

2006-1177: AN INTERDISCIPLINARY BIOENGINEERING BASED BUSINESS AND INSTRUMENTATION DEVELOPMENT PROJECT

Alyssa Caridis, Harvey Mudd College

Colin Jemmott, UVP, Inc.

Darius Kelly, UVP, Inc.

Deb Chakravarti, Keck Graduate Institute

Ekaterina Kniazeva, Harvey Mudd College

Erika Palmer, Harvey Mudd College

Jeremy Bolton, Keck Graduate Institute

Laura Moyer, Harvey Mudd College

Linda Chen, Keck Graduate Institute

Patrick Little, Harvey Mudd College

Qimin Yang, Harvey Mudd College

Sean Gallagher, UVP, Inc.

Dr. Sean Gallagher is the Chief Technology Officer for UVP, Inc., an instrumentation and specialty light source company that manufactures and distributes a broad spectrum of innovative instrument and imaging system solutions for the academic, clinical, medical and industrial laboratory. In his role as CTO, Sean oversees technical operations of UVP, including research, product and applications development, engineering, and technical support. Prior to UVP, Sean held leadership roles in instrumentation, fluidic packaging, and applications development with Hoefer Scientific Instruments, Pharmacia Biotech and Motorola Labs, where he established the microfluidics laboratory and was a founding director of Motorola Life Sciences.

Stephanie Bohnert, Harvey Mudd College

**An Interdisciplinary Bioengineering Based Business and
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Abstract

In 2003/4 and 2004/5, UVP sponsored Clinic projects that combined students and faculty from both the Keck Graduate Institute of Applied Life Sciences and Harvey Mudd College. Strategies developed for the first year were critical to the success of the program, and included weekly joint team meetings with all participants, both on campus and at the UVP, Inc. site in Upland, California. In addition, strong participation by the UVP engineering and management staff in the activity was needed. For the students, exposure to multifunctional team environments - where marketing, sales, manufacturing, engineering, chemistry and biology come together to explore new technology and apply that knowledge to product development - was critical for learning about the business of science. It provided students with insights into instrumentation and product development approaches and challenges, enabling them to make informed decisions about future careers. The company benefited from fresh ideas, lateral thinking, innovative design and fundamentally-new approaches to developing instrumentation. In addition, the company not only had access to a pool of highly-trained talent during the project, but potential hires and consultants after the students graduate. The project described in this paper was funded in the 2004/5 year to develop strategies for the uniform illumination of biological samples for fluorescent digital imaging applications in genetic analysis.

Introduction

Harvey Mudd College (HMC) is an undergraduate engineering school that emphasizes an integrated approach to engineering education while the Keck Graduate Institute of Applied Life Science (KGI) offers a bioscience Masters degree that emphasizes both business and technology in the life sciences. HMC pioneered the use of an industry-sponsored Clinic program as a capstone course to gain practical engineering experience in a team environment, while KGI students participate in a Team Masters Project (TMP), in lieu of a Master's thesis, that provides them with practical biotechnology industry experience. The joint Clinic/TMP project described here offers a wealth of practical experience to students. Other advantages include¹:

- Helping students to observe the nature, demands and ramifications of real-world problems;
- Assisting students in the development of teamwork and leadership skills;
- Increasing student understanding of engineering design processes;
- Enhancing students' ability to practically apply course material;
- Assisting schools in achieving their educational goals

Combining students from both programs in an applied development project mirrors real-life instrumentation development projects in the Biosciences/Life Sciences Industry, providing students with an interdisciplinary product-development team experience. We will report on the experience and give recommendations for further development of combined undergraduate and graduate student team-development projects.

UVP Inc. has been the leading developer and manufacturer of ultraviolet products since 1932. The company designs and manufactures innovative and applicable products across a broad spectrum of industries. UVP roughly is divided into three groups: an Ultra-Violet Products Group, a BioImaging Systems Group and a Light Source Group.

In vivo imaging is an ideal market segment for UVP, because of the company's expertise in lighting, its longstanding UV experience, and its greater focus on the bio-imaging sector of the company. In addition, UVP is an excellent provider of resources for this project, as the R&D and manufacturing plants are located conveniently in Upland, CA, minutes away from the campuses of HMC and KGI. Consequently, the project team has convenient access both to the UVP facility and to personnel for prototyping and collaborative communication with their customers.

Project Background

HMC contacted local businesses, including UVP, in an effort to involve the local manufacturing, instrumentation and technology firms in their Clinic programs. One of the liaisons at UVP had previously sponsored a HMC Clinic project in microfluidics while at Motorola Labs and found the opportunity to participate in Clinic program important to the company's R&D effort. Key for the industrial sponsor was to identify a crucial area for focused research, while at the same time allowing students to develop their own direction.

Optical fluorescence in vivo imaging is emerging as a useful tool to evaluate biological changes in living specimens, which can be utilized for research in areas like tumor biology and drug discovery. A bioimaging system with both spatial and temporal illumination uniformity is needed to accurately determine fluorescence levels. Unfortunately, existing optical fluorescence bioimaging systems do not focus on illumination uniformity, despite its critical importance to quantitative analysis.

The recent growth of molecular and genetic research has led to the need to evaluate biological changes in vivo (non-invasive testing on living specimens). For this reason, several imaging technologies have been adapted for plant and small animal research, including X-ray, magnetic resonance imaging, computed tomography, radioisotope, and optical fluorescence imaging. Among these visualization techniques, optical fluorescence in vivo imaging has emerged as an important alternative, because of its operational simplicity, safety, and cost-effectiveness².

The process of fluorescent imaging is centered on the use of naturally-fluorescent proteins as genetic markers or reporter genes. Typically, the gene expressing the fluorescent protein is introduced into an organism, such as a mouse. If the gene is permanently incorporated into the cellular genome and expressed, then the cells expressing the protein will fluoresce at a specific wavelength when the appropriate excitation light is used. The reporter gene can be placed next to another gene of interest, e.g., a biomarker for cancer, so that both the fluorescent reporter protein and the gene of interest are expressed at the same time. Such molecules come in different colors, which correspond to the wavelengths of light that the proteins emit when illuminated by incident light. Examples of such proteins are green fluorescent protein (GFP). The light penetrates through the surface levels of the specimen and hits the targeted tissue, exciting the fluorescent marker. The tagged part of the specimen then emits a characteristic wavelength of light corresponding to the fluorescent molecule (typically visible or near infrared).

In vivo imaging, by definition, is non-invasive, allowing researchers to analyze biological and drug activities in live animals under normal physiological conditions. The same specimen can be

analyzed over time, since it does not need to be destroyed to identify the location or activity of a tumor or drug. In addition, a fewer number of animals are necessary for each experiment, which in turn lowers the cost of experimentation.

Current Applications for In vivo Technology

Optical fluorescence in vivo imaging has a variety of applications. In the field of biomedical research, scientists can use in vivo imaging devices for the localization and tissue distribution of cancerous tumors³. It also can be used in neurobiology to track the activities of the brain, such as tracking the effect of the transplantation of bone marrow stromal cells into the brain of living animals subjected to cerebral infarction⁴. Another common application of in vivo imaging is drug development. Key areas in drug discovery and development in which in vivo imaging can make an impact include biological screening, pharmacokinetics and bioavailability, safety and toxicological testing, drug dosage, and formulation⁵.

Why Uniformity Matters

Obtaining quantitative results from optical fluorescence in vivo imaging requires scientific cameras, high precision lenses, accurate analytical software, and uniform excitation lighting. Unfortunately, lighting uniformity often is ignored in the development of biological sample imaging systems, but is critical to gathering quantitative data. With uniform lighting, a single specimen will receive the same amount and intensity of light, regardless of its placement or orientation. This ensures quantitative, reproducible measurements of a single specimen over time, which is especially important in tumor progression or drug efficacy research. In addition, quantitative measurements can be generated for multiple specimens in the same space.

The amount of light incident on each marker determines, in part, how brightly the marker fluoresces. With uneven illumination, a marker may appear to fluoresce more or less, depending upon local lighting conditions. The lack of spatial or temporal illumination uniformity in current commercially-available imaging systems results in:

- Qualitative or pass / fail results only;
- Fluorescent tags that appear to grow or shrink from day to day, due to varying incident light, rather than biological changes;
- Larger error margins and greater uncertainty; and
- Smaller signal-to-noise ratios.

Engineering Objectives

The HMC and KGI students worked on the project cooperatively. However, the engineering objectives were managed by HMC, while the business development and biological testing objectives were managed by KGI. The combined results of the business and engineering objectives were reported at weekly concurrent engineering meetings, an effective way to communicate business, project, and engineering objectives to the whole team at one time. This meeting format also encouraged a free exchange of questions, clarifications, and ideas for the next week's work.

The team, in consultation with the liaisons, chose to focus on four main objectives in order to provide an end product that is most useful to the customer. Uniform illumination was the most important objective for this team to realize in its designs. More specifically, the five spatial degrees of freedom were focused on for this project. Currently, UVP believes that small organisms comprise the primary application for in vivo technology. In the future, scientists may wish to study primates or other larger organisms. Therefore, the team looked to create a system that could be scaled up or down as the application requires. Ideally, the cabinet will be easy and straightforward to use. The team worked to create a product that required only knowledge of how to properly load a specimen, and how to control the camera. Additionally, the user should not experience any exposure to harmful light when working with the apparatus, which means the cabinet will be equipped with proper safety mechanisms. Depending on the application, different specimens require different intensities of incident light. Because of this, the team will maximize the range of intensities available.

Business Objectives

The intention of the UVP TMP team of the Keck Graduate Institute was to investigate business development topics and aid in the biological testing of the instrument. Business topics included market research, product development, competitive intelligence, intellectual property and marketing strategies. The market environment and relevant competitor information will aid in the design of the device and product positioning in the marketplace. In addition to the business component, the team emphasized the experimental side of the in vivo imaging component. Specific responsibilities of the TMP team included:

- Analyzing current in vivo technology and applications;
- Identifying market segments and target markets for the device;
- Evaluating the competitive environment;
- Intellectual property research on in vivo and uniform imaging applications;
- Identifying product applications and investigating new applications for uniform imaging;
- Strategic product launch and marketing plans;
- Investigating possible funding options;
- A biological component;
 - Locating a potential partner lab to perform biological testing; and
 - Testing the implemented lighting with live animals/plants.

Engineering Constraints.

UVP's main goal for the team was to develop two or more rough proof of concepts into prototypes for a new lighting design. Because the final product is a prototype of a future product, and because six-dimensional uniform illumination is not a subject matter that is well documented, UVP chose to place as few constraints on the team as possible. Rather than worry about the constraints of price and size, the client sought something that works, which can be modified into a marketable product later. The first constraint was that the lighting system had to illuminate a volume that is 25cm x 25cm x 35cm. This volume was agreed upon by the team and the liaisons as being a suitable starting point for the project. One of the team's objectives was to

have their concept be scalable, so that the volume was somewhat arbitrary. The new lighting system also had to be able to incorporate the emission of a large range of wavelengths, with the help of filters or other such devices. The necessity of different wavelengths correlates to the different fluorescent molecules that are used during in vivo imaging. Lastly, the liaisons wished to see an incident light intensity coefficient of variance of less than 7%.

Test Plan

Given the particular complexity of the characterization of a 3-dimensional volume of uniform light, novel approaches to equipment, procedures, and analysis are needed. Two different mechanisms were devised by the team to measure illumination, one for planar uniformity and the other for angular uniformity. Planar uniformity was measured by placing a sheet of white paper in the imaging system and taking a picture. The resulting grayscale image was then converted into a 2-dimensional matrix of data points by importing it into Matlab, where uniformity was calculated. Angular uniformity was measured with a custom hemispherical device in which a series of photodiodes are arrayed and shielded so as to only be sensitive to light incident from certain directions (1). Each of the photodiodes emits a distinct voltage, which was acquired via a laptop computer and processed by a project-specific LabView program.

Computer Simulation

In order to minimize the amount of physical testing needed during the design process, as well as minimize the total number of manufacturing iterations needed for a complete design, the team decided to purchase a computer simulation package to aid in their efforts.

Validation of Ray Tracing Software

The team obtained TracePro, Lambda Research's analytical ray tracing program, and a dedicated computer in order to simulate the behavior of a lighting system directly from a computer aided drafting (CAD) model rather than building and measuring each iteration of the design. After a CAD model has been developed for a particular illumination design, it can be directly imported into TracePro, where more than two million individual ray paths can be calculated in a matter of minutes. The result is an illuminance map that shows the amount of light passing through any point, plane or arbitrary shape in the simulation.

Uniformity Metric

Angular and planar illumination data were evaluated using the same statistical analysis. The statistical quantities of kurtosis and coefficient of variance (CV) were chosen to characterize how well uniformity has been achieved. The coefficient of variance is the industry standard for measuring two-dimensional lighting uniformity. It is defined as

$$CV = 100 \left(\frac{s}{X} \right) \quad (1)$$

where s is the standard deviation and X is the mean, and measures the distance from the mean to each data point relative to the mean. For biological illumination uniformity, UVP has adopted a value of 7% as acceptable for quantitative work. Kurtosis is based on the size of the distribution tails of a set of data; the smaller the distribution tails, the lower the kurtosis value. Kurtosis is defined as

$$K = \frac{\sum (X - \mu)^4}{Ns^4} \quad (2)$$

where X is the individual data points, μ is the mean, N is the total number of samples, and s is the standard deviation. A value of -1 is considered acceptable for quantitative work⁶.

Other statistical measures considered and rejected include the uniformity gradient, Laplacian, lighting uniformity ratio and point spread function.

Flat image analysis

In order to test the planar uniformity in these designs, a flat piece of white paper was placed on the imaging surface, and a image was taken via a CCD camera. Typical white paper is a useful test target due to the high uniformity of reflectance and color. These pictures are then loaded into Matlab as a monochrome image and are analyzed pixel by pixel. A Matlab script was written in order to automate the reading and statistical analysis of the data.

Hemisphere testing device

It was necessary for the team to fashion a custom device in order to characterize the uniformity of incident light across the two angular degrees of freedom, θ and ϕ . These measurements would have to be repeated over the entire volume of interest in a short period of time.

A double-arc hemisphere was chosen for this purpose, as seen in figure 1. Each of the arcs serves as housing for a number of photodiodes positioned at increments of 16° on the perimeter of each arc. The holes are countersunk to shield the photodiodes so as to be sensitive only to light incident from a narrow range of angles. In this way, four values each of θ and ϕ are explored at once for an approximation of a single (x, y, z) point in space. Then, by positioning this device at a number of different positions (varying x , y , and z) in the volume of interest, a map of angular dependence over the whole volume can be generated.

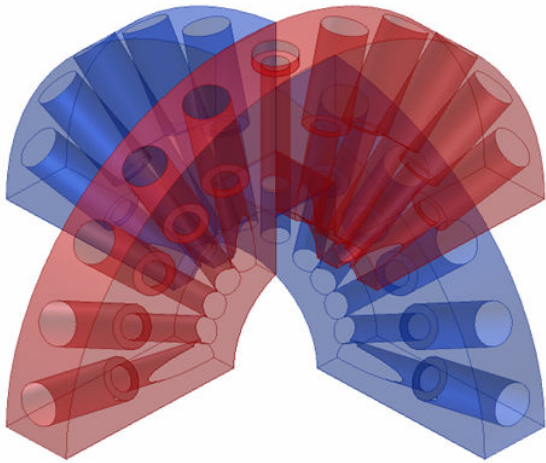


Figure 1: Design drawing of the photodiode double arc light intensity testing hemisphere

Each photodiode emits a distinct voltage dependent on the amount of incident light it receives. A data acquisition system then collects all the individual readings. These can be viewed in real time for troubleshooting or saved for future data analysis by way of a project specific DasyLab program on the testing laptop.

The collected measurements may be used to directly calculate values for the kurtosis and the coefficient of variance. However, it is also useful to visually display the data, to fully understand the physical meaning of the information. This manipulation is performed in Matlab, and represents each diode's reading as a vector with the corresponding direction and magnitude.

The uniformity metrics researched and utilized by the team have proved to effectively quantify uniformity. The use of two statistical quantities instead of just one corrects the problem cases observed early in the project. The coefficient of variance and the kurtosis will be useful for continued research in this area.

Conclusion

This project opened the door for considerable future work and numerous potential innovations. The team developed four feasible designs and working prototypes which are being evaluated and advanced as commercial products, due to the identification of potential clients and system features performed early in the project. In addition, parts from certain designs, such as the fiber optic panels, may be developed to serve other purposes, beyond uniform lighting, for in vivo imaging. The project also provided the foundation data for submission of a Small Business Innovation Research (SBIR) proposal to continuing research in this area.

7 students and 2 faculty members participated in the joint Clinic/TMP project over the academic year. Each project was funded by the company at a cost of \$45,000. Compared to the expense of a company staffed project of similar size, the Clinic/TMP program was a fraction of the cost and did not divert resources from more immediate short term development projects ongoing at the company. Furthermore, because the generated intellectual property (IP) from the project is the

property of the company, this is an effective way to teach students about the importance of IP and also file several key disclosures. An IP attorney hosted a question and answer session with the team to provide basic background in IP as part of the program.

The business objectives were also key in providing the background market and competition analysis for an effective launch of an in vivo imaging product in 2006 (currently in development at UVP). In addition, students were able to shadow UVP employees, attend key trade shows and meet with end users to further understand how their engineering development efforts should be tailored to a customer's needs. While some of the students went on to graduate school, others went on to industry in a variety of roles, including business and product development where the experience provided by the Clinic and TMP project has immediate relevance.

Although the needs of individual projects will vary, UVP identified several factors for a successful industry sponsored academic Clinic/TMP project. These include:

- Carefully chosen projects that are not on an immediate critical path. However, exploring potential new areas or instrumentation concepts and designs in depth are ideal projects;
- Internal company engineering staff with student project experience to help draft the key problem statement for the project;
- Frequent (e.g., weekly) concurrent meetings with all participants and liaisons were key to motivating the team toward success;
- Multiple active liaisons from the company;
- Active technical participation by the company, with personnel and manufacturing support to show how theory is reduced to practice in a commercial development company
- Interaction with key customers to understand the customer requirements
- Attendance at related industry trade shows to further understand the markets for the instrument.

Exposure to multifunctional team environments - where marketing, sales, manufacturing, engineering, chemistry and biology come together to explore new technology and apply that knowledge to product development - is critical for learning about the business of science. It gives students in engineering and life sciences a great deal of insight into instrumentation and product development approaches and challenges, enabling them to make informed decisions about future careers. The company benefits from fresh ideas, lateral thinking, innovative design and fundamentally-new approaches to developing instrumentation. In addition, the company not only has access to a pool of highly-trained talent during the project, but potential hires and consultants after the students graduate. A unique feature of the project described here is the combination of both undergraduate and graduate students with complimentary backgrounds. Business school programs that focus on high technology markets, which generally are engineering intensive, would benefit greatly from such a joint partnering with an undergraduate or graduate engineering school in a variety of disciplines well outside of the Bioscience/Life Sciences. This includes telecommunications, aerospace, defense, environmental, etc.

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