Multidisciplinary Requirement
Mentor Statement Example

DEPARTMENT OF MEDICINAL CHEMISTRY AND MOLECULAR PHARMACOLOGY and
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To: College of Science Undergraduate Educational Policy and Curriculum Committee
Re: Multidisciplinary Experience for Ms. Sheryl Woods

During the summer of 2008, Sheryl Woods pursued research with an interdisciplinary team focused on developing optical and physical methods to recognize specific species of secondary metabolite producing bacteria from mixed culture soil samples. The overall project aims to address the central problem of prediction of chemical diversity and de-replication in natural product drug discovery by using advanced technologies. This project is closely integrated with an effort to increase information content regarding microbial diversity. The application of these new experimental and informatics technologies is aimed at establishing a direct relationship between environmental microbe populations, the production of secondary metabolites, and their pharmacological activities. A specific aim of this project is to find high-speed, accurate physical methods to identify specific strains of Actinomycetes actively producing useful secondary metabolites for isolation.

Summary:
In effort to find new identification methods for large scale classification of Actinomycetes Sheryl was part of an interdisciplinary team which included a natural product chemist, biophysical scientist, engineer and a marine microbiologist, and industrial microbiologist. Three separate methods were used which centered on the use of technology to provide physical characterization of microbes. She was enabled to pursue this project through the direct guidance from team members lead by Professor V. Jo Davisson (Medicinal Chemistry and Molecular Pharmacology). Dr. Gerald Gregory, (marine microbiologist) visiting from Géochimie et Ecologie Marines CNRS Marseille (France), Dr Bartek Rajwa (biophysics/informatics), Dr. Valery Patsekin (physics/engineer), and Mr. Ray Fatig (microbiologist/cell technologist) all were critical in guiding the work. Using different fluidic and optical technologies, the team is working on three different methods described below to achieve the goals. Over the summer, Sheryl read and came to understand the detailed content of at least seven key papers related to the areas of technology being used to help explain the theory (physics and mathematics) behind our research. The first method employed was flow cytometry in which light refraction patterns and unique florescence properties of individual cells are used to identify the species. The multidisciplinary principles she learned from this portion of the project were fluid dynamics, fluorescence properties of individual dyes, and light refraction principles. The second method we used was laser scanning in which we used a laser at a specific wavelength to measure the light scatter properties of whole colonies. The light scatter data were then analyzed with a proprietary algorithm which was able to detect unique patterns among species in order to identify specific Actinomycetes from a mixed culture plate. The multidisciplinary properties she learned in this portion of the project were optical physics, light refraction, and the use of a mathematical algorithm to detect patterns. In the third portion of this project we used a scientific grade CCD camera to image Actinomycetes colonies at specific growth times to measure unique Properties between different species. This technology is very similar to facial recognition software in which millions of features of the face can be measured and used for identification. The primary multidisciplinary principle she approached in this portion of the project was the use of millions of measurements to mathematically find a unique fingerprint in an image. In this experiment she became very familiar with the technology of the Canadian Facial Recognition Project and the principles behind it.

The following sections were prepared by the student. I typically request similar documentation (including references) of undergraduate research students involving credit. The content is offered as further documentation of the nature of the engagement and learning experience.

Sincerely,
Professor V. Jo Davisson, Ph.D.
davisson@purdue.edu

Background
Finding a specific secondary metabolite producing species of bacteria is an important aspect of drug discovery. Currently researchers must isolate thousands of species of bacteria from mixed colony plates and then purify and test each isolate individually to find a species known to be a source of a specific secondary metabolite. This method is very time consuming, costly and does not insure the isolated bacterial species will produce the secondary metabolite of interest even if it has produced the metabolite previously. The aim of this project is utilize advanced physical technologies to screen for bacterial strains that are actively producing the desired secondary metabolites. These data will be combined with biological activity profiles and phylogenetic information to create a unique classification system that will allow for increases in the efficiency of current discovery methods. Our team worked as a component of this broader project utilizing three different physical technologies to identify specific species by flow cytometry, colony imaging, and laser scatter patterns.

Flow cytometry:

The goal of the flow cytometry portion of this project was to use optical multi-parametric analyses of the properties of a cell such as excitability at certain wavelengths, light absorption, light refraction, and cell density as a means to classify organisms using a flow cytometer. For this portion of the project she worked closely with Dr. Gerald Gregory. This process involved staining the cells with specific dyes and then running them through a flow cytometer (Beckman Coulter FC500) in an attempt to identify based upon their fluorescence and light scatter. Using the following dyes, biochemical aspects of the bacteria were tested for the degree of differential staining to provide a simple classification for the species of Actinomycetes in local environmental samples. Nile Red: Stains the lipid rich cell membrane of the Actinomycete cell. Fluorescein isothiocyanate (FITC): Reacts with proteins within the cell. Propidium Iodide (PI): Stains the DNA of the cell. Flow cytometry works by passing a stream of cell suspension through a higher osmolarity liquid, usually phosphate buffered solution which moves faster around the stream of cell suspension. The differences in speed and osmolarity prevent the two liquids from mixing and maintain a consistent stream of particles through the machine. The stream of cells passes through a blue argon laser which emits light at 488 nm frequency. This allows cell characteristics to be classified in two primary ways. The first uses the principle that certain frequencies of laser light excite the dyes on the cell and cause them to fluoresce; this is measured as side scatter. When a molecule of dye absorbs a photon of light emitted from the laser at the given frequency, the dye molecule enters a higher energy state. Florescence occurs when the cell returns to its ground state by releasing the absorbed energy at a longer wavelength. The florescence is emitted at an angle (depending on the cell characteristics the dye is bound to) and then travels linearly until it is picked up by a photo detector which converts the florescence into an electrical signal recording the angle, intensity, and frequency of photons hitting the photo detector. These data are then organized by the computer by dividing the data points into gates to be analyzed. We analyzed our samples using many different angle photo detectors (gates) and different dyes to compare a variety of characteristics of the cells with the goal of finding a unique florescence pattern within each species to use the flow cytometer as a means of cell identification.

The second way the laser beam was able to classify cell species was through forward light scatter. This measurement depends on size, shape, and internal properties of the cell which influence the path of light. The light is measured by photo detectors and then organized into gates which can then be analyzed to discern the optical characteristics of the cell. Her role in this experiment was to prepare and run the samples through the cytometer followed by data analysis with Dr. Gerald Gregory explaining the properties and meaning of the various data points.

Multidisciplinary principles learned from this portion of the project:
- Excitatory properties of the three dyes, Nile Red, PI, and FITC.
- Properties of fluid dynamics
- Laser light refraction principles
- Light reflection patterns and principles based on density and consistency.

Laser light scattering measurements and identification of bacterial colonies
The goal of the light-scattering portion of this project was to identify and classify Actinomycetes from a mixed culture agar plate using the light scatter patterns formed upon irradiation of colonies with red laser light. Light scattering is one of the most fundamental optical processes whereby electromagnetic waves are forced to deviate from a straight trajectory by non-uniformities in the medium that they traverse. The project involved application of light-scatter measurements paired with machine learning and pattern recognition methodology for label-free classification of bacterial colonies. This process is very similar to the concepts of light-scatter measurements in flow cytometry; however for this experiment the optical characteristics of a whole colony were measured rather than single cells. For this portion of the project she worked alongside Dr. Valery Patsekin and Dr. Bartek Rajwa.

By growing purified Actinomycetes colonies on a low nutrient agar plate and then scanning them with a laser emitting a beam at 540nm, we were able to obtain light scatter patterns dependant on the internal characteristics of the colony. A pattern-recognition algorithm was used which detected unique characteristics of light scatter formed by different strains of the Actinomycetes to differentiate isolates. Her role in this experiment was to isolate, purify, and then prepare the plate samples as well as execute data collection. Dr. Rajwa then explained the meaning of the data collected and explained how the data was analyzed using the algorithm he has created.

Multi-disciplinary principles learned from this portion of the project
- Principles of light scattering and other optical phenomena
- Applied optical engineering
- Basics of machine learning, pattern recognition and automated classification

Imaging
The goal of this portion of the project was to identify specific strains of Actinomycetes from a mixed culture plate on the basis of colony morphology. For this experiment she again worked with Dr. Pateskin and Dr. Rajwa. The classification of microorganisms via measurement of their colonies is based on the concept that complex colonies of bacteria can be considered to have “multicellular-like” properties. Consequently, it was postulated that unique colony morphologies could be recognized, classified, and linked with the genotypic information. Using a scientific-grade CCD camera, colonies of Actinomycetes were imaged after a specific duration of growth and the data were then analyzed for recurring, species specific colony morphologies. This technology works in a similar way to the new facial recognition technology which is being developed by other groups to recognize differences between faces with nearly perfect accuracy. The principle behind this experiment is that each species of Actinomycetes will have unique morphological properties that are too minute to be seen by the naked eye but that can be detected by image analysis. A scientific grade CCD camera was used in grey scale because it detects light linearly rather than logarithmically, is able to reduce noise caused by ambient temperatures and cosmic rays, and uses millions of micro lenses to increase precision of data collected. Her role in this portion of the project was to isolate, purify, and prepare the sample plates and then collect images followed by data organization for further analysis and classification. Dr. Rajwa and Dr. Patsekin guided her through the meaning of the data and how the data was analyzed with the proprietary algorithm.

Multidisciplinary principles learned from this portion of the Project:
- She became very familiar with the research of and principles behind the automated image processing
- The mathematical framework employed for pattern recognition and classification
- She learned the scientific principles behind a scientific-grade CCD camera and why it was important for data collection in this project.

Dr. Bartek Rajwa
Dr. Valery Patsekin
Dr. Gerald Gregory
Sheryl Woods