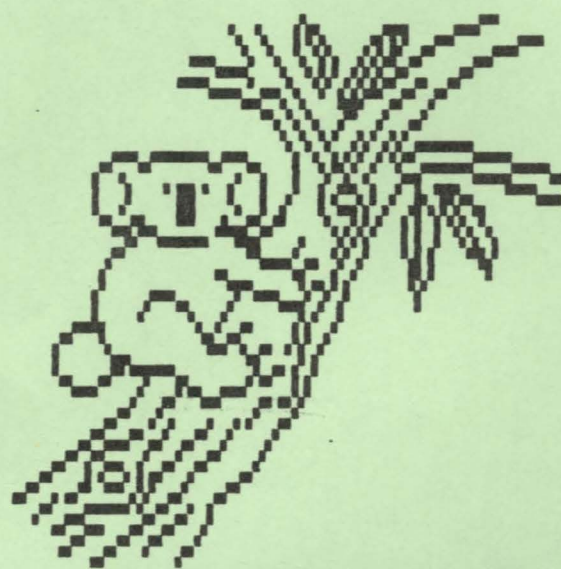


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## NOTES FROM THE EDITOR

Four years ago in Pella, Iowa, I agreed to serve as an interim editor for the BIOSCENE. I have enjoyed serving you but now I must resign and pass the pen to one of you. Between now and our annual meeting, I urge one or more of you to volunteer to serve as the editor. I know that you will find the job rewarding and frustrating. Hopefully, you will have the support that I have had during my term as editor from the membership and from my colleagues at Wabash College.

Particularly, I thank Mrs. Sandy Roth, the Biology Department Secretary and Mrs. Marcia Caldwell. Without their help, there never would be a BIOSCENE. Indeed, they did most of the work. Sandy typed all of the manuscript and Marcia printed each issue.

I also thank Austin Brooks and my other colleagues at Wabash College. All of them, being so close, have contributed to this publication on short notice when the issue was a bit thin.

Bob Buchholz, Chair of the Editorial Board, always was supportive. In this issue, all of the articles about CSIP programs are a result of his initiative.

I am sure that one of you will accept this responsibility. I urge you to write to Ed Kos, Executive Secretary of AMCBT or to Bob Buchholz at Monmouth College.

Finally, I want to thank all of the contributors. Without their effort, there would never be a BIOSCENE. I hope that everyone will support the new editor as they have supported me. For my part, I plan to be a regular contributor.

Bill Doemel

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## ADVANCED MICROSCOPY IN UNDERGRADUATE BIOLOGY

By

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Microscopy is an indispensable and visually exciting component of modern biology. The Department of Biology at Nazareth College in Rochester, New York obtained a National Science Foundation grant (CSI 8551166) under the College Science Instrumentation Program (CSIP) in order to purchase a research-quality epi-fluorescence photomicroscope. The Department argued that this instrument was necessary for two educational goals: 1) to serve as an integral component in laboratory courses in cell biology, genetics, immunology, and botany; and 2) to provide equipment with which students could design and carry out independent research projects. This article will briefly describe the equipment obtained under the CSIP grant; its laboratory education applications thus far; and the effect that this equipment has had on scientific education and research within the department.

**Equipment.** The CSIP grant proposal documented the immediate need for a high-quality microscope with both epi-fluorescence and photographic capabilities. The department chose a Zeiss Standard 16 microscope with Planachromat objectives, a 100 W halogen incidence fluorescence system with filter sets appropriate for fluorescein, rhodamine and acridine orange fluorochromes; and a MC63 automatic 35mm camera system. This system best met the Department's needs for a proven system, with good local service available, that would be relatively easy for students to operate. In addition, elements in this system would be interchangeable with an existing Zeiss phase-contrast microscope in the department.

Through a slight revision of the initial equipment specifications, and

with a favorable package price from the vendor, the department was also able to use grant funds in acquiring a Dage-MTI VC65 monochrome video camera and a Koyo monitor. This expanded the usefulness of the microscope for both laboratory education and student research applications. The result of the CSIP grant has been an integrated, research-quality fluorescence, photo- and videomicroscope yielding excellent images in student hands. The total NSF contribution to this project was \$8,853- Nazareth College provided slightly more in matching funds.

**Applications.** In the past two years, 43 biology students have used this microscope system for photomicrography, fluorescence microscopy, and/or videomicroscopy. An additional 40 students have benefitted from laboratory demonstrations enabled by videomicroscopic displays of living specimens.

Photomicrographic laboratory projects - conducted as integral parts of courses in Cell Biology, Genetics, Immunology and Botany - have included analysis of chromosomes prepared by students from plants, cultured cell lines, and from students' own blood cells; the immunohistochemical localization of fibronectin in human kidney thin sections; and the study of raphides released from plant idioblasts. Independent student research projects used photomicrography for the karyotyping of a human lymphoid cell line (1), and to document the ability of caffeine to release cultured cells from chemically-induced mitotic inhibition (2).

Fluorescence microscopy has been essential in the immunology laboratory course, enabling students to distin-

guish B and T lymphocytes through double-label immunofluorescence, and to quantitate mixed lymphocyte reactions (MLR) by using fluorescein diacetate (FDA) uptake as an index of immune response to foreign antigens. In the cell biology laboratory, students used the uptake of two fluorescent dyes, FDA and propidium iodide (PI) (3) to distinguish living (FDA-labelled green) from dying or dead (PI-labelled red) cells. This same technique was then applied to measuring the effects of ouabain (which inhibits the Na/K membrane pump), nutritional depletion of culture medium, and osmotic stress on cell viability. Other fluorescent microscopy exercises by students included fluorescent banding of human chromosomes and the detection of bacterial DNA using the fluorochrome acridine orange. An independent student project used the FDA/PI technique described above to demonstrate the significantly enhanced survival of lymphocytes cultured in medium supplemented with ascorbic acid (4).

Videomicroscopy was used by students in Botany to observe the actual discharge of idioblast raphides under varying conditions. An independent research student is using videomicro-recordings to study chemotaxis in Amoeba (5): these recordings make it possible to determine the net rate and direction of cellular movement.

Although this microscope system was acquired primarily for hands-on use by upper-level biology students, the video component has provided a convenient and effective basis for laboratory group discussions of protozoan diversity, amoeboid motion, Paramecium conjugation, and Elodea guard cell activity in courses for majors and non-majors.

**Educational Results.** This microscope system has yielded several significant educational results for students. First, they have had "hands on" experience in operating a research-quality instrument. Second,

students have successfully interpreted visual data from this instrument in quantitative terms. Third, the fluorochrome uptake assays have enabled students to literally see that differential absorption and intracellular location of labelled molecules depends upon metabolic and environmental conditions of the target cells. Finally, the presence of this instrument has enabled four students to design and perform independent research projects which otherwise would have been inconceivable in this department.

This system has enabled recording and analyzing microscope images on either photographic film or videotape; and using sensitive fluorescence assays to characterize specific molecular and functional features of cells and tissues. Videomicroscopy has allowed effective examination of cells in motion, and provided high-contrast images for group discussion among students and teachers.

**Effects on Faculty Research.** The successful use of this microscope has also brought about changes in the scientific thinking, reading, and research of this investigator. The ease and effectiveness of the first epi-fluorescence techniques tried with this instrument prompted a search for additional methods. The result was the adoption of the FDA/PI cell viability assay for student experiments concerning osmosis, cell nutrition, membrane transport, and immunological reactions. Similarly, the high-quality videomicroscopy images stimulated this investigator to obtain college funding for a summer research project to design a videomicroscope—microcomputer interface. This interface will incorporate microscope images directly into computer files for image analysis and the generation of lab reports by students and faculty alike.

In summary, the Biology Department realized the need for this equipment, obtained NSF funding for it, and dis-

covered applications beyond those originally envisioned. This has significantly improved the content and variety of laboratory courses, expanded opportunities for student research, and enhanced the expertise and enjoyment of the Department's faculty.

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**SUNY - ONEONTA BIOTECHNOLOGY STUDY OPTION**

By  
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research and clinical/commercial applications of biotechnology. In order to help accomplish these objectives, an ultracentrifuge and a cell fusion system were requested through the College Science Instrumentation Program of the National Science Foundation.

The ultracentrifuge system will be used to isolate and purify proteins and other biologically important biomolecules, cellular organelles, cell surface receptors, cytoplasmic receptors, and will be used to measure molecular densities by means of density gradient centrifugation. Laboratory exercises will include formation of linear sucrose gradients, isolation of plasmid DNA, rate zonal separations on sucrose density gradients, and separation of high density lipoprotein fractions from blood samples. Lectures, discussions, and demonstrations by the instructors will supplement the students' laboratory experience.

Among the newest pieces of equipment available on the market today are cell fusion systems. These systems have been used to fuse many cell types including plant, animal, yeast, and bacterial cells. They have also been used to create hybridomas for monoclonal antibody production and for inserting genetic material into a cell. Their fusion efficiencies are much higher and less destructive than current chemical methods. They can be a very valuable tool having both research and commercial applications. For these reasons, our students should be familiar with a cell fusion system's operation and its potential uses. The students will perform various protocols including isolation and fusion of plant, bacterial, and yeast protoplasts; production of mouse hybridoma cells, liposome formation, and studies of membrane phenomena, using for example, red blood

The State University of New York - College at Oneonta is a four-year, liberal arts and sciences college located in the western foothills of the Catskill Mountains. The college enrolls approximately 5,600 full-time undergraduate students and offers over 60 degrees in the liberal arts, teacher education, home economics, business areas, and several preprofessional areas. The science departments (Biology, Chemistry, Earth Science, and Physics) at SUNY-Oneonta have a tradition of excellence and quality in education.

In response to the rapidly expanding fields of biotechnology, the Biology Department at SUNY-Oneonta has developed a Biotechnology Study Option which is an addition to the Liberal Arts Biology major currently being offered. The Biotechnology Study Option is designed to offer qualified students a sequence of courses which will make them aware of and appreciate the potential benefits of these newly emerging areas of science. The key course in this study option is a one-semester course entitled, "Introduction to Biotechnology." This course will be taught each spring semester beginning in 1988. The course will provide students with an introduction to the basic techniques of biotechnology and the theory behind them. In order to accomplish this, a plant cell biologist, a microbiologist, a geneticist, and a biochemist will team-teach the course.

The major objectives of Int.D. 382 - Biotechnology are (a) to introduce and use in a laboratory situation some of the basic techniques of biotechnology including cell culture, separation techniques, cytogenetics, photomicroscopy, genetic manipulation and analysis in microbial, plant, and animal cells and (b) to inform students of current

cells. Where applicable, comparisons will be made between the efficiencies of traditional chemical methods of fusion and electrofusion. Using a microscope, the students will be able to verify the results of their procedures within minutes, thus getting almost immediate feedback on their technique in following the protocols.

Both the ultracentrifuge and the cell fusion system will be used to familiarize the students enrolled in the biotechnology course with the latest in scientific instrumentation. It is the intent of the course to provide hands-on experience with as many different methodologies used in the fields of biotechnology. Exercises and protocols will be developed by the instructors specifically for the students enrolled in the course, subject to the constraints of the instruments available.

The equipment requested represents some of the newest instrumentation available today. They were chosen because of their ease of operation, versatility, and convenient size. Students will be able to understand the theories upon which the instrumentation is based and then apply those principles to actual experiments. Because of their versatility, numerous procedures will be developed thus affording the students a broader background in the use of the instruments and allow us to fulfill the major objectives of the course.

**COLLEGE SCIENCE INSTRUMENTATION PROGRAM GRANT TO UPGRADE  
LIQUID SCINTILLATION SPECTROMETRY IN BIOLOGY**

By

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Perhaps the most difficult task facing most of us trying to upgrade our instrumentation for teaching cellular and molecular biology and biochemistry is finding money for an instrument priced in that "awkward range": too costly for our colleges to fund alone, and yet not justifiable on a research grant. This is particularly true when the instrument desired is not one which will permit entry into a flashy new curricular area, but is "merely" a newer model of an instrument, such as a liquid scintillation spectrometer, which is already on hand. I have found, however, that the National Science Foundation's College Science Instructional Program (CSIP) is just the place to go for funding such instrumentation.

In the course of a complete revision and restructuring of Grinnell College's biology curriculum to create a concentration in cellular and molecular biology and also to add a research requirement for the major, one deficiency became forcefully apparent to us. The use of radioisotopes and scintillation spectrometry is so wide-spread in cellular biology and biochemistry that our students began learning about many types of experiments which involve the use of such techniques virtually from the first day of the introductory course, but only a very few senior research students were getting any "hands-on" experience with radioisotopes or scintillation spectrometry.

The reason was our inadequate liquid scintillation spectrometer. The twelve year old instrument had become increasingly unreliable and subject to frequent breakdowns. In addition, the instrument was inadequate for the types of experiments we hoped to include in our revised courses: the counting efficiency was poor, so low-level

samples had to be counted for long times to get any degree of precision; the machine counted only a single channel, so double-labeled samples had to be counted by two separate passes; it held only 100 samples; and only the counting window could be changed automatically which limited the flexibility of the machine for multiple users counting overnight or on the weekends when the machine would be unattended.

We decided to apply to the CSIP program for funds to purchase a Beckman 5801 Liquid Scintillation Spectrometer which would remedy all those deficiencies. The 5801 is computer controlled for all counting functions, can hold 300 standard or 360 minivials interchangeably, and can count up to three isotopes simultaneously. In addition, a port to a dedicated IBM PC permits data capture, analysis and graphing at the instrument, or uploading of data to the college's mainframe computers.

In justifying this instrument the Biology Department examined all our course offerings to plan how the use of radioisotopes and liquid scintillation spectrophotometry could be developed. The following are specific examples of how we plan to utilize the new, more powerful instrument in our classes.

**Cell Biology:** This sophomore level required course is our major course for teaching lab techniques such as pipetting, spectrophotometry, aseptic technique, and dilutions. Students in this course will be introduced to the proper handling of radioisotopes, the techniques of liquid scintillation spectrometry, and the ways in which data from such experiments are analysed. The exercise planned is to dilute two isotopes, prepare double-labeled samples, and determine a quench curve.

**Genetics:** This course and those described below are elective, upper-level classes. Students normally take Genetics immediately following Cell Biology, so its experiments are designed to reinforce the technical skills they have just developed and to introduce the use of radioisotopes in actual experiments. Students will compare the rates of synthesis and degradation of RNA and proteins in both prokaryotes and eukaryotes by following the incorporation of  $^3\text{H}$ -uracil and  $^{14}\text{C}$ -leucine into trichloroacetic acid precipitable materials before and after the addition of various antibiotics. They will also determine the degree of relatedness of two bacteria using nucleic acid hybridization on filters.

**Microbiology:** This course and those described below are elective upper-level classes. Two experiments will be done by students in Microbiology. The first is a study of the relationship between oxidative metabolism (establishing a proton gradient across the plasma membrane) and the transport of nutrients by proton cotransport systems. The students determine the rates of uptake of various amino acids by starved cells, by starved cells when glucose is added, and by starved cells in the presence of glucose and either uncouplers or ionophores. The second experiment is to compare the physical and infective titers of New Castle disease virus. Physical titers will be determined by radio-immunoassay and by hemagglutination, and infective titers by hemadsorption to infected tissue culture cells.

**Plant Physiology:** Students in this course will study ribulose diphosphate carboxylase activity by monitoring the incorporation of  $^{14}\text{CO}_2$  into carbohydrates.

**Biochemistry:** Radioisotopes will be used in both semesters of this year course. In the first semester when students are learning analytical procedures, they will determine the

molecular weights of labeled mouse mammary tumor virus virion proteins by molecular sieve chromatography. In the second semester, where the emphasis is nucleic acid biochemistry, they will use a reticulocyte lysate system to study protein synthesis in vitro.

**Student Research Projects:** The Biology Department requires that every student undertake independent research at the college or as part of an approved off-campus program. Our pedagogical objective is not that the students necessarily complete an entire "publishable unit", but rather that they have the experience of designing and executing their own experiments which address an original research question. Many students undertake a project which is part of a faculty member's research program, while others do a project entirely of their own devising. Some examples of recent projects are studies on the aminoisobutyric acid transport systems in primary cultures and cell lines of normal and malignant mouse mammary epithelial cells, and investigations of how intercalating anticancer drugs affect the interaction of topoisomerase II with tritiated DNA.

Our first proposal to CSIP was favorably reviewed but not given a high enough priority to be funded. CSIP encouraged us to strengthen the proposal by considering the reviewers comments and then to resubmit it. The new instrument was funded in April and installed in July, 1986. We began its utilization "from the top down" with faculty and research students learning its capabilities during the summer. New experiments were introduced first into Microbiology and Plant Physiology during the 1986-87 academic year. The limited enrollments in these classes made them suitable places to try out pedagogical approaches for the use of radioisotopes in course labs and to see what unexpected logistical problems we would encounter with increased radioisotope use. Since these trials proved very successful, we plan to introduce the full range of proposed experiments utilizing the instrument during the 1987-1988 academic year.

## CSIP FUNDING FOR A CELLULAR AND MOLECULAR BIOLOGY LABORATORY

By

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Bates College is a small, liberal arts college located in Lewiston, Maine. A graduating class of approximately 250 students includes 35-45 biology majors each year. The biology major is organized around a required "Core curriculum" of four, semester-length courses including Biological Principles, Plant and Animal Biology, Ecology and Evolution, and Cellular and Molecular Biology. The College Science Instrumentation Program (CSIP) Grant the College received was for instrumentation improvements for the Cellular and Molecular Biology course. The biotech revolution in this area has brought many of the once esoteric techniques, such as restriction mapping, to a plebian level. This change has occurred so rapidly that our Department found itself equipped only to demonstrate many of these "hands-on" techniques. The other difficulty we encountered was the time requirements for many of the protocols we sought to teach. While much of the preparation can be accomplished prior to the lab period, we wanted to avoid "push-button" laboratory experiences. Thus, with the goal of providing a modern lecture-laboratory course in cellular and molecular biology, we set about designing a new laboratory offering.

Our first step was to select laboratory techniques appropriate for sophomore biology majors regardless of the time demands entailed in these procedures. The final list of techniques is presented in Table I.

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### Table I. Selected Techniques

Protein determination  
SDS-Polyacrylamide electrophoresis  
Column chromatography, ion exchange and affinity  
Radiation safety  
Autoradiography  
Synthesis of RNA and protein  
Isolation and restriction mapping of DNA  
Mammalian cell culture  
Enzyme kinetics and enzyme immunoassay  
Western gel transfers/detection

---

The topics selected demand more time than the usual three-hour laboratory allotment. To circumvent this problem, the cellular and molecular biology course was moved to the Short Term. This term runs for five weeks in April and May after the close of the winter semester. Students take only one course or Short Term Unit and are thus available from 8 A.M. until 4 P.M., Monday through Friday. Short Term 1986 saw fifty-one students enrolled in Cellular and Molecular Biology. Lectures were scheduled for 9-11 A.M. each weekday. To accommodate this unexpectedly high enrollment (sixty students had taken the course in the 1985 fall semester), the class was divided into three groups. Labs were held from 11-4:30 on Tuesdays, Wednesdays, and Thursdays. Each group was assigned to one primary lab day with the following day for any carry-over procedures. The normal Bates semester affords thirty-nine hours each of lecture and lab time. This Short Term schedule provided fifty hours of lecture and fifty hours of lab. Student response to this Short Term format was extremely positive, laboratories proved to be a source of enjoyment.

The entire program is directly attributable to the support provided by the National Science Foundation through the CSIP program. The College was unable to provide the funds required for multiple sets of lab equipment, the preparative centrifuge, the CO<sub>2</sub> incubator, tissue culture hood, and refrigerated chromatography cabinet, all essential components of the project. The mechanics of the CSIP proposal submitted by Bates included the following:

1. detailed description of the Biology Department course offerings and recent enrollments,
2. a list of recent senior thesis topics and research presentations demonstrating independent student research,
3. a menu of seventeen proposed labs indexed to the techniques required and the equipment to be purchased,
4. a plan for using the equipment during the two regular semesters in upper level electives thus putting the equipment to its fullest use, and
5. a proposal for generating a new laboratory manual for publication.

Our initial proposal was not funded, but the rejection carried with it some very helpful comments from the reviewers. Educated by the review, I rewrote the proposal and it was funded upon resubmission.

In January, 1987, I had the privilege to participate in the panel review of proposals submitted to the CSIP program. This program enjoys increased funding this year with predictions for further budget increases next year. THE CSIP is managed with enthusiasm by Dr. Alexander Barton, Program Director, and his excellent staff. At the opening session, Dr. Theodore Reid, Associate Program Director, made the following points to the assembled reviewers:

1. look through the proposal to the project, fund the project;
2. look for quality, is it within the grasp of the college? does the project make sense for the department at this time?;
3. look for improvement, not maintenance of the existing tools for science education;
4. focus on undergraduate instruction alone, be it research, independent study, etc...;
5. projects for special audiences - the handicapped, women, minorities, are of special interest to the NSF;
6. programs that prepare students to teach at the pre-college level;
7. equipment must be the key missing ingredient, document high level of assurance of immediate progress if granted.

These items may prove as useful in the preparation of a successful CSIP proposal as they were in the review process.

## FIELD COURSES STILL HAVE EDUCATIONAL MERIT

By

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In the late spring and early summer a group of land-locked Northern Kentucky undergraduate science students from the University of Kentucky-Maysville Community College leave the standard college classroom behind and head south for around ten days of intensive on-site marine and environmental instruction. The course is an introduction to the marine environment and exposes these students to every aspect of this new environment that time will allow.

The course is planned for January when the class is opened for enrollment and for February when the orientation begins at the local college setting. The first orientation session is to explain the physical mechanics of the course. This will include travel arrangements, cost, tentative itinerary, and syllabus and safety instruction. The next session on the local campus will be a more in-depth treatment of the academic expectations for the course.

Once the day for departure arrives, students will be assigned to a particular van and the two vans will leave the local campus around 6:30 a.m. for a day and one-half through the southern countryside. Stops will be made periodically as the vegetation changes so that each student will have an opportunity to observe new organisms and new landforms. On arriving in Long Beach, Mississippi the students will check in dormitory rooms at the Gulf Coast Branch Campus of the University of Southern Mississippi. This will serve as the center for instruction.

Monday morning breakfast is served at 7:00 a.m. and the official class is off and running. First on the agenda is an on-site orientation to the campus and the region as a whole. The rest of this day will include a field trip and lecture at the Gulf Port International

Harbor. This will be followed by a trip and lecture at the new Gulf Coast Marine Education Center. Behind the scene treatment of some of the largest aquaria will be explained as well as the twenty-four hour care of the animals. All of the animals housed in the Marine Education Center are endemic to the area thus this tour provides an excellent introduction to organisms that may be observed during the next few days. At this site oyster research is being done as well as other studies on marine populations and larva growth patterns.

In the late afternoon just before the dinner meal, the instructor of the course will take the students to the beach and demonstrate techniques of seining and screen dipping to examine the fauna in the shallow waters close to the shore line. Following the afternoon activities, the group will eat dinner together and then return to the lab for a short lecture to tie the events of the day together and make preparations for the coming day. The day will end around 10:00 p.m. and the students will be free to swim, play tennis, walk on the beach or rest for the coming day. Each day is filled with new activities and excitement keeps the adrenelin flowing in each student for the entire time. They become a close knit group of individuals just like a large family group.

Another very important aspect of this course work is that the students live with the two faculty members for this time period and they have an opportunity to observe their faculty as real people in a real world. This is not always the case in professor/student relationships.

From the time the students leave the home campus until the time they arrive back they must keep a journal of the events of the days. This is kept

in the form of a field logbook and is carried with them every place they go. At night they are asked to bring the logbook up to date before they retire for the evening. When the participating students return to the main campus they will be given one month to formulate an official paper covering all aspects of the field experience and turn in to the instructor of the course. At the same time they are required to turn in the logbook in its rough form.

Other activities that the students will be involved in during the time spent on the coast will include: one day on one of the Barrier Islands, a shrimping experience in which they will be able to assist in lowering the nets and sorting the catch, a visit to a coastal terrestrial site that is adjacent to a bayou, observations of swamps and wetlands, observations of insectivorous plants of the region, an on-site lecture and tour of Ingalls Shipyards in Pascagoula, Mississippi, and many chances to experience fishing cast-net style, crabbing, and flounder-ing.

Terrestrial communities of several kinds will be visited and the participants will make comparisons among them. Environmental factors will be assayed to highlight the importance of factors such as salinity and dissolved oxygen on the presence or absence of organisms in a community. Lecture periods will concentrate on the interaction of man and the coastal biological communities. Importance of the marine environment to the nation as a whole will be discussed and consideration will be given to the interior of the continent and its affect on the coastal region.

While on the beach students will have an opportunity to see many animals and plants that they will have only seen in textbooks. Many specimens will be brought back to the lab so that classification can be conducted and life cycles studied. Being a beachcomber will be a favorite activity. This is a natural instinct and is excellent for the instructor because all of these collected items serve well as the classification activities unfold. Each student ends the class with a collected and identified shell collection to take back home as a souvenir.

Evaluations from students on this course have been overwhelmingly positive. We hear such things as "I have learned more in this class in this short time period than in any other class that I have taken." The course is physically very rigorous and students have to be on constant guard for sunburn. The class is so popular at our school that the course is never published in the schedule because it is already full from year to year.

The faculty are now working on evaluation materials to determine if a measurement of the learning that takes place on such a field course can be determined. Pre- and post-tests are being developed to access the degree of marine awareness before and after exposure to this type of instruction.

## THE ULTRACENTRIFUGE IN THE UNDERGRADUATE LABORATORY

By

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A prominent trend in biological research has been toward an understanding of biological events at the sub-cellular and molecular levels. This type of experimental approach is no longer restricted to the areas of cellular and molecular biology, but is also being applied to other areas such as biosystematics and ecology. The laboratory experiences which students have in undergraduate cell biology and molecular biology courses are no longer necessary only for the understanding of material in these disciplines but also for understanding much of what is occurring in the other areas of biological inquiry.

Thus, the Department of Biology at Saginaw Valley State college is upgrading the laboratories in the Cell Biology and Molecular Biology Courses in order to expose the students to methodology which is becoming fundamental to an education in biology. The Department decided that the acquisition of an ultracentrifuge would be a necessary part of this modernization. In August, 1986 an ultracentrifuge was purchased (partially supported by NSF grant CSI-865 1009) and the Department is in the midst of incorporating its use into the curriculum for biology majors.

The Cell Biology course, which is a required course for all biology majors, provides the first point in the curriculum for the use of the ultracentrifuge. Currently, two laboratory exercises in this course make use of this instrument. These two exercises concern the isolation of organelles on a sucrose gradient and the purification of plasmid DNA on a cesium chloride (CsCl) gradient.

The isolation of some organelles can be achieved in low speed centrifuges, and this was the method used in this laboratory exercise prior to the acquisition of the ultracentrifuge. The use of an ultracentrifuge allows much better separation of the organelles. Also, by incorporating ultracentrifugation into this laboratory exercise, the students gain confidence in their ability to do advanced work with the type of equipment used in graduate courses and research.

For the organelle isolation experiment, both plant and animal material is used. The plant source for organelle isolation is castor bean endosperm and the animal source is rat liver. First, the tissue is homogenized and spun on a low speed centrifuge. Then the samples are placed on a sucrose gradient and spun in the ultracentrifuge for five hours. The gradient is then unloaded in 2 ml fractions that are assayed for enzyme activity. Various marker enzymes for particular organelles are used for this part of the procedure.

In the organelle isolation experiment, it is important to plan a schedule for the various steps, since the work cannot all be done within the normal three-hour laboratory period. Some students must be assigned to come in early to prepare the castor beans and rat liver samples and load them on the ultracentrifuge so that the five-hour run is completed by the start of the laboratory period. The gradient can then be unloaded and the enzyme assays conducted during the scheduled laboratory time. To give all of the students exposure to the use of the ultracentrifuge, it is necessary to provide time at

the end of the period for the entire class to do the homogenization work and restart the centrifuge.

Another experiment in the Cell Biology course which uses the ultracentrifuge is the isolation of plasmid DNA from bacteria. This procedure is also used in several experiments in the Molecular Biology course which is an elective. The techniques which the students learn in this exercise are fundamental to the area of genetic engineering. For this procedure, bacteria (Bacillus subtilis) cells containing the plasmid pBR322 are grown overnight and then collected by low speed centrifugation. The cells are broken open by incubation with lysozyme. The sample is then spun on a low speed centrifuge and the supernatant containing the plasmid is saved. Cesium

chloride is then added and the sample is spun in the ultracentrifuge for 48 hours. A gradient of CsCl can only be formed on an ultracentrifuge. A low speed centrifuge cannot substitute for this step of the procedure. The plasmid DNA forms a band in the CsCl gradient and can be collected as a fraction from the centrifuge tube. The plasmid DNA is then used for other experimental work which includes the use of restriction enzymes, agarose gel electrophoresis and gene cloning.

In summary, the ultracentrifuge is becoming a fundamental tool for use in the undergraduate curriculum. The use of this instrument allows the students to do a more professional job in the laboratory and to conduct experiments which would otherwise be impossible.

## A CSIP-FUNDED PLANT PHYSIOLOGY PROGRAM BASED ON STUDENT-INITIATED PROJECTS

By

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Trinity College is a small, liberal arts college with about 1800 students, in Hartford, Connecticut. Plant physiology is an entirely new program at Trinity, and the college was willing to commit considerable resources to equipping the new teaching laboratory. However, because we started with little and wanted our students to use up-to-date equipment, matching funds from the College Science Instrumentation Program (CSIP) of the National Science Foundation were invaluable.

An undergraduate plant physiology laboratory can have many goals: to demonstrate basic principles of plant physiology, to help students gain confidence and expertise with laboratory techniques, to give students an appreciation for the workings of science, and to allow students to practice experimental design. Our program emphasizes experimental design and experience with modern instrumentation. We want the students to become familiar with a variety of common plant physiological techniques so that they know the sorts of questions answerable by each and are able to frame meaningful research questions. In addition, we want them to use instruments similar to those they may later find as graduate students or research assistants.

We do not try to cover all of plant physiology in the lab; instead, we emphasize selected topics and allow more time for student projects. We focus on four major areas: tissue culture, photomorphogenesis, photosynthesis, and water relations. Students start carrot callus cultures at the beginning of the semester, then spend the rest of the first half of the semester using a variety of physiological measurements to compare high-light and low-light grown sunflower seedlings. The high-light:low-light

comparisons assure that all the students learn to use all the equipment, and they give some structure to the curriculum. Although students are not necessarily working on the same phase of the project at the same time, every two weeks they must turn in a brief report on one of the four areas. The minor reports on the high-light:low-light comparisons (photomorphogenesis, photosynthesis, and water relations) are corrected and returned to the students, and later in the semester they turn in a formal report synthesizing these results. During the second half of the semester, the students design and carry out their own projects, using the techniques they have just learned or other techniques they want to try (we give lots of individual help at this point), and they write a formal report on their research. We will discuss each part of the course in more detail below.

### The First Half

1. **Tissue culture.** Many plant physiology lab classes have an exercise on initiation of carrot callus. We chose it because of the importance of tissue culture in plant biology, because the students need to practice sterile technique, and because it ties in nicely with sections on mineral nutrition, plant growth regulators, and biotechnology in the lecture part of the course. Students start carrot callus cultures on media with different concentrations of auxin and cytokinin, and they also design and carry out a simple experiment to test another variable (students' choice: light, temperature, anatomical origin of explant, etc.). We purchased a portable laminar flow hood, which will also find use in other departmental lab classes. After a short demonstration, students sign up for hood time and start their cultures on their own. Later in the

semester, they subculture their callus and try to initiate growth of plantlets.

**2. Photomorphogenesis.** During the second week, the class works together pooling data on the growth and chlorophyll content of the high-light and low-light grown sunflowers. We have two temperature- and humidity-controlled growth chambers (one purchased on the CSIP grant), one with all its lights on and one with only the incandescents on during the photoperiod. The students count leaves and measure hypocotyls, and also use a computerized digitizer to measure leaf area. They also use this area-measurement technique for later experiments, for example to express photosynthetic rate on a leaf area basis. We purchased an Apple Macintosh computer and video digitizing system (Magic from New Image Technology). The students place the leaves (and some standards with known area) on a light table and photograph them with an inexpensive video camera. The Magic system digitizes and stores the image on disk. Later, the students can retrieve the image and run a Pascal program that we wrote to calculate leaf area. The program counts black bits in the stored document, compares the result with that for known area standards, and calculates leaf area. This is a rapid process, and the students find it much more engaging than older manual methods. In addition, the students can see the power of computers to expedite simple, repetitive laboratory measurements. In the future we will expand the capabilities of the image analysis system so it can measure lengths and angles for analysis of other photomorphogenesis and tropism experiments that students might devise.

During the same laboratory period, the students extract chlorophyll quantitatively from leaves of the two populations of plants and measure the amounts of chlorophyll a and chlorophyll b spectrophotometrically. We purchased a Hewlett Packard diode array spectrophotometer; with this instrument, a

student can acquire and print out a 400-700 nm absorbance spectrum within a minute. This speed makes it possible for all the students, even in a fairly large class, to have experience with a research-quality spectrophotometer.

From the pigment and growth analysis, students see obvious (hypocotyl length, leaf area) and more obscure (chlorophyll a/b ratio) effects of etiolation. They also learn to measure light (with a Skye Instruments quantum meter and/or a thermopyle) and they get some idea of the types of experiments one might do to study specific photomorphogenic responses in more detail.

**3. Photosynthesis.** During the next several weeks, students work independently and come in any time the building is open to learn about photosynthesis and water relations. For the photosynthesis exercise, they use a Hansatech gas-phase oxygen electrode to measure oxygen evolution at different light levels for the high-light and low-light grown sunflower seedlings. They then construct light curves for the two groups of plants.

**4. Water relations.** We have several instruments for studying plant water relations: a Scholander bomb (PMS Instruments), a thermocouple psychrometer (Decagon Devices), and a null-balance porometer (Li-Cor). We chose the Decagon psychrometer because nine samples can equilibrate at once in the sample changer. This is good for student use because most of the time involved in psychrometry is equilibration time. We chose the null balance porometer because it is accurate and because the null-balance concept is used in many kinds of experiments and many fields of biology.

Again, to become familiar with the instruments, the students compare the high-light and low-light grown sunflower seedlings. They measure stomatal conductance (with the porometer), then

water potential (with both the bomb and the psychrometer) and osmotic potential (on frozen/thawed leaf samples with the psychrometer). They use a linear regression program we wrote for the Macintosh to plot their psychrometry standards and give them an equation with which they can calculate water potential of their unknown leaf samples.

### The Second Half

As they are learning to use the instruments, students begin to approach us with ideas for their individual projects. We juggle demands for growth chamber conditions; we order supplies, and most importantly; we advise the students on experimental design; and encourage them to tackle doable projects. Student projects have included: analysis of plant sugars by thin layer chromatography, comparison of photosynthetic rate in C<sub>3</sub> and C<sub>4</sub> plants grown with different water availability, effect of soil salinity on plant water relations, and analysis of herbicide effects on photosynthesis. Both the bomb and the porometer are portable, so that students may take them into the field for individual projects, though

none have done so yet. The difficulties we encounter are predictable: some students want to do Ph.D. projects in 6 weeks; some procrastinate until the last week of class.

The overall organization of our laboratory program is suitable for small classes of motivated students. It requires substantial funding to start, but not much to sustain. Because students can work on their own, we only needed to purchase one of each piece of expensive equipment. However, this means that students must have almost unlimited access to the laboratory; we have a combination lock on the door to allow students to get in when they want. They generally try to learn the equipment during working hours when we are likely to be available, but they also need demonstrations and detailed handouts on operating each instrument.

We have been pleased with student performance in the laboratory. Most students enjoy the autonomy and the individual attention. They appreciate being able to use the fine equipment that the CSIP grant enabled us to buy.