations of dissolved water + CO_2 (air entrained as the beaker initially was inverted) and liquid CO_2 below it, and 2) a lower interface between liquid CO_2 and seawater

below it. Although the density of the CO_2 continuously increased as the ROV moved deeper in the ocean, for the depth and temperature range of this experiment, the CO_2 phase always was less dense than the seawater (thus, the inversion of the beaker).

During the entire descent and ascent, the laser beam was focused into the same small volume within the beaker. Raman spectra were taken at a total of 13 intervals (spaced by 40-100 m) along the descent and ascent of the ROV. A marked increase in signal intensity and downshift in band positions accompanied the CO_2 phase change from vapor to liquid state **(Fig 6. : Spectra from CO2 in jelly jar at different depths).**

It is well known that the physical parameters of CO_2, such as density and solubility in (sea)water, are a function of temperature and pressure. The ability to monitor changes in these fundamental parameters with the DORS is important in experiments that test the feasibility of sequestering CO_2 on the ocean floor. The Raman spectrum of CO_2, in turn, is a moderately accurate monitor of its density (Wright and Wang, 1973, 1974; Schrötter and Klöckner, 1979; Garrabos, et al., 1980, 1989; Seitz, et al. 1996; Hacura, 1997; Maté, et al., 1998; Olijnyk and Jephcoat, 1998; Blatchford and Wallen, 2002), and Raman spectroscopy can be used to determine the concentration of CO_2 (above an instrument-specific detection threshold) dissolved in water (Berger, et al., 1995; Sloan ??; REFS??).

As our laboratory work (Seitz, et al. 1996) and that of others has shown, as CO_2 density increases, the Raman band positions of both the v_1 and $2v_2$ bands of the Fermi diad downshift, whereas the spectral separation increases between these two bands (CHECK these: Wright and Wang, 1973, 1974; Schrötter and Klöckner, 1979; Garrabos, et al., 1980, 1989; Hacura, 1997; Maté, et al., 1998; Olijnyk and Jephcoat, 1998; Blatchford and Wallen, 2002). We used the ??? (Ed??) equation of state to calculate the density of the CO_2 in our previous laboratory experiments (Seitz, et al., 1996) and in the ocean experiment. **Figure 7** compares the spectral separation in the Fermi diad bands as a function of calculated CO_2 density for both our laboratory

data and ocean data. The laboratory and DORS curves are, for the most part, parallel but offset by more than a wavenumber, suggesting that the dispersion function applied to the DORS was inappropriate for the conditions during the dive. The marked non-parallelism in the low-density (i.e., low-pressure, shallow-ocean) data suggests that the CO_2 had not come to thermal equilibrium when the spectra were acquired. Such a mistake in the input temperature would lead to a miscalculation of the density of the CO_2.

In other experiments testing the possibility of ocean sequestration of CO_2, liquid CO_2 was analyzed at 3600 m depth on the seafloor. The Raman probe head was focused onto/into liter-scale immiscible blobs of CO_2 in several different configurations: a) laser projecting through a glass vessel into which CO_2 had been injected from the delivery system on the ROV, b) laser projecting downward directly into CO_2 in such a filled vessel, and c) laser projecting onto a CO_2 blob placed directly on the sediments of the ocean floor, typically inside of a ??-cm-diameter PVC "corral." **[Figure 8: small photos of those 3 configurations] Figure 8** shows a typical spectrum acquired on (which configuration??): The CO_2 bands are superimposed on the background seawater spectrum.

Gases.

This may not be an appropriate heading, because sufficient pressures here make the volatile species supercritical.

SHERi's N2?CO2 dives???? What depth? Liquid, gas, or supercritical fluid? One spectrum.

Fig. 10.

Solids.

To test our capability in manipulating the probe head into focus for analysis of a rock or mineral sample fully immersed in seawater, we strapped several samples of carbonate rocks and

carbonate minerals to the outside of a ??-cm-diameter PVC ring and took them to the ocean floor. **[Fig. 12. Photo of corral with rock and mineral slabs fixed to it; laser irradiating calcite rhomb].** In one geometrical configuration of the DORS probe head with respect to the target sample, the laser was projected perpendicular to the approximately 7 x 10 cm face of a translucent, yellow, cleaved rhomb of calcite ($CaCO_3$). This configuration readily produced a strong Raman spectrum of calcite superimposed on the background spectrum of seawater. **[Fig. 11. Spectrum of calcite rhomb on ocean floor.]**

The superposition of the seawater spectrum does not appreciably degrade the signal:noise ratio of spectra taken of minerals on the ocean floor: The water causes little elevation in spectral background, and the positions and strengths of the OH-bands of water are such that they do not interfere significantly with the bands of most minerals. Even hydrated and hydroxylated minerals tend to have OH-stretch bands that are much narrower than and readily distinguished from those of water.

To date, there has been only one instance in which very high fluorescence precluded useful Raman measurements. This effect occurred when the laser was directed onto clay-rich, organic-rich sediments of the ocean floor. Such fluorescence occurred both during *in situ* measurements and in measurements made in the laboratory on samples retrieved from cores.

ADDITIONAL CHALLENGES

Selection of Better Calibration Standards.

Our challenge is to find suitable calibration standards that can be analyzed on the ocean floor, thereby permitting back-correction of the recorded spectra to proper band position and relative intensity. The criteria for such ocean-floor calibration standards are that they: 1) should have numerous, narrow bands distributed across the entire 100-4000 Δcm^{-1} region, 2) should be stable and transportable to depth in seawater (if a container is required, it must be transparent to

the laser), 3) should either not be spectrally sensitive to changes in temperature and pressure, i.e., density, or should have a very well-known spectral response to changes in density.

The selection of such standards has been difficult. Some liquids that are commonly used as laboratory standards, such as isopropanol (which we did take to the ocean floor in our first deep ocean dives), are much more compressible than water; this means that they undergo significant changes in density, which can be assumed to be accompanied by significant changes in band positions.

Minerals, in principle, should be better calibration standards. Diamond, which we used so far, unfortunately has only one Raman band. This band is very strong, however, and its 1332 Δcm^{-1} position was very useful for our first study on CO_2, because it falls right between the positions of the Fermi diad of CO_2 at about 1285 and 1388 Δcm^{-1}. We currently are investigating the suitability of hydrous silicate minerals as standards that could be exposed directly to the ocean and taken down on dives

Improvement of Control on Laser Focus.

Laser Raman spectroscopy is a scattering phenomenon that calls for the laser to be focused on a relatively small volume of material in order to create sufficient power density. The same optics used to focus the laser also permit the spatial isolation of the scattering signal; with appropriate optics, one selectively can retrieve the back-scattered radiation and thereby isolate the signal of the desired target from that of its background/matrix.

Whether the Raman spectrometer is configured with an optical microscope (in the laboratory) or with a hand-held probe head (for sea-floor operation), there are three important issues to consider in the selection of a focusing lens: power-density at the sample, stand-off (i.e., focus) distance, and depth-of-field. These parameters, of course, are not independent of each other (REFS??). For instance, the power-density of the laser irradiation, which is inversely

proportional to the size of the focused beam spot, is controlled by the magnification and numerical aperture of the lens.

In our first six deployments of the DORS to the deep ocean floor, it has not been possible to finely control the position of the Raman probe head with respect to the target object. So far, our ability to control the position has been limited to ± a few millimeters. Positioning depends mostly on the fine-motor skills of the individual pilot who maneuver the robotic arms of the ROV from the control room on the mother ship. Under such conditions, it is better to use a lens that offers a large stand-off distance (to prevent the need to bring the probe head so close as possibly to impact the sample). Large stand-off distances, however, typically correlate with a large depth-of-field. Depending on the nature of the target object, this correlation can be useful. Even if the distance between the probe head and the sample is difficult to control at the sub-millimeter level, a lens with a large depth-of-field permits reasonable laser-focusing and recovery of the Raman signal. Thus, the f/2 lens of the dry optic (stand-off distance in water increases to ?? cm) permitted focus through sea water onto an immiscible bleb of carbon dioxide on the ocean floor, through a glass beaker into the liquid CO_2 within it, and through sea water into a transparent fragment of the mineral calcite.

??Do we NEED a TABLE with optical parameters for the two optics: focal length/stand-off distance (in air, in water), diameter of focused beam, depth of field??

Our underwater test, however, demonstrated two drawbacks to the large stand-off distance (focal length) of the focusing lens used so far. Firstly, the laser has a long path length through the ambient medium (sea water) before it reaches the target at the laser focal point. Although this surrounding medium is not at the point of laser focus, it does scatter the beam (thereby decreasing the laser intensity on the intended target), and some of that back-scattered light is accepted by the lens. This means that the spectrum of the sample is always superimposed on that of the background (Figs. 3, 9, 12, and 10??). Secondly, this spectral

superposition eliminates the ability of the Raman probe to spatially resolve compositional differences along the laser beam path, e.g., changes in concentration of dissolved components.

For the above reasons, we have begun testing the use of a high-pressure immersion tip, also with an f/2 lens, but with only an 11-mm stand-off distance. This immersion lens has a focal depth of about 0.2 mm as compared to about 1.0 mm depth of focus for the dry lens. The smaller beam spot (greater focusing) of the immersion lens produces higher signal:noise ratios, and the shorter focal length produces better rejection of the sea water signature (SHERI??) than does the dry optic. However, the smaller depth of field of the immersion lens requires better focusing of the probe head onto the sample in order to achieve a reasonable signal:noise.

Another challenge to the focusing capability of our DORS comes from optical interfaces. Because Raman spectroscopy is a scattering phenomenon, the retrieved signal is affected by all aspects of optical scattering within the sample and on the sample's surface. The case of two transparent liquids with a smooth (although curved) shared interface, as between immiscible liquid CO_2 and water, creates no strong optical problem, as long as the laser is aimed perpendicular to the interface. Samples that are milky translucent or extremely fine-grained (whether as powders or consolidated solid masses), however, cause almost the same responses as opaque phases. This non-penetration of the interface is due to light scattering, whose intensity tracks with the difference in refractive index between the two (admixed) substances. Thus, even with the relatively large depth of field of the dry optic in its pressure housing, we were unable with the DORS to obtain a good Raman signal from the fine-grained, milky translucent limestone and marble samples (both rocks are calcium carbonate, i.e., calcite) fixed onto the PVC corral, although the signal from the transparent calcite was very strong (Fig.11).

These optical challenges provided the impetus for additional engineering upgrades to the instrument and the testing of additional optical devices, as described below. [Note: brief

references appear at end of next section.] Peter?? Sheri?? We could leave the discussion simply as it appears below, or you could add more information here on the upgrades.

SUMMARY AND CONCLUSION

We have demonstrated that it is possible to obtain high quality Raman spectra on the seafloor at 3600 km depth. There are three main reasons that Raman spectroscopy has not previously been used in this environment. Only recently have the following essentials become available: 1) Sophisticated carrying platforms such as research ROVs; 2) small, light-weight, portable Raman instruments; and 3) small stable solid-state lasers. Third-generation Raman spectrometers are now available with holographic gratings, high throughput, and very sensitive CCD detectors that provide the small size and the stability that allowed us to construct the Deep Ocean Raman System (DORS).

During construction and implementation of the DORS, there were many difficulties that needed to be overcome: limitation of size and weight, elimination of fragile optical components, building of pressure-tight housings, and the establishment of electronic communication and control over a 4-km-long tether from the ship to the remotely operated vehicle and the deep ocean Raman system. (??Add reference to manuscript by George, et al.)

We have accomplished the first feasibility tests, but we are still in the early stages of solving problems. We have been able to carry out some aspects of calibration (with a diamond chip in the optical path), but we need to improve our methods of both wavelength and intensity calibration. The challenges of precise positioning and focus of the laser have limited our successful analyses to large, transparent objects on the first dives.

In the future, ??plans for various targets?? In response to the needs for accurate laser positioning, focus, and manipulation in the Raman analysis of solid phases (rocks, precipitates etc.), we are designing a precise X-Y-Z positioning system for measurement of solids in the deep

sea. We also plan to add "through-the-lens visualization" to guide our positioning of the laser beam on the target of interest.

??Final comments?? Peter??

Acknowledgments

We thank the following people for providing carbonate samples that were taken to the sea floor: Bob Cradock, Cheryl Seeger, Jere Cadoret, and Larry Nuelle. DoE acknowledgment – Peter. Others??

Figure Captions

Fig. 1. Box sketch of ship, ROV, drawer, Raman probe head, with dimensions sketched in. **Request MBARI to make this illustration and provide caption**.

Fig 2. Cut-away diagrams of the 3 pressure-cannisters with the Raman components **Request MBARI to make these illustrations (in black and white with WHITE background) and provide caption.**

Fig. 3. Comparison of isopropanol spectra in the low-wavenumber (a) and high-wavenumber (b) spectral regions. The spectra were acquired with DORS on deck in the Pacific Ocean (DORS Deck), with DORS at 3600 m ocean depth on the ROV Tiburon (DORS 3600 m), and with the duplicate laboratory Raman system at Washington University (Laboratory). For these spectra, a 100 -μm spectral slit was used on the laboratory instrument, but no slit on the DORS.

Fig. 4. : Comparison of spectral quality and peak position measured with DORS and with the well-calibrated duplicate laboratory Raman system at Washington University. (a) Blow-up of one of the many Raman peaks for isopropanol (b) Blow-up of the only Raman-active peak for diamond. The spectra were acquired with DORS on deck in the Pacific Ocean (DORS Deck), with DORS at 3600 m ocean depth on the ROV Tiburon (DORS 3600 m), and at Washington University (Laboratory). The laboratory instrument had a 100 -μm spectral slit, whereas the DORS had no slit for the acquisition of these spectra.

Fig 5: Comparsion of the Raman spectra of deionized water (acquired with the laboratory system at Washington University (at room temperature) and ocean water (acquired with the MBARI Deep Ocean Raman System at 3600m depth and 4 °C). Insert shows enlargement of 800 to 1900 Δcm^{-1} spectral region. The laboratory system had a 100 -μm spectral slit , wheras the DORS had no slit.

Fig. 6. Raman spectra of liquid and gaseous CO_2 collected during small-scale CO_2 injection experiments on the ROV Tiburon. Raman spectra were acquired during ascent from the ocean floor while DORS was sitting in the ROV's toolsled. Difference in phase is recognized by strong increase in intensity and spectral downshift in the liquid compared to the gas. In this figure only, the spectra have uncorrected peak positions. The spectral separation between the two CO_2 peaks (more accurately measurable than the absolute peak positions) is shown in Fig.7.

Fig. 7. Plot of the Raman spectral separation between the two peaks of Fermi diad of CO_2 versus CO_2 density (see text). Laboratory data were collected at 23°C at well calibrated pressures; Raman peak positions were corrected based on gas emission lines from calibration lamps (see Seitz, et al., 1996). DORS data (uncorrected peak positions) were collected in

ascent from 664 m depth in the ocean. Parallelism of laboratory and DORS data indicates appropriate spectral response to changes in density. Offset between the two curves suggests that the dispersion function that was applied to the DORS was inappropriate under the specific dive conditions.

Fig. 8. Three types of experimental configurations during Raman analysis on the deep ocean floor: a) CO_2-water-hydrate "slush" in a beaker on sea floor; probe head focused horizontally into beaker; b) probe head focused vertically down onto large, clear blob of CO_2 that almost completely covers bottom of corral; c) robotic arm (vertical, black) holds probe head and brings the laser into focus on (and through) CO_2-water-hydrate slush in graduated cylinder; large beaker of similar slush lies on its side on ocean floor (bottom, left).

Fig. 9. Raman spectrum taken by DORS on a large liquid CO_2 blob on the sea floor at 3600 m depth. Although only CO_2 was within the focal volume of the probe head, Raman scattering also was detected from the intervening sea water due to the long focal length of the probe optics.

Fig. 10: N2/CO2 gas from Sheri's dive.... Please provide GRAMs files ...

Fig. 11. a) Several sawn slabs of calcite and carbonate rocks (some shown by arrows) are strapped to a PVC corral at 663 m depth on sea floor. Probe head has been guided into focus on the large calcite rhomb with a white vertical stripe. b) Raman spectrum of that calcite rhomb (1 second acquisition).

Fig. 12. Series of Raman spectra (each with 1 second acquisition time) as the probe head's focus is adjusted on a CO_2 blob on the ocean floor. As explained in Fig. 9, both sea water and

CO_2 are in the laser's beam path. Spectra of both phases are recorded, but the CO_2:H_2O band ratios vary with focus.

REFS

Berger, A.J., Wang, Y., Sammeth, D.M., Itzkan, I., Kneipp, K., and Feld, M.S. 1995. Aqueous dissolved-gas measurements using near-infrared Raman spectroscopy: Applied Spectroscopy, 49, 1164-1169.

Blatchford, M.A. and Wallen, S.L. 2002. Development and validation of spectroscopic methods for monitoring density changes in pressurized gaseous and supercritical fluid systems: Analytical Chemistry, vol. 74, 1922-1927.

Buffett B. A. (2000) Clathrate hydrates. Annual Review of Earth and Planetary Sciences 28, 477-507.

Garrabos, Y., Echargui, M.A., and Marsault-Herail, F. 1989. Comparison between the density effects on the levels of the Raman specgra of the Fermi resonance doublet of the $^{12}C^{16}O_2$ and $^{13}C^{16}O_2$ molecules: Journal of Chemical Physics, 91, 5869-5881.

Garrabos, Y., Tufev, R., and Le Neindre, R. 1980. Rayleigh and Raman scattering near the critical point of carbon dioxide: Journal of Chemical Physics, 72, 4637-4651.

Hacura, A. 1997. High pressure Raman study of Fermi resonance in CO_2 in gaseousCO_2-N_2 mixtures: Physics Letters A, 227, 237-240.

Maté, B., Tejeda, G., and Montero, S. 1998. Raman spectroscopy of supersonic jets of CO_2: Density, condensation, and translational, rotational, and vibrational temperatures: Journal of Chemical Physics, 108, 2676-2685.

Mernagh, T. P. and Wilde, A. R. 1989. The use of the laser Raman microprobe for the determination of salinity in fluid inclusions: Geochimica et Cosmochimica Acta. 53, 765-771.

Olijnyk, H. and Jephcoat, A.P. 1998. Vibrational studies on CO2 up to 40 Gpa by Raman spectroscopy at room temperature: Physical Review B, 57, 879-888.

Pasteris, J.D., Freeman, J.J., Goffredi, S.K. and Buck, K.R. 2001. Raman spectroscopic and laser scanning confocal microscopic analysis of sulfur in living sulfur-precipitating marine bacteria: Chemical Geology, 180, 3-18.

Schrötter, H.W. and Klöckner, H.W. 1979. Raman scattering cross-sections in gases and liquids, *in* Weber, A., editor, Raman Spectroscopy of Gases and Liquids: Berlin, Springer-Verlag, pp. 123-166.

Seitz, J.C., Pasteris, J.D., and Chou, I-M. 1996. Raman spectroscopic characterization of gas mixtures. II. Quantitative composition and pressure determination of the CO_2-CH_4 system: American Journal of Science, 296, 577-600.

Sitter, D.N. and Gelbart, A. 2001. Laser-induced fluorescence imaging of the ocean bottom: Optical Engineering, 40, 1545-1553.

Sloan E. D. (1998) *Clathrate Hydrates of Natural Gases*. New York: Marcel Dekker, Inc

Villagarcia, M.G., Llinas, O., Reuter, R., Rueda, M.J., Zielinski, O., Godoy J. 2002. Distribution of gelbstoff fluorescence in the Northern Canary Box: Deep-sea Research Part IIBTopical Studies in Oceanography, 49, 3497-3511.

Wright, R.B. and Wang, C.H. 1973. Densityr effect on the Fermi resonance of gaseous CO_2 by Raman scattering: Journal of Chemical Physics, 58, 2893-2895.

Wright, R.B. and Wang, C.H. 1974. Effect of density on the Raman scattering of molecular fluids. II. Study of intermolecular interaction in CO_2: Journal of Chemical Physics, 61, 2707-2710.

Identifying the Scientific Targets