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Converting a Cell Biology Laboratory Course from Cookbook Labs to Guided Inquiry Investigations

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To provide cell biology students with opportunities to design experiments and organize their own data, a guided inquiry experience was designed using only one model organism for the entire semester. Instead of step by step instructions, students are given a brief introduction to the topic and a research question to be addressed. Students are also given 'How-to guides' for techniques that may be useful to them in addressing the research question. Pairs of students brainstorm experimental designs, identify missing information, and present their ideas. Students determine the appropriate controls, number of replicates, and the data that should be collected. This laboratory format is amenable to a variety of model organisms and techniques, but has been developed using *Tetrahymena pyriformis* and inspiration from published ABLE labs. The first two guided-inquiry investigations of the semester are presented here. Students are first asked to determine if an unknown organism is prokaryotic or eukaryotic by characterizing its size, shape and internal structure. Then, they determine the doubling time of the organism by growing cells in culture, counting cells, and quantifying total protein.

Keywords: laboratory revision, guided-inquiry, cell biology

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Introduction

Cookbook-style lab activities tend to minimize students' opportunities to practice experimental design and determine the best ways to record, organize, and interpret their data. The cell biology laboratory curriculum at Western New England University originally consisted of activities that allowed students to gain experience practicing important techniques and exposed them to standard cell biology concepts, but spelled out specific instructions for how to perform each step, and how to record data. These lab activities also used different model organisms for each experiment. The goals for this laboratory revision were to involve students in the process of experimental design without changing the topics or learning objectives. To encourage students to see connections from week to week and technique to technique, each topic was modified to incorporate the same organism for the entire semester. Table 1 shows the topics covered for the first 5 weeks of the semester, the different organisms that were used in the cookbook-style lab, the learning objectives for each lab, and the research questions that were developed to transform each lab into a guided-inquiry investigation.

Table 1. Cookbook-style labs were converted to inquiry investigations using the original learning objectives and a common organism

Cookbook-style Labs			**Inquiry Investigations**
Topic	Organism	Learning Objectives	Research Question
Light Microscopy	Various bacteria, protists, plant, and animal cells	• Use common microscopic techniques to examine cells • Make microscopic measurements with an ocular micrometer	Are the unknown organisms prokaryotic or eukaryotic?
Growth of cells in culture	*Chlorella vulgaris*	• Explain the parts of a logistic growth curve • Measure the growth of cells in culture and determine the cell titer	What is the doubling time of *Tetrahymena pyriformis?*
Analysis of protein	Egg white from chicken	• Relate protein concentration to absorbance • Use the Lowry assay to determine protein concentration in a cell homogenate	Can protein concentration be used to determine the doubling time of cells in culture?
Density Gradient Centrifugation	Spinach chloroplasts	• Perform differential centrifugation to isolate organelles	Can lysosomes and mitochondria from *Tetrahymena pyriformis* be cleanly separated by differential centrifugation?
Enzyme Kinetics	Human Lactase	• Explain, mix, and perform enzyme catalyzed reactions *in vitro*. • Plot enzyme velocity as a function of substrate concentration	Determine the Vmax and Km for the succinate dehydrogenase enzyme from *Tetranymena pyriformis.*

Short student handouts were developed for each lab that included learning objectives, basic background information, available materials, and the experimental question. Students achieve the learning objectives just as they did in the cookbook version of each lab. However, in the guided inquiry versions, students must use the organism and techniques to answer an important experimental question. This cultivates more interest and enthusiasm among students and better simulates the scientific process. Students are instructed that even in a graduate research lab, they will always have access to protocols describing how to perform various procedures. Therefore, in the guided-inquiry approach, students are given how-to guides for basic procedures; some are used over and over throughout the semester. Students are instructed that these procedures can be used in many different experimental situations. The student handouts for the first two labs of the semester are included below, along with a list of the how-to guides that are distributed for these topics.

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Student Outline

Week 1: Techniques in Light Microscopy and Observation

Learning Objectives

1. Use common microscopic techniques to examine cells.
2. Make microscopic measurements with an ocular micrometer.

Introduction

The development of the light microscope permitted the exploration of a world never before imagined possible. It took 150 years of observations for biologists to reach the conclusion that all organisms are composed of cells. The importance of the cell as the basic unit of life cannot be overstated: all physiological and behavioral activity of an organism is a consequence of the activities and interactions of individual cells. Everything you say, do, and think has a physiological basis in cells.

Materials

You will be provided with a live culture of single-celled organism(s), a microscope with ocular micrometer, a stage micrometer, blank microscope slides, coverslips, Vaseline, protoslo, and various solutions:

1. Lugol's iodine solution (I_2KI): forms a purple-black color when it reacts with starch
2. India ink may be ingested by the organism
3. Methyl green-pyronine stains nuclei green and cytoplasm pink to red.
4. Neutral red is red at acidic pH and yellow at basic pH.
5. Janus green B stains mitochondria.
6. Nigrosin is a negative stain that stains the surface details by darkening the field around cells.

For each of the solutions 1-5, combine one drop of cells and one drop of stain on a slide, and add a coverslip. For Nigrosin, combine one drop of cells and one drop of stain on a slide. Mix with a toothpick or pipette tip and spread into a thin layer. Let the slide dry before observing. You do not need a coverslip.

Experimental Design

Your task is to describe the organism(s) in your culture as specifically and accurately as possible. How many different types of cells do you see? Are they prokaryotic or eukaryotic? Describe all of your evidence. What is their average size and shape? Take an average of several cells and report this with standard deviation. Do the cells move? If so, how? (cilia, flagella, other motile structures?) What types of subcellular structures do they contain? What can you conclude about the intracellular environment? For each solution, what do you see? Do cells survive exposure to each solution, or are they 'fixed'?

Data Collection and Analysis

Collect as much data as possible to answer the questions posed in the experimental design section. Record this data in the form of tables, drawings, photos, and descriptive paragraphs in your lab notebook.

How-To Guides for Week 1:

How to make a wet mount
How to use the microscope to measure the length and width of specimens
How to observe cells using Dark-field Microscopy

Week 2-3: The Growth of Cells in Culture

Learning Objectives

1. Explain the various parts of the logistic growth curve.
2. Measure the growth of cells in culture and determine the cell titer of a culture.
3. Make a growth curve from data you collect.

Introduction

Cells grow with a characteristic sigmoid (sometimes called an S-shaped curve or a logistic curve) growth curve. If a new culture is seeded with a few cells, growth begins slowly as cells gear up metabolically. This is called the **lag phase**. Within a few hours or days, depending on the organism, growth is at its maximum rate in the so-called **exponential phase** (or **logarithmic phase**). The term 'exponential' is used because during this period each cell gives rise to two; then those two give rise to four, then eight, sixteen, thirty-two, etc. As nutrients in the culture are depleted and as wastes accumulate, growth slows to a standstill. This is the **stationary phase**. During the stationary phase cells are dividing only occasionally, and cell death is counterbalancing cell growth.

Cell biologists are able to grow many kinds of cells in culture and large numbers of cells can readily be made available for experimentation. Organelles can be isolated, enzymes can be extracted, and genes can be altered, all using cells in culture.

Materials

You will be provided with live cultures of the ciliated protozoan, *Tetrahymena pyriformis*, a microscope with ocular micrometer, a hemocytometer, coverslips, a Spec-20 Spectrophotometer, cuvettes, pipettes and tips, Pasteur pipettes, a vortex mixer, and Lugol's iodine solution (I_2KI).

Experimental Design

Your task is to design two experiments to answer the following questions: 1) What is the doubling time of *Tetrahymena pyriformis*? 2) Which method for determining doubling time is more accurate – measuring absorbance, or counting cells? Think about how growth rate can be measured, what controls are necessary, and any additional questions you may have for the instructor.

How-to Guide for Week 2

How to count cells using a counting chamber (hemocytometer)

Notes for the Instructor

Students are generally surprised to learn that there can be multiple ways to approach a scientific question, and that sometimes, experiments don't work out exactly as they expect. During their first experiences with a guided-inquiry investigation, students generally need to be presented with several questions to get them started. Examples include "What will be your first step in this experiment? Do you have all the information you need to answer this question? What will you measure? How will you measure it? What will your control be? What should it look like if x happens?"

In the week 1 experiment presented here, the students are given cultures of the unicellular ciliated protist, *Tetrahymena pyriformis.* Students should determine that the cells are eukaryotic based on size and the presence of organelles. Representative student data shows an average length of 44.5 ± 5.4 µm, and an average width of 31.5 ± 5.8 µm. Generally, students can detect the presence of the nucleus using methyl green pyronine, the presence of mitochondria using janus green, and the presence of cilia using the dark-field technique. The absence of starch indicates that these cells are not photosynthetic, and extended observation of cells mixed with india ink shows that they ingest particles by phagocytosis. If students misinterpret the results of certain stains and don't calibrate the micrometer properly, they may come to the conclusion that the cells are prokaryotic. This is an excellent opportunity for discussion about the ambiguities that can arise when interpreting data and the importance of double checking calculations.

In the week 2 experiment, students need more direction, especially since they have no basis for determining how often to collect samples for absorbance measurements and cell counts. Students are encouraged to determine what information they are missing as part of the brainstorming process, how they might design an experiment to determine the doubling time of this organism, and then the instructor can relay the relevant information to fill in the gaps. For example, students realize that a spectrophotometer can tell them how dense a growing culture has become, but must also think about how to blank the spectrophotometer, and realize that they need to set the spectrophotometer to a particular wavelength. This experience thinking about the experimental process and identifying missing information helps students later on in the semester when they design more sophisticated experiments. In week 3, students determine if the total protein concentration of cells can also be used to determine doubling time using the Lowry Assay. For detailed information on how to culture *T. pyriformis,* and for several experiments that inspired the use of this organism, see Bozzone (2005).

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About the Author

Jessica Rocheleau joined the faculty at Western New England University as an Assistant Professor of Biology in 2010. She teaches courses in cell biology, recombinant DNA technology, nutrition, genomics, and introductory biology for pre-pharmacy students.

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