SRI REU 2012 STUDENT PROJECTS AND PROGRAM ACTIVITIES

SRI REU 2012 STUDENTS

Student Research Projects and Accomplishments.

Below is a summary of each student's project at the Molecular Physics Laboratory (MPL) at SRI International during the summer of 2012 in their own words with some editing of the text as appropriate.

Elizabeth Case (University of California, Los Angeles)
Mentors: Drs. Kostas Kalogerakis and Oleg Kostko
Project Title: Important Ionospheric processes: Temperature dependence of O(1D) relaxation by O(3P)

The upper atmosphere is home to a phenomenon called airglow, the de-excitation of atoms such as oxygen and nitrogen release photons that produce a beautiful lightshow visible from space, and additionally affect aeronomical observations and experiments. This summer, my primary research goal was to investigate one of these processes: the relaxation of $O(^1D)$ by $O(^3P)$

at room temperature, to test and confirm previous REU experimental results that had determined this process' rate coefficient.

The first part of the summer was spent becoming familiar with the lab and the tools, including lasers, gas tanks, the LabView program, photon counters, etc. Later in the summer, we began the main experiments. We fired a 157 nm fluorine (F2) laser into an airtight cell that contained various concentrations of oxygen, helium, argon, neon, and nitrogen. The laser's photons dissociated and excited oxygen atoms; we looked at the relaxation of two of the excited states: $O(^1D)$ and $O(^1S)$. When they collided with other species or underwent natural radiative decay, they released light at certain wavelengths. We observed the time decay of these processes using photomulitiplier tubes and oscilloscopes. The data was then analyzed in Origin (data processing software), in order to determine the rate constants, indicative of the lifetime of these emissions. We also observed the emissions using a monochromator, which allowed us to take a spectrum of the photon emissions (from the ultraviolet to infrared).

Through our experiments, we obtained rate constants that were about 30% slower than previous studies had found. Consequently, we spent much of my last month testing our set-up in an effort to determine where errors, if any, were causing the experimental aberrations. This was the most interesting part of the summer – there's nothing like testing all the variables to realize how many there are, and how important it is to make the experiments as uniform as possible. These tests gave me a much deeper understanding of the experiment. We calibrated the gas flow meters, investigated the rate constant dependence on pressure and temperature, tested all of our cables, resistors, and gasses, worked with different laser powers, worked to better understand the degree of dissociation of the oxygen gas inside the chamber, etc. All in all, the last month was a hands-on introduction to the importance and difficulty of experimental design.

In the end, my time at SRI introduced me to the rewards of a career in research including obtaining results that have never before been found, and difficulties, like inconclusive results.

Patricia Ingraham (Grove City College)
Mentor: Drs. Hua Lin and Tom Shaler
Project Title: A novel method for isolating and characterizing histone proteins for biomarker discovery

Histones are a type of protein found in the nucleus of cells that aids in the compaction process of DNA. These proteins often have post-translational modifications (PTMs) – biochemical modifications that alter a function or property of the protein. Due to histones' unique association with and close proximity to DNA, we believe that there may be certain PTMs on histones that are a biomarker for radiation toxicity. To test this, my project this summer involved writing and developing a protocol to extract histone proteins from whole blood samples.

I began this summer by performing a literature search of previously used histone extraction methods noted in various journal articles. All extraction methods, though similar to the extraction we hoped to perform, involved starting with a great quantity of cells, not from a small quantity of whole blood. This difference – great vs. small, cells vs. whole blood – is what made our extraction unique. After searching the available literature, I created a tentative extraction protocol by combining a number of different extraction methods. I also chose three different promising-looking mild lysis buffers that I wanted to try in my extraction method (called Abcam, Shechter, and Chadee, based off of the primary author of the journal article in which I found them).

The extraction protocol we decided upon involved an initial wash with a lysis buffer (Abcam, Shechter, or Chadee), then an overnight acid extraction using 0.2M sulfuric acid, followed by a 30min acid extraction using Trichloroacetic acid. Finally, all samples were washed repeatedly with pure acetone to remove any residual acid, then treated with the protease, trypsin and acidified using 0.5 HCl to prepare it for mass spectrometric analysis.

The blood samples we used were taken from healthy donors at the Stanford Blood Bank and were a mixture of red blood cells (RBCs) and PBMCs (peripheral blood mononuclear cells). The plasma in these blood samples had previously been removed by another department for other experimentation. Because PBMCs are the only component of blood that have nuclei (and thus histones), our first task was to separate out and discard the red blood cells, leaving only the PBMCs, by using a BD Vacutainer Cell Preparation Tube. This method allowed us to easily pipette out the PBMCs. Some samples were mixed with PBS (phosphate buffered saline), and all samples were frozen at -80^{o}C until needed.

Next, we began experimenting by using my extraction protocol with the various lysis buffers we had decided upon. All three of our chosen lysis buffers gave good results, although the final pellets of the samples using the Abcam buffers were always much smaller than the other two buffers. After running our samples in the mass spectrometer, the MS data reflected the poorer performance of the Abcam buffer: both the Shechter and Chadee buffers purified the histones very well, and had a low residual content of hemoglobin, while the Abcam buffer purified histones less well.

We also tried using a total lysis buffer called Lysis-M, and performing a similar acid extraction with it, both with and without the addition of a Protein Precipitation Kit. We quickly found that neither of these methods worked as well as the mild lysis buffers. The Protein Precipitation Kit did not generate a high enough yield to be run using the mass spectrometer, and the acid extraction did not purify the histones nearly as well as the previously mentioned buffers.

To end the summer, we began analyzing the mass spectrometer data. We separated all of the peptides identified as histones that contained modifications, and chose a few of these peptides to look at in a more detailed manner. One of the peptides we chose, which had an acetyl modification, was a known histone with a modification that was listed on an online database. We also looked at an additional peptide, again with an acetyl modification, that – as far as we can tell – is a new discovery. Both of these are encouraging finds, and we will continue to track these modifications in future experiments.

We hope to continue this work by using blood from radiation therapy patients before and after radiation therapy treatments with the aim of detecting some change in the PTMs of these patient blood samples after radiation. This process may distinguish a biomarker for radiation toxicity. Discovering such a biomarker will enable doctors to give a more optimal radiation dose to cancer patients, and hopefully save lives.

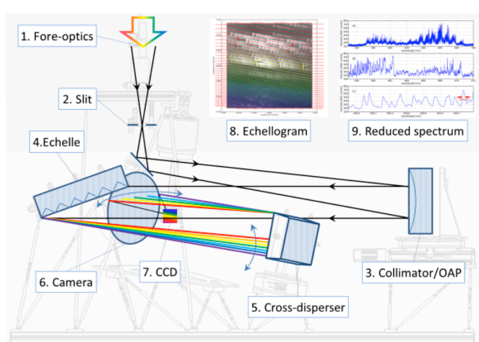
Stefan Mellem (St. Olaf College)

Mentors: Drs. Deepali Saran and Daniel Matsiev
Title: CESAR: Simulation-based Development and Image Processing

Light spectra obtained from the middle and upper atmosphere can be valuable tools for analyzing the atomic and molecular makeup of these regions, as well as characterizing the energy transfers and reactions that occur within them. The importance of spectra as a tool for aeronomical research is compounded by the difficulty of taking other types of measurements, such as sample collection, that require close proximity. Currently, however, the only spectral data from the upper atmosphere with high resolution and broad wavelength range comes from echelle spectrographs that are permanently housed in large telescope observatories (e.g. HIRES at the Keck Observatory). The goal of CESAR (the Compact Echelle Spectrograph for Aeronomical Research) is to provide data of comparable quality (high sensitivity, broad spectral coverage, and high resolution) to large, stationary spectrographs, but in a portable unit that is specifically designed for aeronomy research.

CESAR's portability will allow for study of atmospheric spectra from different physical locations, and its focus on aeronomical research will provide the aeronomical community with the ability to relocate the instrument to interesting geophysical locations (e.g. for the study of meteorites entering the atmosphere or nightglow and auroral features at middle or high latitudes)—something not possible with the limited and in-demand instruments associated with large telescopes.

CESAR Schematic



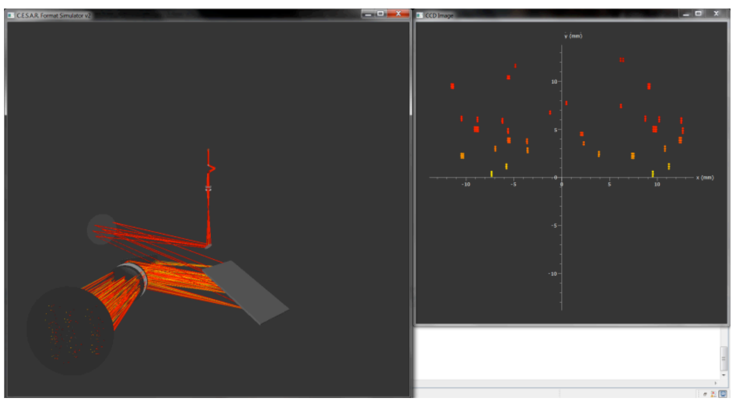
Our overarching project goal was to be able to remotely deploy CESAR and use it to take high-resolution, high-sensitivity data in the 300-1000nm range of wavelengths. This goal involved three major components: instrumentation, instrument control, and data analysis.

The most pressing need at the summer's outset was to reassemble and realign the instrument, which had been previously assembled but performed below specifications when tested. In order to address this need while also building a foundation for later data analysis, I wrote a new Python-based computer simulation of the CESAR optical system, known as the CESAR Format Simulator 2 (CFS 2). The major purpose of the simulation was to characterize the performance of the instrument and how misalignments would affect that performance. It could thus serve as a diagnostic tool in aligning the instrument.

To that end, I designed my simulation with three important traits in mind: simple and flexible data extraction, detailed and accurate physics, and a modeling scheme that could be easily tweaked to mimic real-world placement of instrument components. In concert, these allowed me to reduce the 'black box' effect present in previous calculations and in models where results were not easily connected to the fundamental physics behind them.

I was able to use CFS 2 to characterize a number of different problems we've seen in spectra from the instrument itself. For example, a shift in the positioning of the rays incident on the CCD detector (as compared to where they ought to strike the detector nominally) would tend to indicate a problem with the angle of the echelle grating or the cross-disperser. Poor resolution would be indicative of a problem with the focusing of our collimating mirror, or a problem with the position of the CCD detector within the camera system. Thanks to this new tool for characterizing optical components within CESAR, we now have a much better understanding of how each element of the instrument interacts with the others.

In the process of creating and using the simulation, I learned quite a bit about optical design as well. I was able to see firsthand how the use of multiple lenses in the camera allows us to minimize aberrations in the image such as Petzval curvature, for example. By isolating individual optical elements or sets of optical components such as the camera, CFS 2 can inform our intuitions and calculations about how each component should behave individually, which should be useful for component-by-component performance checks.



A CFS 2 simulation of the Neon spectrum, in progress.

We have now reassembled the instrument to the point that it is capable of taking spectra, and as we continue our efforts to realign it properly, CFS 2 will work in concert with predictions by Zemax models to direct our efforts toward the most likely problems and most critical alignments. Already, in the last few weeks of the summer, I've been able to verify a number of results from my simulation (such as CESAR's spectral resolution) with Zemax, and translate a Zemax model of the fore-optical system into my own simulation. By cross-referencing between the two programs, we hope to take advantage of both the detail of Zemax and the flexibility of CFS 2.

Our new focus for the future is to codify procedures for aligning the instrument so that its performance can meet specifications no matter where in the world it is deployed. Although the instrument is not yet in the final, deployable state that we're working toward, we've made significant progress in understanding the underlying issues. Moving forward, CFS 2 should continue to be a valuable tool all the way up to and beyond CESAR's deployment.

Cinthia Padilla (California State University, Fullerton)
Mentor: Drs. Ryan Leib and Jason White
Title: Environmental Monitoring using Mass Spectrometry

My summer began with learning about time-of-flight mass spectrometry. I was able to see how it separated molecules in gas samples so that we can see what our sample is made up of. Along with mass spectrometry, I also learned about different types of ionization such as single photon ionization, electron ionization and multi-photon ionization. Understanding these was crucial to determine which method was best suited for my proposed research project.

The overall goal of my summer project was to design and conduct an experiment that would allow us to see the capability of the mass spectrometer (MS) for environmental monitoring. The project had to be a case where the environmental sources could be monitored over time. Therefore, we decided on analyzing samples of air in the SRI campus parking lot throughout the day.

The first thing to be done was learn how to use the MS and see what the output was like. Dr. Leib taught me how to use the instrument as well as how to analyze the data. This was done using standard samples of which we knew the contents and their concentrations.

For air samples from the parking lot, signals were expected to be much more concentrated at 12pm and 4 pm due to higher traffic expected at those times, meaning that a direct correlation between lot air and car traffic is expected.

Once the protocol was put together, the experiment could begin. Duplicate samples were taken every two hours with the first at 8am and the last at 4pm. This amounted to a total of 10 collected samples. Samples straight out of a car exhaust were also taken in order to look for correlations. When taking the samples, things such as temperature, humidity and wind speed were noted (obtained via google.com). The samples would be compared to a sample of a different time of day to track how compound concentration would change throughout time as well as standard air samples, so that the compounds found can be quantified.

Once all of the samples were collected and put through the MS, they were calibrated and compounds that we are familiar with were identified. After being identified in the mass spectrum, they were integrated to obtain a value and this was repeated with every sample at all of the different times.

It was found that the time with the highest concentration was 2pm. This was also the time that had the most cars in the parking lot as well as the most trucks having passed by between 12pm and 2pm. Our hypothesis was correct in the aspect that most of the compounds come from car exhausts, but wrong in what time our signals would be strongest.

Matthew Ferranti (Stanford University)
Mentor: Dr. Joe Marschall
Title: Oxidation of Carbon in Dissociated Oxygen

Above Mach 5, vehicles moving through the atmosphere create a hypersonic flow. The work done to compress the air ahead of the vehicle heats both the gas and vehicle. This work also provides energy to dissociate the air molecules, making them about a thousand times more reactive. Carbon – as a light, strong material with a high sublimation point – is often used in ablative heat shields for these types of vehicles. My project focused on how carbon oxidizes in the presence of dissociated and molecular oxygen in upper atmospheric conditions (temperatures from 600-1500K, pressures around 1-2 torr).

In particular, we attempted to calculate the reaction efficiency of the oxidation reaction between carbon and oxygen. Cylindrical carbon samples (40mm long) were inserted into a quartz sample holder, which was then loaded into the furnace. A microwave discharge could be turned on to dissociate a fraction of the oxygen molecules. We could measure the upstream and downstream pressure, the temperature at four locations, the forward discharge and reflected power, gas flow rates, and the sample mass loss.

We assumed that the Hagen-Poiseuille flow equation described the gas flowing through our system. This model assumes one-dimensional flow for a laminar, continuous, ideal gas. We tested this assumption with different gases at various temperatures and flow rates and verified it within one percent under most conditions.

We used a nitric oxide titration (titration equation $N+NO \rightarrow N2+O$) to determine how much atomic oxygen is coming from the discharge. Beyond the titration endpoint, NO starts appearing in the gas phase. We can look for NO and find the endpoint by using a mass spectrometer. Because the nitric oxide titration produces a known amount of O atoms, the laser induced fluoresence (LIF) signal from these atoms can be compared to the signal from the atoms coming out of the discharge in order to determine the dissociated oxygen concentration. We created a computer model solving chemical rate equations to model the nitric oxide titration conditions.

As expected, we found a linear relationship when the mass change per unit area of the sample was plotted against time for a specific temperature, with the discharge both on and off. We also found an exponential relationship between the temperature and overall reaction efficiency, consistent with the exponential Arrhenius rate equations.

Interestingly, some of the carbon samples at very high temperatures (above 1300K) appeared ashen and flaky when removed from the furnace. This texture change seemed to partially shield them from oxidation. Future work could involve examining these samples under a microscope to understand their composition.

Jocienne Nelson (Oberlin College)
Mentor: Dr. Gregory Faris and Ashot Markosyan
Title: Stimulated Scattering in Fluids

This summer I worked with Gregory Faris and Ashot Markosyan on stimulated scattering in fluids. We worked with two types of scattering: Rayleigh and Brillouin which are used to determine various physical properties of a fluid. Our goal was to refine the detection of Brillouin scattering. My first few weeks at SRI were spent learning the experimental setup and the science behind the experiment. Even learning how to turn the system on and off was a complicated process where I could damage expensive and important equipment if I did something wrong. So it was quite a responsibility. I learned how to take both Rayleigh and Brillouin spectra. During my time at SRI I spent much time testing the system for various types of noise. I got comfortable using the Windows-based oscilloscope to look at magnitude spectra and learned how to make changes in the RF electronics setup. We made an important step in determining that the laser was shot noise limited and therefore ready to use to take real meaningful data. My time at SRI has made me appreciate the complexities that go into the design of the experimental setup. Unfortunately I had to leave early because of an unforeseen illness but I enjoyed my time at SRI and value it for the science I learned and the insight I gained into the inner workings of experimental physics.

SRI 2012 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 at the Molecular Physics Laboratory (MPL), 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.cafescipa.org). Below, is a list of seminars attended by the REU students during the summer of 2012.

Date	Time	Seminar Title and Speaker
6-01-2012	10:00-11:00 am	*Secret Ingredients in Peptide Lead Optimization* Amit Galande, Ph.D. Director, Proteomic and Protein Biochemistry, Immunology and Inflammation SRI International
6-04-2012	1:15-2:00 pm	*The Outlook for Energy and Technology Implications* P. Hugh Helferty, Ph.D. Manager, Corporate Strategic Research ExxonMobil
6-04-2012	6:00-7:30 pm	*Growing Brain Cells to Study Autism* Ricardo Dolmetsch, PhD Assistant Professor of Neurobiology Stanford University
7-11-2012	11:00 am-noon	*Cubic Mile of Oil* Ripudaman Malhotra, Ph.D Associate director of the Chemical Science and Technology Laboratory SRI International

Academic/Industrial Visits, Seminars and Presentations

1. On July 13th 2012, the REU students attended a presentation by fellow REU student, Elizabeth case, on the discovery of the Higgs Boson. Elizabeth, wrote an article on this discovery for the Daily Bruin, UCLA's campus newpaper (http://dailybruin.com/2012/07/08/scientists-announce-finding-of-higgs-boson-particle-responsible-for-all-mass-in-universe/).
2. On July 19th 2012, the REU students toured SRI's Robotics labs. SRI engineers Dr. Roy Kornbluh demonstrated various robotics technologies being developed in their

laboratories including technologies in the fields of medicine and hydropower technology. The students particularly enjoyed demonstrations of the wall climbing robot.

3. On July 24thth 2012, the REU students attended the poster day event hosted by the Biosciences division at SRI international. The students were able to learn about the various research areas being pursued in the biosciences division and interacted with several scientists in the division.
4. On July 25th 2012, the REU students participated in an online webinar hosted by the *Institute for Broadening Participation* on Tips on Funding Opportunities and Applying to Programs for graduate school.
5. On August 2nd 2012, the REU students toured the SLAC Algae facility on campus and were hosted by Dr. Bedwell from SRI's Materials laboratory.

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. MPL'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. The 2012 REU students gave the following presentations on August 7 and 8, 2012:

Student	Seminar Title
Cinthia Padilla	Environmental Monitoring with Mass Spectrometry
Elizabeth Case	Important Ionospheric processes: Temperature dependence of O(^1D) relaxation by O(^3P)
Patricia Ingraham	A novel method for isolating and characterizing histone proteins for biomarker discovery
Stefan Mellem	CESAR: Opening the Black Box
Matthew Ferranti	Carbon Oxidation in Dissociated Oxygen

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 MPL.

Social Events

Students were invited to attend SRI and Molecular Physics Laboratory events during the course of the REU program. MPL hosted bi-weekly payday meetings for students to learn about current news from the MPL and enjoy bagels/donuts in a congenial atmosphere with other lab scientists. They also attended farewell celebrations for MPL 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.

Students ate together regularly at SRI’s cafeteria and were joined once a week by Jacqueline Kritzer, Administrative Assistant, Molecular Physics Laboratory. Before the REU students left MPL, a farewell celebration was given in their honor.

James R. Peterson Award for Excellence in Undergraduate Research

During its 50th anniversary reunion in 2006, the Molecular Physics Laboratory 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 MPL'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 2012 winner was Stefan Mellem of St. Olaf 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), and Michael Rodriguez, California Lutheran University (2011).