

SRI REU 2014 STUDENT PROJECTS AND PROGRAM ACTIVITIES

Student Research Projects and Accomplishments:

Below is a summary of each student's project at the Molecular Physics Program in the Materials Research Laboratory (MRL) at SRI International during the summer of 2014 in their own words with some editing of the text as appropriate.

REU STUDENTS:

Holden Parks (Swarthmore College, Swarthmore, PA)

Mentors: Drs. Jochen Marschall and Jason White

Project Title: Laser Induced Fluorescence Measurements of Boron



Hypersonic vehicles travel in environments characterized by extreme temperature and continuous particle flows. Heat-resistant materials are therefore used on the exteriors of these vehicles to protect their payloads. ZrB_2 and HfB_2 , both diboride compounds, are promising candidates for use as surface components due to their high melting points of around 3000°C . When these compounds, however, are placed in hypersonic environments, they oxidize and produce boron monoxide (BO). My project involved using laser-induced fluorescence to study the emissions of this molecule, work that will contribute to future research in quantitative, in situ detection of BO formation in diboride samples. This information can subsequently be used to determine the rate at which the sample is oxidizing in a hypersonic environment.

In order to study the emission of BO, we first had to excite the molecule to higher energy states using a laser. We used the 532 nm output of an Nd:YAG laser to excite Pyridine 1 laser dye, which emitted light in the visible range (about 690 nm). This dye output was frequency doubled and tripled using BBO crystals and directed into a cell into which we flowed BO. To produce the BO, first we used a microwave discharge to dissociate diatomic nitrogen. These nitrogen atoms reacted with NO molecules to produce oxygen atoms, which subsequently reacted with boron trichloride to produce BO. Photons from the laser excited the BO molecules, which consequently emitted photons as they relaxed to lower energy states. These emissions were collected using a spectrometer and PMT. To calibrate the optical response of the system, we also collected the emissions of NO, a molecule with widely studied and well-known emission spectra.

My work this summer involved exciting the BO to the $B^2\Sigma^+$ ($v = 1$) electronic-vibrational energy state and detecting the photons emitted from the molecule as it relaxed to states lower in potential

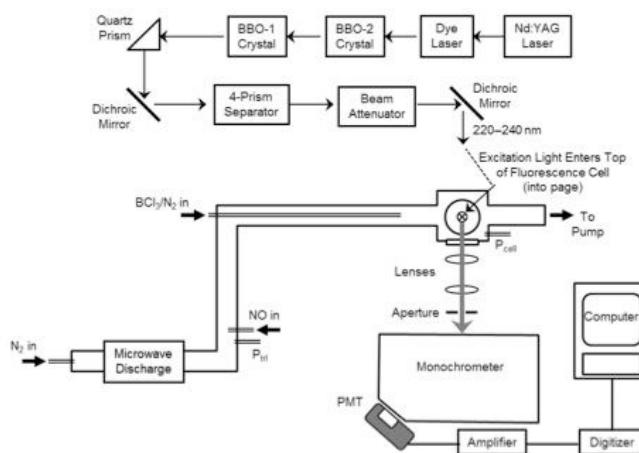


Figure 1: Full experimental setup

energy. I found quickly that the peak furthest to the blue end of the spectrum was about 8 times larger than expected, based on a pGopher simulation. My mentors had found similar results when studying the emissions of the $B^2\Sigma^+$ ($v = 0$) state. The photons forming this peak are of the same wavelength as the laser photons, so we hypothesized that this problem was likely due to light pollution from the laser.

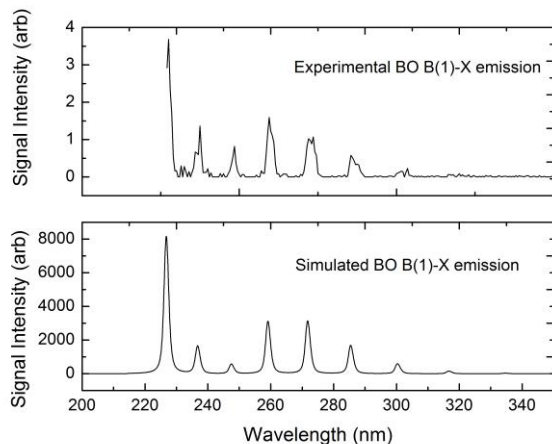


Figure 2: Experimental (top) and theoretical (bottom) BO B(1)-X emission spectra

We attempted to remedy this pollution problem in several ways. By varying the amount of reactant gases placed into the cell, I attempted to increase the amount of BO gas produced, which would increase the intensity of BO emission. Increased emission intensity would give our data a better signal-to-noise ratio, but I found that any increase in BO signal was marginal. I also measured BO fluorescence response as a function of laser power to determine if the laser was causing a nonlinear response in fluorescence at different emission wavelengths, but found the relationship to be linear and essentially identical for the emission wavelengths observed.

In addition to these experiments, we attempted to reduce light pollution by changing our data processing procedures. I eventually found a method that effectively subtracts the laser light contribution from the BO fluorescence signal, resulting in spectra similar to the PGOPHER simulations (an example of which is shown in figure 2). Using this data, the transition moment functions and Einstein coefficient for the BO $B^2\Sigma^+$ state, both necessary for the aforementioned quantitative BO detection, will be determined.

Colleen Werkheiser (Reed College, Portland, OR)

Mentors: Drs. Gregory Faris and Jian Peng

Project Title: New Nonlinear Optics Methods for Stimulated Scattering



My project looked at stimulated Rayleigh and Brillouin scattering. The goal of my project was to advance the techniques previously used for this project in order to perform microscopy for biological applications. This meant that much of my apparatus was already set up when I arrived. My first week consisted of various orientations and trainings, as well as reading SOPs and manuals for many of the pieces of equipment that I would be using this summer. I then focused on reading past publications in order to understand the theory behind my experiment and become familiar with the methods used previously. Once this was done, I was able to get into the lab and begin working.

I first had to learn about our complicated setup with many optical and electronic components. I learned how to turn the system on and off, and how our LabView programs worked to control our electric and acoustic modulators. I also became comfortable using the oscilloscope and its various onboard math functions, such as the Fourier transform that I would use extensively throughout the summer. I then learned how to perform alignment in order to optimize our signal.

My next task was to test the two lasers that we would be using in order to determine whether they gave stable frequencies that would allow us to take measurements over extended periods of time. One issue we were concerned with was mode hopping, which could cause uncertainty in the frequencies of the lasers. I first measured each laser separately, looking at how stable the frequencies were and whether we could determine the frequency based on the parameters that we could set manually. Once I found stable regions for both lasers I took measurements of the beat frequency between the two lasers using a new detector. I measured the drift of this beat frequency and determined that this signal was stable enough for us to use these two lasers together for our experiment.

With this information, we were able to begin looking for our signal. Unfortunately, the fiber amplifier we were using was damaged and had to be sent out for repairs. We set up a free space amplifier and changed our beam paths accordingly. From there, we began looking for the Rayleigh and Brillouin signals, which we were able to find fairly easily. We then began testing different modulation methods in order to determine which one gave us the best signal to noise ratio. For each method, I looked at the dependence of the signal shape on the phase of our modulation and recorded this data. We then began focusing on getting the setup ready to do imaging.

My first job was to set up the stage that would move our sample. I read the manuals for the stage and controller and did some preliminary testing of moving the stage. From there, I focused on writing some LabView codes that would move the stage both manually and in a predetermined pattern that we had chosen. I was able to finish these codes and test them, making sure that the movement of the stage could be accurately timed with our pulsed laser signals. My work here was very successful, as the system is just about ready for imaging, and I greatly enjoyed this experience.

Michael Sandoval (San Jose State University, San Jose, CA)

Mentors: Drs. Sanhita Dixit and Gregory Faris

Title: Optical Microfluidics Methods and Artificial Bilayers



This summer my goal was to create a reproducible method to create artificial lipid bilayers that could be automated because that would be quite useful from an analytical standpoint. At the beginning of the summer, I had to learn the basics of how fluids behaved at the micron length scale. This was done by analyzing droplets of a saline solution being ejected out of a glass tip triggered by a LabVIEW program. A microscope pointed at a cuvette of mineral oil was used to observe the droplets, as they were ejected into the oil. Two of the main things trying to be analyzed at this point were to see how to achieve a desired droplet size, and what was the behavior of this droplet leaving the tip.

The next step in the project was to move from a cuvette to a petri dish, so we could see how droplets behaved as they rested at the bottom of the dish, and in the long-term, to see how bilayers would form. With this came a large amount of troubleshooting. It turned out our valve was broken the entire time, so I had to go back and re-analyze old experiments. On top of that, the entire physical setup of rods and clamps holding things together fell apart. Once all of these issues were dealt with, I started to develop a method that seemed promising with the saline solution, so it was time to move on to an aqueous lipid solution. First I had to learn how to make this type of solution. This involved learning how to dry lipids out of chloroform, and turning the

lipids into a solution of vesicles. To achieve this, we utilized an extruding machine, which filtered the lipid solution through an artificial membrane, which in turn helped the formation of lipid vesicles.

I soon learned that electric charge and fluid flow in the petri dish is extremely important. This began another long period of troubleshooting, for if I could not deal with electric charge, the droplets moved out of view as they were formed. Towards the end of the summer, I was successful at determining how to handle charge on the droplets and produce droplets reliably under the mineral oil in the Petri dish.

Gabrielle Abraham (University of Arkansas, Fayetteville, Arkansas)

Mentors: Drs. Eric Hall and Gregory Faris

Title: Laser Heated Microdroplet PCR



Polymerase Chain Reaction (PCR) is a very common method used for studying which genes are present or expressed within a cell, amplifying a genetic sequence, and quantifying the amount of DNA originally present in a sample. Our goal for this project was to create a method of performing PCR in an environment more similar to that of a cell. We did this by placing small samples of hydrophilic PCR mix within hydrophobic oil. This caused the sample to ball up, creating a droplet. The higher the surface tension between the oil and the droplet, the more spherical the droplet becomes. The droplet was heated to the optimized temperatures for PCR by a 1.5- μ m diode laser. This methodology created faster means for PCR, but had the fault that different sized droplets heated differently.

An error of droplets shrinking during PCR arose earlier in the year, which led to the droplets not reaching the temperatures necessary for PCR to occur. We hypothesized that this was caused by water solvating out of the droplet and into the surrounding oil. To fix this, we first began altering the medium of the droplets. Initially, mineral oil was used because of its low water solubility; however, it is an impure substance and there was no way to examine how it changed from stock to stock. Because of this, we switched to squalane, a pure alkane, as the medium. In the end, we found saturating the squalane to be the most effective means for lessening the droplets shrinking. This was done by heating and stirring a combination of squalane and water for at least half an hour, then heating the saturated squalane during PCR. This reduced shrinkage resulting from 45 cycles of PCR from about 30% of the initial area to 15-20%.

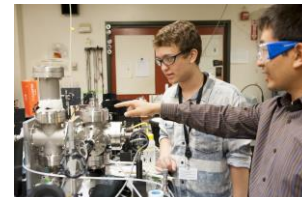
At this point, we addressed the speed of PCR with the intent of decreasing the time PCR took so the droplets wouldn't have enough time for significant volume loss to occur. The first step we took was to switch from using DNA to using amplicons, the resultant strands of DNA from PCR. This allowed us to increase the concentration of template, lessening the time it takes for the reaction to begin. We then found that we could decrease the denaturation step of PCR from 3 seconds to 1 second. This was sufficient for the amplicons to separate and short enough to somewhat lessen droplet shrinkage. We then addressed the annealing and extension steps of PCR. This involved increasing the amount of DNA polymerase and primer in the mix. This decreased the amount of time it took for the PCR to begin and allowed us to lessen the overall time for the process to occur. These efforts made a significant impact on the amount of time PCR took, and combined with the changes made to the oil, droplet shrinkage was reduced to only 10% of the original area lost.

Because PCR was occurring more consistently than before, we turned to a more complex addition to PCR-multiplexing. Multiplexing is studying the expression of multiple genetic sequences at the same time in the same sample. In this experiment, biomarkers in circulating tumor cells (CTCs) were examined. CTCs are cells which separate from the original tumor and enter the vascular system, allowing cancer to spread throughout the body. There are different kinds of CTCs which react differently to different medicines and can be identified by the biomarkers they express. We hope our findings create a more effective means for identifying CTCs in cancer patients.

James Reppert (North Carolina State University, Raleigh, NC)

Mentors: Drs. Jian Peng and Gregory Smith

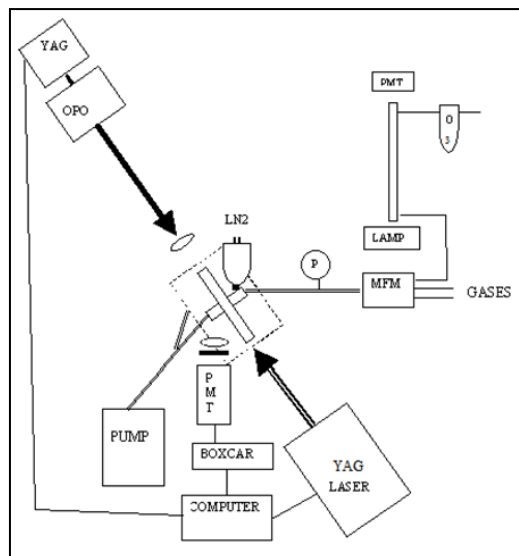
Title: Rate Constant Measurement of the Atmospheric Reaction $H + O_3$



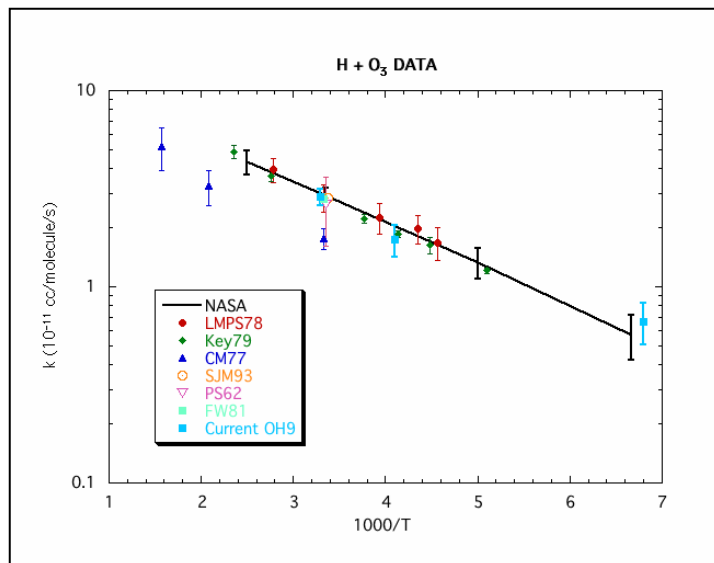
In order to ascertain knowledge about the Earth's upper mesosphere and lower thermosphere, we must do calculations using data from optical emissions of excited states. For instance, the TIMED satellite detects vibrational emissions from $OH(v=8,9)$. To use this data to determine the concentration of different elements in the atmosphere, we would need to know the rate constant for the $H + O_3$ reaction.

The rate constant for $H + O_3$ has been measured in the past; however, these measurements were taken decades ago at higher temperatures (200-400 Kelvin). Since temperatures go as low as 150 Kelvin in the upper mesosphere and lower thermosphere, we must measure this rate constant at lower temperatures while simultaneously not increasing uncertainties.

To do this, we set up a gas chamber that would simulate the reactions that occur in the upper mesosphere and the lower thermosphere. To the right is a diagram of our experimental setup. A computer triggers and controls the delay between the lasers. The ozone, hydrogen, nitrogen, and argon gases flow into the center chamber and are pumped out continuously on the left. One of the lasers photo dissociates about 1% of the ozone into O_2 and oxygen atoms. These oxygen atoms then react with the hydrogen gas to produce the H atoms required for the $H + O_3$ reaction to occur. To measure the observed rate of reaction, we used the other laser to perform laser induced fluorescence to monitor the creation of the product OH, $v=9$. From the reaction rate, we can calculate the rate constant from the known ozone concentration.



Mathematically, we can show that the concentration of OH, $v=9$ should be a bi-exponential function of time. However, we must consider the effects of secondary chemistry. Left over O atoms react with different species of OH to form H atoms and O₂. This effect will change the concentration of OH, $v=9$, especially at later times, which causes us to get a lower rate constant measurement. To compensate for secondary chemistry, we ran an extensive model of the chemical reactions in our experiment with a program called CHEMKIN. This allowed us to compare the



measured reaction rate to the theoretical reaction rate of the model. Using this information, we were able to calculate a correction factor to offset the effect of secondary chemistry on our rate constant measurement. This correction factor adjusted our rate constant measurements 5-15%.

To determine if our findings were sensible, we compared our values for the rate constant to those of a NASA publication that lists several different past studies of this rate constant. This data is shown in the Arrhenius plot above. As shown in this plot, nobody else has taken a rate constant measurement at upper mesospheric temperatures (~150K) besides us (shown in cyan on the Arrhenius plot). For this reason, in addition to measuring the rate constant at room temperature, we measured the rate constant at around 150K and 250K by using a liquid nitrogen bath and a dry ice mixed with isopropanol respectively to model the temperature dependence of this rate constant.

Our rate constant measurement shows that the rate constant for H + O₃ has the same exponential dependence on temperature, given by Arrhenius' equation, at upper mesospheric temperatures.

Allison Mis (Harvey Mudd College, Claremont, CA)

Mentors: Drs. Hua Lin and Thomas Shaler

Title: Novel methods for isolating and characterizing biomarkers: Experiments and analysis using high-resolution mass spectrometry



This summer, I worked with Hua Lin and Tom Shaler on using mass spectrometry to characterize the binding between proteins and weakly-binding molecules. We developed and refined an automation method for this process and also discovered an interesting interaction between myoglobin and the sugar NAG3. I really enjoyed working at SRI this summer, but it did not come without its challenges.

I think most of my moments of frustration came from feeling like I never fully took ownership of my project. As a physics major, I did not come in with much biology or chemistry background, so many of the basic concepts of the interactions we were studying were foreign to

me. I did read up on proteins, but it's difficult to know what concepts out of the whole of biochemistry were the ones pertinent to our project.

Additionally, because I haven't worked in a chemistry lab since freshman year, I was occasionally frustrated by the work involved with preparing samples and making dilution calculations. Finally, I often found myself without work to do while I waited for the next instruction from Tom. Some of this could have been alleviated if I had understood the overarching goal of our project enough to think of and run experiments on my own, but in reality, even when I did have a firm grasp of what we were studying toward the end of the summer, I didn't feel comfortable running my own experiments because I was afraid I would study something useless or waste a large amount of sample.

I do think that most, if not all, of these challenges resulted in something positive. Overall, I feel more confident in my lab skills because I know I was able to do good work in a biochem lab, studying things I hadn't ever seen in a formal classroom context. It is comforting to know that I can handle a wide variety of work, and I think this REU has helped me prove that to myself. Also, because I was unfamiliar with many of the concepts fundamental to our research, I got very comfortable asking Tom "stupid questions," a practice I think makes me a better researcher because I'm no longer too shy to take charge of my own learning.

I really enjoyed working at SRI this summer, and am grateful for the exposure it has given me to this type of work environment. I am still unsure what my future holds career-wise, but this summer I have discovered things about SRI that I hope to find in my future workplaces—a sense of camaraderie among employees, being surrounded by people who are enthusiastic about their projects, and a focused but casual work environment. On a personal note, living in the Bay Area over the summer has been amazing, and I hope to return here after graduation. This summer has been one of great personal and professional growth for me, and I am glad it happened at SRI.

SRI REU Program Activities:

Regular meetings with the REU students were scheduled to gauge student progress and address any concerns. In addition, several activities were included in the 12-week program to provide a well-rounded REU experience.

1. Seminars

Several opportunities exist for the REU students to attend seminars on the SRI campus. Besides the staff in the MRL, staff members from across the campus routinely give seminars. In addition, there are invited speakers visiting the campus as well. For example, SRI is the venue for seminars hosted under the Café Scientifique Silicon Valley initiative (<http://www.cafescipia.org>). A list of seminars attended by the REU students during the summer of 2014 is included below.

Date	Seminar Title and Speaker
6-02-2014	<i>Argus: A millimeter-wave Heterodyne Spectroscopic Mapper</i> Kiruthika Devraj, Ph.D. Kavli Institute of Particle Astrophysics and Cosmology, Stanford University
6-11-2014	<i>Research at LLNL</i> Dr. Anantha Krishnan, Ph.D. Lawrence Livermore National Laboratory
6-16-2014	<i>Cubic Mile of Oil</i> Ripudaman Malhotra, Ph.D. Associate director of the Chemical Science and Technology Laboratory SRI International
6-20-2014	<i>New Consequences of Maxwell's Equations</i> Felix Smith, Ph.D. Emeritus, SRI
6-24-2014	<i>Innovation Workshop</i> Curt Carlson, PhD. SRI
6-30-2014	<i>Miniature Sensors for Geospace Research</i> Rick Doe, Ph. D. SRI
7-11-2014	<i>Synthesis, Folding and Assembly of Peptoid Nanostructures</i> Ronald Zuckerman, Ph.D Lawrence Berkeley National Laboratory
7-14-2014	<i>Engineering The Musculoskeletal Machine</i> Melinda J. Cromie, Ph.D. Stanford University School of Medicine, VA Palo Alto
7-18-2014	<i>Roadmaps for transitioning all 50 U.S. states to wind, water, and solar power for all purposes</i> Prof. Marc Jacobson Stanford University
7-28-2014	<i>Recent Asteroid and Comet Results from Arecibo Observatory Planetary Radar</i> Alessandra Springmann University of Arizona

2. Academic/Industrial Visits, Seminars and Presentations

1. On June 18th 2014, the REU students participated in a tour of the SLAC National Accelerator Laboratory and were accompanied by Drs. Dixit and Faris. Dr. Carsten Hast hosted the group visit at SLAC.
2. On June 27th 2014, the REU students participated in a tour of the Lawrence Berkeley National Labs and were accompanied by Dr. Faris. Dr. Oleg Kostko hosted the SRI group at Berkeley.
3. On July 17th 2014, the REU students participated in a tour of the Vincent Coates Foundation Mass Spectrometry Laboratory at Stanford University and were accompanied by Drs. Dixit and Faris. Dr. Ryan Leib hosted the group visit at Stanford.

3. Student Presentations

Around the 11th week of the program, each REU student is required to give a presentation outlining the research they conducted over the summer. MRL's lab director and other associated or interested staff attend. Presentations last approximately 20 minutes with an additional 10 minutes reserved for questions and discussion. Prior to these presentations, each student hosts the remaining group for a tour of their lab and experimental set up. The 2014 REU students gave the following presentations:

Student	Seminar Title
Holden Parks	<i>Laser Induced Fluorescence Measurements of Boron</i>
Collen Werkheiser	<i>New Nonlinear Optics Methods for Stimulated Scattering</i>
Michael Sandoval	<i>Optical Microfluidics Methods and Artificial Bilayers</i>
Gabrielle Abraham	<i>Laser Heated Microdroplet PCR</i>
James Reppert	<i>Rate Constant Measurement of the Atmospheric Reaction $H + O_3$</i>
Allison Mis	<i>Novel methods for isolating and characterizing biomarkers: Experiments and analysis using high-resolution mass spectrometry</i>

4. Career Advice and Graduate School

This year's group of REU students requested a formal information session on graduate school applications and career options after graduation. Drs. Dixit and Faris held an hour-long session with the REU students to answer their questions. There was a lot of enthusiasm from the students about pursuing a graduate degree abroad, and Dr. Dixit provided information on the necessary web based resources for the students to pursue. She also gave the students some information about pursuing a PhD program in the UK, since Dr. Dixit obtained her Ph.D. at the University of Edinburgh.

5. Ethics Training

A formal mechanism to train the students in the ethics of scientific research was put in place in the summer of 2010. As part of this training, the students were required to take an online course to educate themselves about ethics in a research environment. The online course is available freely at: http://ori.dhhs.gov/education/products/montana_round1/issues.html#intro.

The study of the following three sections was mandatory; Section One: Ethical issues in Research, Section Two: Interpersonal Responsibility, and Section Four: Professional Responsibility. At the end of their study of each section, this website provided a test. The students were asked to take the test and furnish copies of their scores to Dr. Sanhita Dixit or Jacqueline Kritzer in the MRL.

6. Social Events

Students were invited to attend SRI and MRL events during the course of the REU program. MRL hosted bi-weekly payday meetings for students to learn about current news from the group and enjoy bagels/donuts in a congenial atmosphere with other lab scientists. They also attended farewell celebrations for MRL staff, seminar presentations given by prospective postdoctoral research candidates and any group meetings that were of interest or relevant to their research. An SRI “All Hands Meeting” given by company President and CEO, Dr. Curtis R. Carlson, provided the students an opportunity to learn about SRI staff and their research activities. Before the REU students left the MRL, a farewell celebration was given in their honor.

7. James R. Peterson Award for Excellence in Undergraduate Research

During its 50th anniversary reunion in 2006, the Molecular Physics Program announced the creation of the James R. Peterson Award for Excellence in Undergraduate Research. This award is given to the summer undergraduate student participating in MRL's NSF-supported Research Experiences for Undergraduates (REU) program that best combines Jim Peterson's technical excellence and spirit of friendliness and cooperation.

REU student nominations determine the winner of the Peterson Award. The 2014 winner was Collen Werkheiser of Reed College. Previous winners include Anand Oza, Princeton University (2006), Zachary Geballe, University of Michigan (2007), Brad Hartl, University of Wisconsin, LaCrosse, (2008), Aya Eid, Illinois Institute of Technology (2009), Alejandro Ceballos, Northern Arizona University (2010), Michael Rodriguez, California Lutheran University (2011), Stefan Mellem, St. Olaf College (2012), and Timothy Weber, Columbia University (2013).