

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



The cell biology course at Western New England University had historically included cookbook-style labs that utilized different organisms for each session. To provide students with opportunities to design experiments and organize their own data, the laboratory was completely redesigned as a guided inquiry experience using only one model organism for the entire semester. Instead of step by step instructions, students are given a brief introduction to the topic (e.g. Microscopy, Growing cells in culture, Organelle separation) 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, determine what additional information they may need, and then present their ideas to the class. For some experiments, we come to a group consensus on the preferred methodology and for others, each student pair designs and executes their own experiment. Students determine the appropriate controls, the appropriate number of replicates, and the important 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.

Cookbook-style labs		Inquiry Investigations	
Topic	Organism	Learning Objectives	Research Question
Light Microscopy	Various bacteria, protists, plant and animal cells	<ul style="list-style-type: none"> 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	<i>Chlorella vulgaris</i>	<ul style="list-style-type: none"> 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 <i>Tetrahymena pyriformis</i> ?
Analysis of Protein	Egg white from chicken	<ul style="list-style-type: none"> 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	<ul style="list-style-type: none"> Perform differential centrifugation to isolate organelles 	Can lysosomes and mitochondria from <i>Tetrahymena pyriformis</i> be cleanly separated by differential centrifugation?
Enzyme Kinetics	Human Lactase	<ul style="list-style-type: none"> Explain, mix, and perform enzyme catalyzed reactions <i>in vitro</i> Plot enzyme velocity as a function of substrate concentration 	Determine the V _{max} and Km for the succinate dehydrogenase enzyme from <i>Tetrahymena pyriformis</i>

Assessment

- Individually, students write formal lab reports for each inquiry-based lab, presenting the data they collected and the conclusions they reached.
- Students are given opportunities to discuss data presentation and figure preparation, and they are given extensive feedback for each report
- After the first series of labs, each pair presents their work in a poster session

Lab 1: Light Microscopy

- Students are provided with a live culture of unknown cells (*Tetrahymena pyriformis*) and the following solutions:
 - Lugol's iodine (I₂KI): stains starch
 - Methyl green-pyronine: stains nuclei green, cytoplasm pink
 - Neutral red: turns yellow at basic pH
 - Janus green B: stains mitochondria
 - Nigrosin: negative stain
 - India Ink: is ingested into food vacuoles

Instructions to Student Pairs:

- 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 stain, what do you see? Do cells survive the staining procedure, or are they 'fixed'? See the "How to" guides for more information.

How-to guides for Light Microscopy

- How to make a wet-mount
- How to calibrate an ocular micrometer and use the microscope to measure the length and width of specimens
- How to perform dark-field microscopy

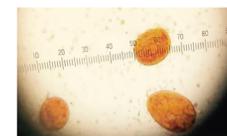
Sample Student Data

Stain	Observations	What it Means
No Stain	- Mobile - Nucleus	- Cilia present - Eukaryotic
Lugols	- Orange/brown color - Fixed with stain - Smaller	- No starch present - Stain demobilized - Some prokaryotic, some eukaryotic
Nigrosin	- Medium to small in size - Visible compartmentation or organelles - Mobile	- Eukaryotic - Cilia present
Janus Green	- Green Organelles – Mitochondria - Fixed due to stain - Medium sized	- Eukaryotic - Cilia still noticed
Neutral Red	- Red color - Fixed, motile structure visible - Organelles visible, medium sized	- Acidic Ph - Cilia present - Eukaryotic
Methyl Green	- Blue to green nuclei - Pink cytoplasm	- Eukaryotic
India Ink	- Mobile - Black inside cells - Other organelles visible	- Cilia present - Ingested carbon - Eukaryotic

Observations and conclusions after observing the unknown organism (*T. pyriformis*) under various stains and conditions

Cell	Length (µm)	Width (µm)
1	47.5	25
2	47.5	27.5
3	35	30
4	47.5	37.5
5	45	37.5
Average	44.5 +/- 5.4	31.5 +/- 5.8

Measurement of *T. pyriformis* cells using a calibrated ocular micrometer at 400X magnification



T. Pyriformis stained with Lugol's iodine at 400X magnification



T. Pyriformis stained with neutral red at 400X magnification

Lab 2: Cells in Culture

- Students are provided with cultures of *Tetrahymena pyriformis*, flasks of autoclaved media, a microscope with ocular micrometer, a hemocytometer, and a spec-20 spectrophotometer.

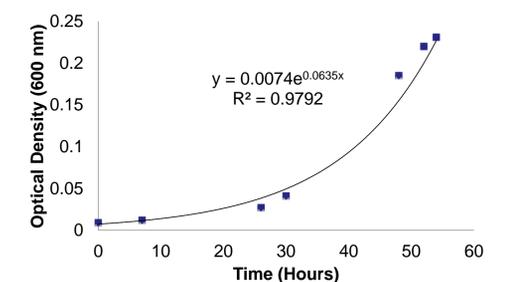
Instructions to Student Pairs

- Your task is to design 2 experiments to answer the following questions: 1) What is the doubling time of *Tetrahymena pyriformis*? 2) How many cells are in each culture flask?
- Think about how growth rate can be measured, what controls are necessary, and any additional questions you may have for the instructor.

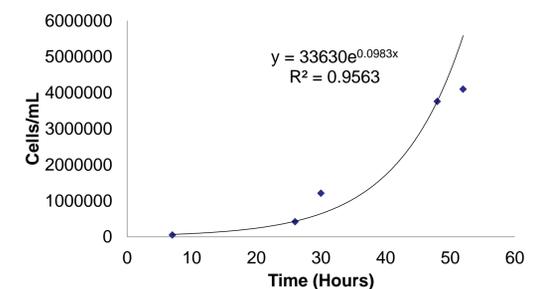
How-to guides for Cells in Culture

- How to count cells using a counting chamber (hemocytometer)
- How to perform the Lowry Assay

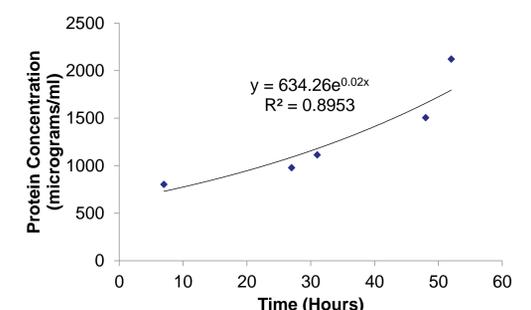
Sample Student Data



Absorbance of *T. pyriformis* cells in culture was measured at 600 nm



T. pyriformis cells were counted using a hemocytometer



Total protein was extracted from *T. pyriformis* and measured using the Lowry colorimetric assay

Experimental Process	Doubling Time (Hours)
Optical Density	3.45
Hematocytometer Counting	3.01
Protein Concentration	6.91

Determination of the doubling time of *T. pyriformis* using three different experimental techniques