Project Manager: Peter Brewer

Acquisition of Laser Raman Spectrometer for Deep-Sea Studies

Requested Incremental Budget: $356,114.86
Total Project Costs, Including Labor:
Duration of Project: Jan. 1, 2000 - Dec. 31, 2002
Date Submitted: June 1, 1999
Lead Scientist: Peter Brewer
Lead Engineer: George Malby
Project Team: Brewer, Malby, Pasteris, Kvenvolden, Buck, Peltzer, Friederich, Paull
Summary of Resources Requested
(Total No. of days per Division for 1999):

R&D Total: 381
TSD Total: 200
DMO Total: 8
ITD Total: 20

1) Abstract

In this new project we seek to extend MBARI's deep-sea measurement capability by adding Raman spectroscopy to the suite of tools available for both laboratory and *in situ* work. This is a powerful, nondestructive, tool for obtaining quantitative molecular data on a wide variety of solutions, and solids, and under extreme conditions (Lyon et al., 1998). It may be used both in the bulk solution mode, and for the microscopic analysis of small particles and fluid inclusions. MBARI's principal observing tools, it's ROV systems, are well adapted to deploy this instrumentation, which is of interest to many scientists. The proposal directly addresses MBARI's mission of better instruments and methods for scientific research in the deep-sea, and exemplifies the peer relationship of scientists and engineers in achieving this goal.

In many ways this technique extends the present broad projection of visible light to interrogate the scene for the scientist to the sophisticated use of the returned scattered (and thereby wavelength shifted) signal from intensely focused laser light to yield specific information on the chemical, and physical, characteristics of the target. Common examples of chemical species detectable by Raman in solution are CO_2, HCO_3, CO_3, SO_4, NO_3, PO_4, $SiOH_4$ etc., although detection limits are now too low for sea water, except for SO_4 which is readily observable. It is an advantage that simple dissolved ionic species (Na, Cl, K, Mg etc.) are not seen. For solids a huge variety of materials have been examined, and it is here that our effort will focus; for example see the recent direct investigation of the specific chemical form of sulfur species within marine bacterial cells (thioploca) collected by MBARI. The investigation of the changing cage occupancy of the hydrates (Seitz et al., 1987) formed in our *in situ* studies is of prime importance. Additionally, raman spectroscopy can provide real-time mineralogy of samples enabling better decisions regarding which samples to collect. Isotopic signatures can also be spectrally resolved (the heavier species lies to the low-Δcm^{-1} side) if the abundance of the trace isotope is artificially enriched so as to permit reasonable detection.

In preparing for this initiative we invited Dr. Jill Pasteris (Washington University), an acknowledged expert in the field (Pasteris,1998), to MBARI for a seminar and advanced consultation. She is formally collaborating on this project, and with 1999 seed money is already working with lead engineer George Malby, to identify instrument specifications and preferred vendors. This proposal capitalizes on the extraordinary advances in technology that have been made in the last few years, and would bring new and very powerful capabilities to MBARI.

2) Proposal

Raman Spectroscopy. The fundamental principle of Raman spectroscopy arises from the nature of the interaction of light with the covalent bond. Most of the light interacting with the sample induces elastic (Rayleigh) scattering of the same wavelength as the incoming radiation. A small fraction, about 1 in 10^8 scattering events, involves inelastic scattering in which the exiting radiation has a different wavelength from the incoming laser light. Most inelastic scattering involves a loss of energy (Stokes Raman scattering), and the amounts of individual energy losses are directly correlated with the energies of the specific vibrational modes of the covalent bonds in the sample. The spectrum therefore contains detailed

information on the bonding configurations and basic structure of a compound. Thus polymorphs of the same composition (e.g. calcite, aragonite) have different Raman spectra.

A laser Raman spectrometer for the laboratory essentially consists of an optical microscope for imaging the sample and focusing the laser beam, a spectrometer system for discriminating the scattered wavelengths, and a detector for recording the intensity of the scattered radiation. Since the beginning of the marketing of laser systems there have been tremendous improvements in capability, and the time is ripe for extension to the deep sea world. For the laboratory a microscope is important, but for the initial deep-sea application to hydrates it is not since it is the changing nature of the bulk phase that is at present unknown, and very large gains in knowledge can be made without microscopically precise placement of the laser beam. However, in a parallel MBARI proposal a deep-sea ROV microscope is planned, and we are well aware of, and pleased by, this initiative.

A critical part of the adaptation to deep sea work is the fiber optic link to the sensor head. This unit basically has to serve three functions: to transmit the excitation light to a precise point on the sample (under fine motor control by the vehicle), to transmit the scattered return signal to the spectrometer, and to image the sample with sufficient resolution so as to enable the scientist to choose and identify the precise phenomenon or region of investigation.

Preliminary work

(Brewer) In preparation for this effort several preliminary steps have been taken. The P.I. visited the laboratory of Dr. Dendy Sloan (Colorado School of Mines) earlier this year to view his efforts. Dr. Sloan will visit MBARI this summer, and has been asked to give a seminar, as part of an externally funded cooperation on hydrates. Figure 1 shows the Raman spectra of both CO_2 and CH_4 clathrates as obtained from laboratory microscopic studies of fluid inclusions (taken from Fig. 9 in Seitz et al., 1987).

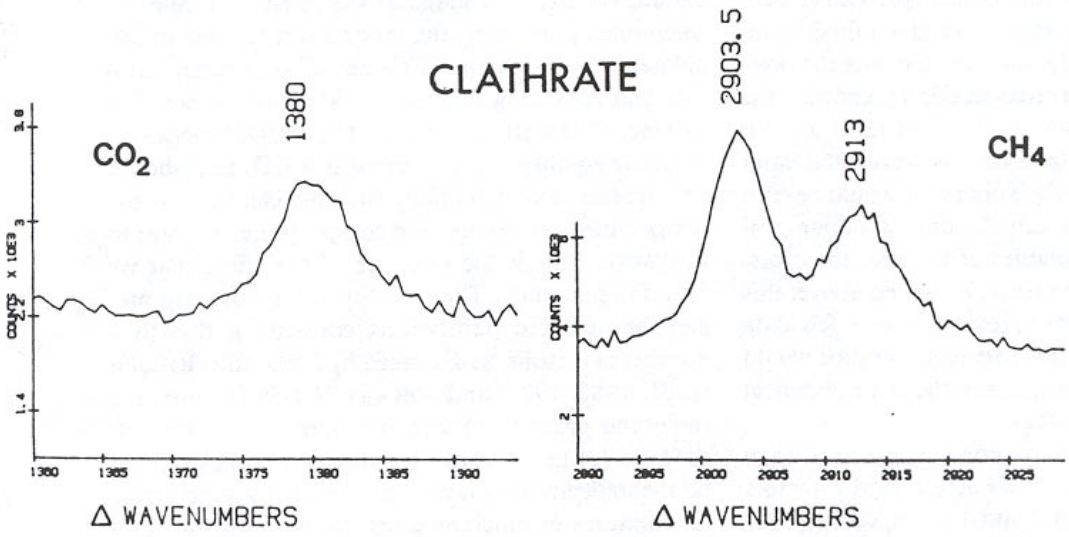

FIG. 9. LRM spectra of clathrate formed in inclusion Jean. Scanned in spectral regions of symmetric stretching vibrations for CO_2 and CH_4. Note double peak for CH_4. Analysis conditions described in text. Sum of 2 coadded scans.

(Malby). George Malby has corresponded extensively with Pasteris, and is taking a key part in vendor site visits in summer 1999. The Raman spectrometer will be configured to operate *in-situ* installed on an ROV (or other remotely operated platform/station). It will consist of, an optical sensing head, an x, y, z head positioner, a laser excitation source, a monochromator with scanning and readout capability, a video camera, the necessary I/O to provide remote readout and control, and a processor and display for observing and recording the data (both spectrographic and visual). Raman spectrometers are available commercially (i.e. from Instruments SA, InPhotonics, Renishaw, Kaiser Optical Systems, and others) – but not in a form suitable for use in the ocean or in configurations that meet the MBARI science needs. In addition, the specific operating parameters of the spectrometer will need to be tailored for use in the ocean. For example, the laser excitation wavelength(s) will need to be chosen so that well known biological fluorescence responses do not fall at the same wavelengths as the Raman shifted spectral lines for the molecular species of interest. As currently conceived, the in water portion of the MBARI Raman spectrometer would consist of two major assemblies – a remotely positionable optical head able to withstand high ambient pressures and an atmospheric pressure container holding the laser, the scanning monochromator, a video camera, and all of the associated local control, power, and I/O circuitry. These two units would be connected together by a triaxial fiber optic cable and a copper cable for positioner power and motion control. Note that a laser is used to illuminate the sample of interest so that only monochromatic visualization is possible. The data processing unit, the computer controller, and the data recorder will be located topside, either on a ship or on the shore. Communications between the in water unit and the topside units will be conducted over a single fiber optic cable which is compatible with those currently installed on the MBARI ROVs. While it is believed that all of the spectrometer components described above exist as commercial units, the specific configuration useful to MBARI does not exist and the engineering effort anticipated is that associated with acquiring, assembling, packaging, and testing the spectrometer.

(Buck). Raman spectroscopy has been used by Kurt Buck in collaboration with Jill Pasteris as a result of her MBARI seminar in March (1999). They have probed the sulfur vesicles of the chemoautotrophic bacterium *Thioploca*. This organism is globally important in both nitrate and sulfur cycles. The sulfur vesicles that are present in the cell walls of this bacterium are thought to be sulfur polythionates and to act as storage products from the oxidation of hydrogen sulfide. This dogma is based predominantly on earlier work with endosymbiotic bacteria of metazoans (clams) (Vetter 1988) or on related freshwater bacteria (Lawry et al. 1981). Various allotrophes of sulfur are susceptible to alteration by organic solvents and temperature so care must be taken when probing these vesicles. Most previous studies did not take this into account. Raman spectroscopy appears to be a near-ideal way to look at these small (2 µm) structures. The non-invasive, non-destructive probing revealed that the sulfur present was an amorphous elemental form rather than the polythionate previously proposed for endosymbiotic bacteria. To suggest that there might be a difference in the storage product between microscopic endosymbiotic and macroscopic free living bacteria is probably not too difficult to swallow. In addition several artifacts associated with the Raman spectroscopy itself have been discerned.

This work will continue through the remainder of 1999 as samples are available and we will expand the compounds probed for to include nitrate and iron, two potential electron acceptors. To compare with earlier (non Raman spectroscopy) results from endosymbiotic

bacteria we have sent away a living vestimentiferans (no sulfur vesicles detected) and plan on also sending away vesicomyid clams. We anticipate that the main body of this research will appear as a manuscript with Pasteris as the lead author, and that the results will also be included in at least two manuscripts currently being prepared at MBARI on *Thioploca* and on endosymbionts of clams and vestimentiferans.

Proposed Research

Schedule. This is intended to be a three year proposal; the overall schedule is as follows. As part of the preliminary phase in 1999 we have carried out the steps outlined in the previous section. This should result in detailed specification of an instrument, capable first of laboratory operation, and secondly configured so as eventually be made field ready for ROV deployment. We anticipate placing a purchase order in early 2000, and taking instrument delivery in the second quarter. Laboratory work will follow with access by several investigators. Design and acquisition of pressure housings, cables and connectors, etc. will follow in late 2000, with tank testing in late 2000/early2001. A set of field experiments on natural samples, and generated hydrates, will be carried out in summer 2001 in local waters, with continuing laboratory work. Papers written will reflect this sequence.

If a purely laboratory study was envisaged then MBARI need not acquire this technology, but could rely on cooperative arrangements with other laboratories, much as we now do for conventional isotope mass spectrometry. It is the intent to proceed with deep ocean deployment that characterizes our efforts. Nonetheless, basic competence in laboratory studies is a prerequisite for this, and we anticipate continuation of microbial studies. For hydrates both CH_4 and CO_2 systems can be created in small sample cells, and reference spectra obtained.

Ocean experiments. In the deep sea the unique signatures of the fluid dynamics are also revealed, as phases consolidate, rejected brine is released, and exposure to the unsaturated external ocean occurs. We have only sketchy ideas of the nature and time scale of these processes. We intend to carry out a set of deep-sea experiments in 2001/2002 on both CH_4 and CO_2 hydrates, using the laser Raman technique to probe the changing geochemistry.

There are many unknowns here. For the formation of deep ocean CO_2 hydrates our recent work has shown that the volume changes were unusually large; it is widely believed that this is due the formation of metastable phases with a large number of vacancies. We can test these ideas; and once saturated phases are formed and identified we can then estimate realistic decomposition rates in the unsaturated ocean. For both CO_2 and CH_4 hydrates a key feature is the rejection of brine during the formation process. The great sensitivity of Raman to sulfate ion, coupled with the fine spatial resolution of the laser dot, should permit detailed examination of boundary layer chemistry associated with salt rejection. In the case of CO_2 hydrates the boundary layer should also be saturated with several weight percent of CO_2, which has a detectable Raman signature. These layers are too small to be probed by other means.

Finally, the prospect of moving beyond controlled ROV experiments within our local waters to natural sites is exciting. It is a goal to devise procedures for in situ identification of hydrates and rocks, minerals, ores and organic material on the sea floor.

One of the problems in the exploration for natural gas hydrate at the seafloor by ROV is differentiating between carbonate hard ground and gas hydrate. The laser Raman technique will facilitate these basic identifications. In addition, the technique will allow, for the first time, the measurement of in situ chemical properties of the natural gas hydrate. All previous measurements have been on samples removed from their natural environment, and thus the unstable gas hydrate has been altered significantly at the time of measurement in laboratory settings. The proposed technique gets around the recovery problems and allows measurements to be made without any change in the physical environment of the gas hydrate.

References

Lawry, N. H., Jani, V. and Jensen, T.E. 1981. Identification of the sulfur inclusion in Beggiatoa alba B18LD by energy-dispersive X-ray microanalysis Curr. Micro. 6:71-74.

Lyon, L. et al. 1998. Raman Spectroscopy. Anal. Chem., v. 70, 341R-361R.

Pasteris, J.D. 1998. The laser Raman microprobe as a tool for the economic geologist. In: "Applications of microanalytical techniques to understanding mineralizing processes" M. A. McKibben, W. C. Shanks, and W.I. Ridley, eds. Society of Economic Geologists, 233-250.

Seitz, J. C., Pasteris, and B. Wopenka. 1987. Characterization of CO_2-CH_4-H_2O fluid inclusions by microthermometry and laser Raman microprobe spectroscopy: Inferences for clathrate and fluid equilibria. Geochim. Cosmochim. Acta, v.51, 1651-1664.

Vetter, R.D. 1985. Elemental sulfur in the gills of three species of clams containing chemoautotrophic symbiotic bacteria: a possible inorganic energy storage compound. Mar. Biol. 33-42.

PROJECT SUMMARY

Notes: Labor, Fringe and Ship Costs will automatically be updated after data input phase.
Total project costs will be calculated at that time
If multi-year project - submit separate file for each year (name yr1, name yr2, etc)

PROJECT TITLE: Acquisition of Laser Raman Spectrometer for Deep-Sea Studies

EXISTING PROJECT?: **YES** ☐ **NO** ☑

PROJECT NUMBER:

LEAD SCIENTIST: Peter G. Brewer

LEAD ENGINEER: George Malby

PROJECT MANAGER: Peter G. Brewer

☑ Multi-Year? **YEAR:** 2000

DATE LAST UPDATED: 6/1/99

PROJECT COST SUMMARY

Revenue			
Revenue / Reimbursement Total	$ -		
Expense			
DIRECT LABOR		$	163,639.76
LABOR FRINGE		$	70,365.10
INTERNAL SHIP	$ -		
CAPITAL	$ 95,000.00		
ALL OTHER EXPENSE	$ 27,110.00		
PROJECT TOTAL		**$**	**356,114.86**

FORM UPDATE
5/18/1999 4:32 p.m.

Acquisition of Laser Raman Spectrome **NOTE: ENTER STAFFING DATA AS DAYS - NOT HOURS!** 11/30/99 10:07 AM

MILESTONE NAME	*Rqmts Doc/Spec*	*Prychase date & subassemblie*	*Elec/Mech Design*	*Fabricate Assemblies & Housings*	*Integrate syste*	*Bench Test System*	*Establish Sci Test Protocols*	*Tank Testing*	TOTAL DAYS	Total Days
DATE OF COMPLETION										
RESEARCH										
Scientist	10.00	2.00				5.00	15.00	3.00	35.00	
Research Specialist	5.00					3.00	10.00	2.00	20.00	
Research Technician	10.00					15.00	30.00	15.00	70.00	
Post Doc - Science									0.00	
Lab Assistant									0.00	125.00
DEVELOPMENT										
Engineer-Research									0.00	
Engineer-Electrical	10.00	5.00	30.00	10.00	10.00	20.00	2.00	10.00	97.00	
Engineer-Software	10.00				15.00	30.00	2.00	10.00	67.00	
Engineer-Mechanical	10.00		30.00	10.00	15.00	20.00	2.00	5.00	92.00	
Post Doc - Engineer									0.00	256.00
IAG										
S/W Applications Developer									0.00	
Software Engineer/IAG									0.00	0.00
ITD										
Research Tech/Video Analyst									0.00	
Editorial Assist/WEB									0.00	
Techwriter/Documentation							20.00		20.00	
CTD Data Tech									0.00	
Licensing Associate									0.00	
Research Tech/Samples									0.00	
AV Technician									0.00	20.00
DMO										
Director/Associate	3.00								3.00	
ROV Pilot/Ventana									0.00	
ROV Pilot/Tiburon	5.00								5.00	
Technician/Marine Ops									0.00	
CTD Tech/Ops									0.00	
Diver									0.00	8.00
TSD										
Technician - EE				30.00	20.00	20.00		20.00	90.00	
Technician - ME				30.00	20.00	10.00		10.00	70.00	
Machinist/Machine Shop				30.00	10.00				40.00	
Technician - Ocean Obs									0.00	
I.S. Administrator									0.00	200.00

PROJECT NAME Acquisition of Laser Raman Spectrometer for Deep-Sea Studies
LEAD SCIENTIST Peter G. Brewer
LEAD ENGINEER George Malby
PROJECT MANAGER Peter G. Brewer

	POINT LOBOS ONLY	POINT LOBOS/ VENTANA	WESTERN FLYER ONLY	WESTERN FLYER/ TIBURON	OUTSIDE SHIPTIME *	OUTSIDE SHIPTIME *
SEA DAYS (insert # of days)	0	0	0	0	0	0
DOCKSIDE / MOBILIZATION DAYS (insert # of days)	0	0	0	0	0	0
TRANSIT TIME : extended cruise time charged to project (insert # of hours)	0	0	0	0	0	0
Preferred Usage: **Examples: a)** 10 consecutive days (Santa Barbara) requiring 2 days each mob/demob; **b)** (2) 3-day groupings in March & September (time series); 1/2 day mob needed in March for software installation; **c)** Outside shiptime: 7 days JOHN MARTIN throughout year for mooring work						
Special Requests (night dives, extended days, etc.)						
PCO/RSC use only: Actual At-sea Days Allocated:						

OUTSIDE SHIP TIME *: All Project Managers are required to secure their own outside ship time, though DMO will assist as applicable.
Outside ship time expense must be budgeted in expense sheet. (See DMO for rates)

Extended Costs		**Rate**	**Extension**
Point Lobos only	$	4,828	$0
Point Lobos/Ventana	$	7,738	$0
Dockside / Mobilization	$	3,869	$0
Western Flyer only	$	11,056	$0
Western Flyer/Tiburon	$	16,499	$0
Extended Hours	$	-	$0

SHIP COST TOTAL (internal) $ -

Project: **0**

Please mark all dates for which shiptime **WILL NOT** be feasible for your project with a bold **"X"** and comment as needed.

Please mark specific dates or time periods for which shiptime is **MANDATORY** for meeting your project milestones with a bold **"M"** and comment as needed.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31
EXAMPLE 1				<	<	**M**	>	>								<	<	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	**X**	>	>			
comments				testing new eqpmt first week of month															tentative cruise on New Horizon												
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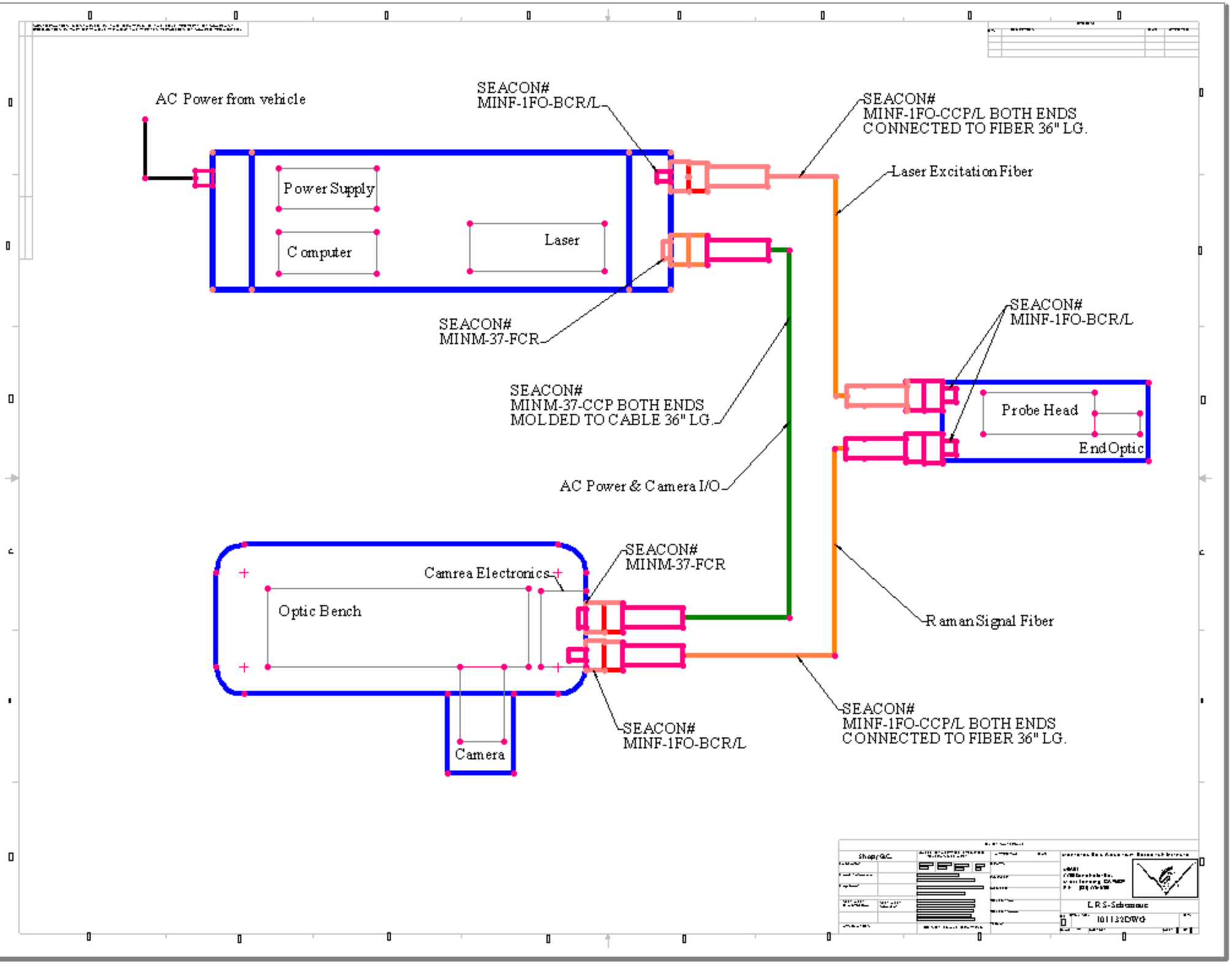
Project-No	Acct-No	Account Name	Line-Item Description	Amount
PROJECT NUMBER:	380	Supplies - Science Lab	Misc. supplies (sample cells, standards)	$5,000
		Supplies - Science Lab Total		$5,000
PROJECT NUMBER:	110	Capital Additions > $5,000 unit price	Laser	$50,000
PROJECT NUMBER:	110	Capital Additions > $5,000 unit price	Monochromator	$5,000
PROJECT NUMBER:	110	Capital Additions > $5,000 unit price	Video Camera	$5,000
PROJECT NUMBER:	110	Capital Additions > $5,000 unit price	XYZ Stage (parts)	$15,000
PROJECT NUMBER:	110	Capital Additions > $5,000 unit price	Control Electronics (Boards & Components)	$10,000
PROJECT NUMBER:	110	Capital Additions > $5,000 unit price	FO/Interface Electronics	$5,000
		Capital Additions > $5,000 unit price Total		$90,000
PROJECT NUMBER:	120	Computer Additions > $5,000 unit pr	Computer and Monitor	$5,000
		Computer Additions > $5,000 unit price Total		$5,000
PROJECT NUMBER:	540	Outside Services - General	Consulting - Dr. J. Pasteries,, Washington Univ.	$10,000
		Outside Services - General Total		$10,000
PROJECT NUMBER:	320	Supplies - Engineering Lab	Misc components, raw stock cabel penetrator	$7,500
		Supplies - Engineering Lab Total		$7,500
PROJECT NUMBER:	410	Travel - Domestic - Lodging	Vendor visits (2 trips - 2 nights each)	$400
		Travel - Domestic - Lodging Total		$400
PROJECT NUMBER:	411	Travel - Domestic - Meals/Incidental	Vendor Visits (2 trips - 3 days each)	$210
		Travel - Domestic - Meals/Incidentals Total		$210
PROJECT NUMBER:	412	Travel - Domestic - Transportation	Vendor visits (2 trips)	$4,000
		Travel - Domestic - Transportation Total		$4,000

Grand Total $122,110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Laser Excitation Fiber
Power Supply
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Laser
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Optic Bench
Raman Signal Fiber
Camera
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CONNECTED TO FIBER 36" LG.