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CourseSource

Lesson

Fatty Acid Induction of Lipid Droplets in Cancer Cells

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Abstract

There is a growing need for the development and communication of cell culture-based laboratory activities specifically designed for undergraduate students. This multi-week laboratory activity allows students to take part in the planning, experimentation, data analysis, and communication of the results of their cell culture-based research project. The laboratory activity specifically uses fatty acid induction of lipid droplets in cancer HeLa cells followed by a novel live-cell staining protocol developed specifically to allow undergraduate students the opportunity to complete a cell culture-based fluorescence microscopy project. This laboratory activity incorporates multiple levels of assessment and allows students to explore the responses of HeLa cancer cells to their environment.

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Learning Goals

◊ Students will use cell biology methods and tools to increase their familiarity of the cell through a mammalian cell culture laboratory.

◊ Students will use cell biology methods and tools to increase their familiarity of data analysis and communication of work through a mammalian cell culture laboratory.

◊ From Cell Biology Learning Framework:

  » How do the methods and tools of cell biology enable and limit our understanding of the cell?

Learning Objectives

1. Students will remember and understand basic fatty acid and lipid droplet biology.
2. Students will demonstrate critical thinking by designing and executing a project in mammalian cell culture biology.
3. Students will analyze and evaluate data.
4. Students will create and publish a website of their project results.

INTRODUCTION

Course-based undergraduate research experiences (CUREs) provide engagement opportunities for authentic, inclusive research experiences at the undergraduate level (1, 2). Recently there has been an increase in the number of these CUREs using mammalian (3–5) and non-mammalian (6, 7) cell culture-based techniques. These cell culture-based CUREs have been effective at helping engage underrepresented diverse populations in STEM fields (8), such as women, ethnic minorities, and persons with disabilities, which continue to be important populations to retain in science careers. Also, these cell culture-based CUREs create authenticity and inquiry (9) and improve overall student engagement and learning (7). This laboratory activity presents a new and engaging cell culture-based CURE using a newly developed live cell co-staining protocol for this experiment specifically with undergraduates in mind. This live cell co-staining protocol is undergraduate friendly and produces reproducible high-quality results. The protocol limits issues frequently witnessed with cell staining protocols with undergraduates with limited cytochemistry experience such as issues with fixation, blocking, and antibody reactions. Additionally, this laboratory activity incorporates a series of short modules (approximately 1–3 weeks each) each called a "Cell Block" along with associated short videos (some by undergraduate students themselves) walking the instructor and students through the process. These Cell Block short videos and associated protocols are described in this article (Supporting Files S1–5) and are a part of a growing international movement to make cell culture protocols more accessible for faculty and undergraduate students (10).

In this CURE laboratory activity, students examine an organelle not frequently discussed in undergraduate textbooks called Lipid Droplets (LDs). Lipid droplets are small storage organelles composed of various neutral lipids and surrounded by a phospholipid monolayer (11). LDs participate in various aspects of energy metabolism (11, 12). For more general background information on LDs see (13, 14) and this iBiology lecture.

LDs changing their size or numbers can indicate everything from metabolic conditions to neurological diseases and even different kinds of cancer (15–17). Recent evidence suggests a role for these LDs in highly aggressive cancer and resistance to chemotherapy drugs (16, 18, 19).

Interestingly, some long-chain fatty acids have been shown to induce LD formation (20–22). However, comparisons of the diverse number of fatty acids have yet to be published and very few studies have been performed in HeLa cells (22). Thus, with the large number of possible fatty acids to choose from and the need for more comparative data in this area, this laboratory activity provides many opportunities for students to explore various fatty acids and their connections with LD formation in HeLa cells. Students often propose projects comparing different fatty acids that are unique by: fatty acid chain length (long- short- or medium-chain fatty acids), saturation status (monounsaturated, polyunsaturated, or saturated), or those that have an interesting background in pharmacology, physiology, or industry. The students enjoy being creative and comparing their selected fatty acids abilities to impact LD formation.

In this laboratory activity, HeLa cells were used as a model of aggressive cancer to examine their lipid droplet formation. HeLa cells are cervical adenocarcinoma cells, which provide a nice model system for this laboratory activity working with students (23). There are many freely available resources about HeLa cells, and additionally as a separate part of this course, students read and discuss the book *The Immortal Life of Henrietta Lacks* by Rebecca Skloot to help students familiarize themselves with the origins and importance of these cells in our society. The use of HeLa cells allows for opportunities to foster questions with students concerning bioethics, informed consent, biospecimen research, and bias in the use of the HeLa cell line. This laboratory activity accomplishes these opportunities through class book discussion. A resource exists that provides an alternative to a traditional book discussion that accomplishes these same goals (24).

Intended Audience

This laboratory activity is intended for application in a research setting that can utilize mammalian cell culture equipment. However, some of the laboratory activity can be incorporated without the need of mammalian cell culture equipment. The laboratory activity is intended for an undergraduate introductory first year biology majors cellular and molecular biology course. However, it has been used successfully with upper division research students as well. This laboratory activity is used in a small enrollment (30 students per semester) divided into two sections (15 students in each section), in small groups of about 4, at a small liberal arts university. A full-time instructor runs both laboratory sections of the course. However, this could be scaled up for larger course enrollments, more sections, or even larger or smaller groups at other institutional types as personnel and mammalian cell culture facilities allow. One method of scale could involve the use of graduate and undergraduate teaching assistants that could help the small groups.

Required Learning Time

This laboratory activity spans ten weeks and is implemented in two-hour lab periods that meet once a week. The laboratory activity is divided into Cell Blocks which can be used together or independently during the semester. Students work independently and with their group outside of lab periods to complete some assigned tasks. One week (Week 5, see Table 1) does allow opportunities for students to gain more experience in the laboratory with extended tasks during the staining protocol week. While this extended learning is optional, many students are very engaged and excited to carry out their proposed group project and thus attend if their schedule permits. Students work in self-selected small groups of four and their work is assessed throughout the course to provide them with feedback and opportunities for growth and improvement.

Prerequisite Student Knowledge

This laboratory activity is designed for students with minimal background in the subject matter. The activity includes all necessary resources including video tutorials that walk students through most of the process. Prior to the Week 5 activity students have been introduced to microscopy and micropipetting as a separate part of this laboratory course and thus will be comfortable with utilizing these instruments. Students learn the background information needed for this project as they progress through the laboratory activity.

Prerequisite Teacher Knowledge

This laboratory activity allows institutions to build and enhance their framework of *Vision and Change* Core Concepts (25) by examining deeper the structure and function of LDs in cancer cells. It also provides a framework for *Vision and Change* Core Competencies (25) by allowing students to apply the process of science, use quantitative reasoning, communicate and collaborate with other disciplines, and understand the relationship between science and society. Thus, this laboratory activity is helpful for teachers trying to enhance their institution's alignment with national biology recommendations.

It is highly recommended that the instructor has previous experience with mammalian cell culture in a BL2 Safety Lab. This is very important for the success of this project and recommend being trained in this area prior to taking on the cell culture portion of the laboratory activity. Alternatively, at some institutions it may be possible to partner with another laboratory that is already doing mammalian cell culture and utilize their facilities for the Week 5 experiment, which is the only time needed at the facility. Additionally, the instructor will need to familiarize themselves with the background on lipid droplets, fatty acids, and HeLa cells for which there are resources. For specific background on fatty acids and LD formation see papers provided in Supporting File S1. For more general background information on LDs see (13, 14) and this iBiology lecture. For more general background information on HeLa cells see online resources and the book *The Immortal Life of Henrietta Lacks* by Rebecca Skloot and potential resources (21). For more general information about culturing mammalian cells see various videos on the CBEC YouTube Channel and other freely available resources (26).

SCIENTIFIC TEACHING THEMES

Active Learning

This laboratory activity has students working collaboratively in small groups of about four to perform all tasks: researching background knowledge, writing their research proposal, completing the laboratory exercise, performing data analysis, and developing their website publication of their results.

Assessment

Assessment of this laboratory activity includes four distinct elements:

Small Group Research Proposal

The first major assessment of the laboratory activity is the assessment of a written small group research proposal based upon the students' background research. The small groups meet with the instructor for formative feedback and an opportunity for revision of their proposal. The instructor evaluates the proposals using the proposal rubric (Supporting File S1). Since this is a group assignment, all students receive the same grade for this assessment. This proposal assesses Learning Objectives 1 and 2.

Image Data Collection

The second major assessment of the laboratory activity is the assessment of the collection of images from their experiment. This experiment was detailed in Supporting Files S2 and S3. Students successfully completing this step are evaluated for completeness. Since this is a group assignment, all students receive the same grade for this assessment. This collected image data assesses Learning Objective 2.

Data Analysis Excel File

The third major assessment of the laboratory activity is the assessment of the small group data analysis Excel file, which includes their graphical presentation of their data and the statistical analysis. The details of this analysis are presented in Supporting File S4. Students take their image data analyzed in ImageJ and then in Excel (or similar software) calculate the mean averages, standard deviations, and standard error of the mean for each condition. Then students compare their conditions using a two-sided unpaired Student's *t* test. They also take the data and construct a graph demonstrating their results. Students frequently request help on knowing how many LDs or cells to count in ImageJ. Often, students will count many more than what is required. Reminding them that usually, 10 cells are sufficient to get enough data on the average number of LDs per cell and 100 LDs are sufficient to obtain enough data on the average area of lipid droplets. Encourage students to count the same number of data points for each condition, when appropriate. Students present their data analysis to the instructor and the instructor provides feedback and allows for revisions of the data analysis prior to publication. Since this is a group assignment, all students receive the same grade for this assessment. This data analysis assesses Learning Objective 3.

Website Publication

The final major assessment of the laboratory activity is the assessment of the small group website publication of their results. The instructor evaluates the website publication using the website publication rubric (Supporting File S5). This is assessed formatively each week with the small groups to ensure that all sections are being completed accurately. Students provide a website with a title, purpose statement, methodology, figures and figure legends, results section, discussion section, and acknowledgements section. The rubric provides guidelines on these sections to assist the instructor with website assessment. The levels of competence are not weighted. These could be weighted if the instructor chooses to provide clarity on categories and quantifying the total score. Since this is a group assignment, all students receive the same grade for a majority of the assessment. However, students complete the individual-based teamwork evaluation based upon contribution to the project and peer-review optional Week 11 activity. This website publication assesses Learning Objective 4.

Inclusive Teaching

This laboratory activity is inclusive because it places students as scientists in a research setting with a novel CURE laboratory activity, which have been shown to better include unrepresentative minorities (8); are more inclusive than independent research experiences (1); and may help reduce opportunity gaps in the life sciences (27). Finding ways to reduce opportunity gaps is an ongoing goal for increasing diversity, equity, inclusion and justice in science higher education. There is a disproportionate number of minority groups leaving science because of the lack of interest and engagement. Therefore, it is important that institutions provide projects and laboratory activities that are relevant so that each student can identify as a scientist (28). Further, this laboratory activity allows students to gain project ownership and to express their creativity through their project proposal and website publication. These types of assessments are creative written works that provide opportunities for students to make mistakes, show growth, and ultimately learn. Their learning is not dependent on exams nor oral presentations, which have shown to increase anxiety for students (29). Thus, this laboratory activity encourages each student to identify as a scientist, allows instructors to connect with students, and fosters inclusive classroom environment by reducing anxiety and stress (30).

LESSON PLAN

This laboratory activity is designed to be implemented in a 10-week period of time. However, in this implementation, the laboratory activity spanned the entire semester around which two other labs were also embedded to help students master skills in micropipetting and microscopy and an important discussion on the book *The Immortal Life of Henrietta Lacks*. Table 1. Teaching Timeline outlines how one might carry out this laboratory activity. The timeline notes reference Supporting Files S1–S5, which are detailed instructions, laboratory protocols, assessment rubrics, and references all provided to students during the course. These files are labeled as "Cell Blocks", which divide the large laboratory activity into five small blocks of experiments so that the students are not presented with everything at once. It is worth noting that we have added a Week 0 option in our lesson where students participate in a book discussion on *The Immortal Life of Henrietta Lacks* to discuss bioethics, informed consent, biospecimen research, and bias in the use of the HeLa cell line.

In summary, the instructor introduces the concept of the laboratory activity with the students in Week 1 and then students are asked to form groups and perform background research, support for which is provided in Supporting File S1. During Week 2 student groups develop a research proposal outlining their specific independent variable (fatty acid[s]) and dependent variable (area of lipid droplets or number of lipid droplets/cell). A rubric for the research proposal is provided in Supporting File S1. In Week 3 student groups meet with the instructor to discuss their proposal and then student groups revise the proposal for Week 4 submission.

Week 5 is when the most preparation work is required for the instructor, when laboratory reagents are prepared and the HeLa cells that are being passed are prepared for students to implement the experiment. See Supporting File S2 for details. Week 5 requires three days of active attention by the instructor and students. Day 1 of Week 5 includes preparation of fatty acid and plating HeLa cells. Some areas to help avoid possible issues with the experiment include limiting the course to sodium-based fatty acid salts and those that have high solubility in ethanol. Check over the reagent information before purchasing fatty acids. These two considerations have shown to be critical for proper fatty acid final concentration in solution and for experimentation success. See Supporting File S2 for details. Day 2 of Week 5 includes adding the fatty acid to HeLa cells (Supporting File S2). Day 3 of Week 5 is the staining protocol found in Supporting File S3. The staining protocol has been worked out to allow for the three co-stains to work together. It is not recommended to use shortcuts (*e.g.*, shortening the time of incubation of the stain) as these tend to not help the clarity of the final images. No overstaining nor understaining have been witnessed with these live cell stains. After the staining of the cells during Day 3, the cells are imaged using a fluorescence microscope (Supporting File S3) and images are collected and shared with the students. It is recommended that instructors attend to the students when taking images on the fluorescence microscope. Students tend to have many questions about using an unfamiliar instrument.

Week 6 is the beginning of data analysis, where students use the images collected and follow a set of tutorial videos for how to gather data on their dependent variables (Supporting File S4). Then students perform the data analysis for Week 7 and submit their data analysis for review by the instructor prior to Week 8. During Weeks 8 and 9, students meet with the instructor to discuss their data analysis and then the students may need to revise their data analysis. Also, during this time, students begin designing websites to communicate their results of their laboratory activity following the guide and rubric found in Supporting File S5. Students publish their websites during Week 10. It is nice to include a Week 11 option to allow students to complete a peer-review of other small groups' websites using the provided rubric (Supporting File S5).

TEACHING DISCUSSION

This laboratory activity allows students to immerse themselves in the process of completing a cell culture research project in 10 weeks. The lesson has four major assessments. Observations from teaching, suggestions for the instructor, and effectiveness of each major assessment is discussed here. Then following discussions on these assessment pieces, extensions and modifications are suggested.

Small Group Research Proposal

Learning Objectives 1 and 2. The first major assessment of the laboratory activity is a written small group research proposal based upon the students' background research. Instructor needs to actively engage students during their initial research. It helps the small groups if the instructor checks in with them weekly to see how they are doing with their proposal writing and provide formative feedback for revisions based upon Table 2. Overall, the first draft proposals have many errors and sometimes have unfinished sections. Students

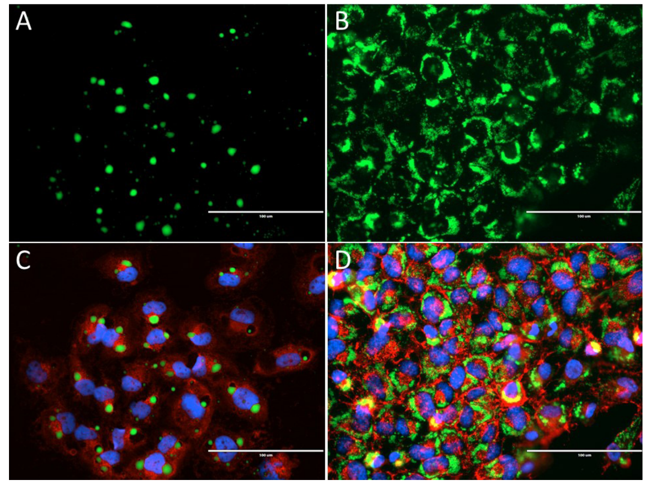


Figure 1. Fatty acids induce lipid droplet formation in HeLa cells. Image of live-stained lipid droplets (green) in HeLa cells after incubation with either **(A)** oleic acid or **(B)** linoleic acid following the protocol described in this article. The same HeLa cell images are merged with the corresponding live-stained cell membranes (red) and nuclei (blue) for **(C)** oleic acid or **(D)** linoleic acid.

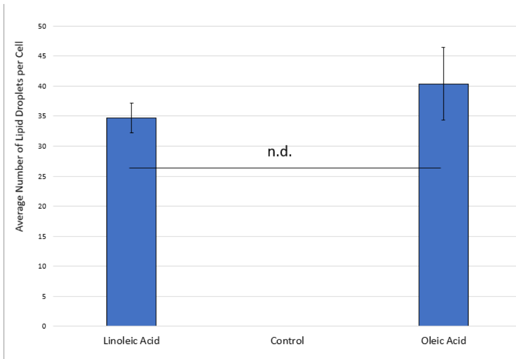
will likely not understand the dependent and independent variables. See Supporting File S1 for the rubric and sections used for the research proposal. After formatively discussing the rubric with the student small groups and going over these points, student small group revisions are usually quite good and effective at communicating their message. The rationales tend to be where students showcase their creativity well as they demonstrate why the small group selected their chosen fatty acid(s) based upon their research and/or interests.

Image Data Collection

Learning Objective 2. The second major assessment of the laboratory activity is evaluation of the collection of images from their experiment. This is one of the most memorable moments for the students to see their hard work come to fruition after weeks of preparation. Students exhibit excitement and disbelief that they were able to accomplish their goal. This moment is called the "aha moment" and has been critical to helping with retention in the life science programs. Images collected usually are of high quality. It is common and should be expected for the negative control (ethanol solvent) to have few to no LDs and for the positive control (oleic acid) to have numerus very large LDs. Experimental fatty acids will have LDs that are variable in appearance; some may not induce any LDs at all; and some more than the positive control. See two examples of fatty acids inducing LD formation in HeLa cells, stained for LDs and those merged with the cell nuclei and cell membranes in Figure 1.

Data Analysis Excel File

Learning Objective 3. The third major assessment of the laboratory activity is the small group data analysis Excel file, which includes their graphical presentation of their data and the statistical analysis. For each condition, calculations of the mean averages, standard deviations, and standard error of the mean were performed. Depending upon the experiment dependent variable, either 10 cells were analyzed for number of LDs per cell for each condition or 100 LDs were analyzed across multiple cells for each condition. Comparisons of conditions for all data were performed using a two-sided unpaired Student's t test to check for statistical significance.



	Linoleic Acid (Experimental)	Control (Negative)	Oleic Acid (Positive)
Cell 1	23	0	21
Cell 2	39	0	69
Cell 3	47	0	26
Cell 4	45	0	32
Cell 5	35	0	51
Cell 6	36	0	21
Cell 7	26	0	72
Cell 8	28	0	24
Cell 9	30	0	39
Cell 10	38	0	49

	Linoleic Acid	**Control**	**Oleic Acid**
Average Number of Lipid Droplets per Cell	34.7	0	40.4
Standard Deviation	7.944949479	0	19.1613036
Standard Error of the Mean	2.512413625	0	6.059336231
Number of Cells	10	10	10

"n.d." represents the p-value of Oleic and Linoleic acid is greater than 0.05, showing there is no significant difference between the two acids.

Student's t-test (unpaired, two-sided)	Linoleic vs Control	Oleic vs Control	Oleic vs Linoleic
P-values:	5.08794E-11	2.95851E-06	0.396307463
	P-value<0.05	P-value<0.05	P-value>0.05
P-value<0.05- there is a significant difference between the two groups	There is significant difference between the Linoleic and Control	There is a significant difference between the Oleic and Control	There is no significant difference between the Oleic and Lenoleic
P-value>0.05- there no significant difference between the two groups			

Figure 2. Quantitative analysis of impact of fatty acid on lipid droplets in HeLa cells. A representative data analysis of different conditions (negative control ethanol solvent, positive control 400 µM oleic acid, or experimental 400 µM linoleic acid) were added to HeLa cells for 24 hours. This was followed by the staining protocol. Then for ten cells for each condition, the number of lipid droplets in each cell were counted using ImageJ. Then those values were placed in Excel and the mean number of lipid droplets per cell were calculated as well as the standard deviation, standard error of the mean, and the total number of cells per condition. The means were then graphed along with the standard error of the mean reported as the error bars. Finally, two-sided unpaired Student's *t* tests were used to determine whether the conditions were significantly different from each other in their induction of lipid droplet numbers per cell. The *p* values are presented as well as a brief interpretation. Visual representation of the *p* values is included in the graph.

Data is reported as a graph along with the standard error of the mean reported as error bars. After students perform this data analysis, work is submitted for instructor review and formative feedback. Students then implement revisions. It is important that the instructor meets individually with each small group to ensure that data analysis is correct prior to publication. See an example of a data analysis for the impact of specific fatty acids on the number of lipid droplets per cell in the Excel file in Figure 2. Implementation of the video tutorials by the instructor has been shown to greatly reduce the number of questions by the small groups and helps with the flow of this assessment. As long as students are following the instructions and the video tutorials, final data analysis is usually very good with very few errors.

Website Publication

Learning Objective 4. The final assessment of the laboratory activity is the small group website publication of their results. Students design a website highlighting their project. The instructor provides frequent formative feedback and then students revise and publish their final website. Students use Supporting File S5 and links to example publications to help with their website design. The examples save time for the instructor to handle more questions specific to the data and not the format of their work. Students often want to know which website design tool to use. Usually there are many options and students should select the one that works best for them. Students are provided with some examples of design tools that have worked in the past (see Supporting File S5). The same file contains the rubric used to assess the website.

Extensions/ Modifications

There are several ways in which instructors can modify this laboratory activity to assist student learning and the overall experience. The instructor could utilize the experience of upper division students that have previously taken this course or similar mammalian cell culture laboratories. One effective way is to provide opportunities for the upper division students to come back as peer leaders or teaching assistants for the introductory laboratory. Often upper division students pass the HeLa cells for the introductory students in a collaborative manner, which helps with all student's learning and growth in cell biology. This engagement with upper division students creates a sense of community and excitement as the introductory students see the potential for them if they wish to continue to learn and grow in cell biology. Thus, allowing the instructor to create not just a single laboratory course experience, but one that allows students to participate in a research community. This creates more opportunities for deeper and more intimate experiences in research.

In light of the recent pandemic, the laboratory activity was adapted for virtual instruction. Details for how this lesson was adapted for the online environment and other similar cell culture-based laboratories can be found in the following resource (10). In short, the instructor posted YouTube and Instagram videos completing the tasks outlined. Students completed all the assessment pieces outlined above including data analysis. This might also serve as a method for incorporation of the assessment items from this laboratory activity for those institutions that lack cell culture resources yet still want to give students opportunities to engage in cell culture project. These types of experiences allow us to continue to offer research at the undergraduate level from all backgrounds and previous experiences. It also helped us serve our online students and keep them engaged in the process of science and authentic research.

It is recommended that during the background research students make a concept map connecting the terminology being used. Students have reported in the past as not being able to make connections between the complex terminology presented in the background research section. This extended activity would help students achieve Learning Objective 1.

In addition to the complex terminology, the Week 0 book discussion allows for gains in student interest in this project. Prior to this 10-week laboratory activity, students participate in a book discussion on *The Immortal Life of Henrietta Lacks* to discuss bioethics, informed consent, biospecimen research, and bias in the use of the HeLa cell line.

Often an extension in Week 11 is to have the students perform a peer-review using the rubric from Supporting File S5. This allows students to view other peer groups' websites and provide feedback and to see how others approached the project.

While a majority of students examine the dependent variables outlined in these protocols (average area of LDs per condition and average number of LDs per cell), some students explored other responses. Some project extensions that could be included would be to examine nuclei deformation, a common response by addition of fatty acids to cells during LD formation (31). Another alternative that students have chosen is to examine cell death as a result of LD formation.

Another modification is the opportunity to use alternative cell lines. Of particular interest would be working with hepatocytes, which have literature exploring LDs formation in response to long-chain fatty acids (22, 23).

Further, instructors can use any of the Cell Blocks as a standalone experience or part of their own course design. For our laboratory activity the Cell Blocks were used together, but providing students with access to video resources about designing an experiment could be beneficial for some instructors. While other instructors may find it valuable to have a resource for data analysis of LDs with ImageJ software. This laboratory activity was used as part of a semester long CURE, but could easily be adapted to a mentored research environment for a few undergraduates or part of a summer research course.

Conclusion

In conclusion, this laboratory activity allows students to creatively design and implement a research project in cell culture fluorescence microscopy and to analyze data and publish their work. Overall, students find this experience one of the most critical aspects of their first-year experience. This has led to the development of CUREs in many laboratory courses and has spread excitement for life science majors across campus.

SUPPORTING MATERIALS

- S1. Lipid Droplet – Background Cell Block
- S2. Lipid Droplet – Experiment Set Up Cell Block
- S3. Lipid Droplet – Staining Cell Block
- S4. Lipid Droplet – Data Analysis Cell Block
- S5. Lipid Droplet – Website Design Cell Block

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Table 1. Lesson plan timeline. The lesson best spans ten weeks of a semester as well as outside of class time for preparation and independent student activity. There is an optional Week 0 opportunity for a book discussion and an optional Week 11 opportunity for peer-review of websites.

Activity	Description	Estimated Time	Notes
Week 0 (optional)			
Book Discussion	Students individually read *The Immortal Life of Henrietta Lacks* and then gather as a lab section to discuss the book.	1 hour + approximately 20 hours of outside of class time	Option to include activity where the instructor assigns students to read the book and then provides students with a set of discussion questions. Students lead the discussion.
Week 1			
Introduce Project	Instructor discusses the opportunities students have to explore the impact of a fatty acid on lipid droplet formation.	15 minutes	Instructor walks through the exciting points from the Introduction section of this article and supplements with images to enhance.
Small Group Formation	Students form a small group of about 3 students and discuss roles and fill out small group contracts.	15 minutes	See example of group contracts in Supporting File S1.
Introduce Background Cell Block	Instructor walks through the Supporting File S1.	30 minutes	See Supporting File S1.
Background Research	Students are provided with a list of "areas of research". Students collect background information in their lab notebook researching these "areas of research" using Google Image Search and supplement their research with pre-selected peer-reviewed publications.	1 hour + approximately 2 hours of outside of class time	See areas of research topics (A–H) and peer-reviewed publications References (1–7) in Supporting File S1.
Week 2			
Background Research	Students will continue to research their areas of interest.	30 minutes	Instructor could suggest that small groups create a concept map of "areas of research". See areas of research topics (A–H) and peer-reviewed publications References (1–7) in Supporting File S1. Many small groups will have questions for the instructor concerning what they are researching.
Outline Group Project Variables	Students will provide the instructor with their specific independent and dependent variables for their proposal.	30 minutes	Students will need approval by the instructor to ensure that their variables are valuable and testable. Instructor encourages students to find a variable that is interesting to them.
Write Small Group Research Proposal	Students will collect their background research and write a project proposal.	1 hour + approximately 2 hours of outside of class time	See Proposal Rubric in Supporting File S1.
Prep for Week 5			
Order Fatty Acids	Instructor orders fatty acids in advance.	1 hour of outside of class time	See recommended source for fatty acids in Supporting File S2. These should be ordered as early as Week 3 to ensure that the reagents are in hand for Week 5.

Activity	Description	Estimated Time	Notes
Week 3			
Submit Small Group Proposal	Student small groups submit proposals prior to the lab session.	approximately 15 minutes of outside of class time	Rubric found in Supporting File S1.
Discuss Small Group Proposal	Instructor meets with small groups separately for 30 minutes each to discuss proposals and suggest revisions.	30 minutes	Rubric found in Supporting File S1.
Revise Small Group Proposal	Student small groups revise their proposals.	1.5 hours + approximately 2 hours of outside of class time	Rubric found in Supporting File S1.
Week 4			
Students Submit Group Proposal	Student groups submit their final proposal prior to the lab session for instructor review and proposal approval.	approximately 15 minutes of outside of class time	Rubric found in Supporting File S1.
Instructor Reviews Group Proposal	Instructor reviews group proposals via rubric and ensures required reagents are in hand.	approximately 2–3 hours of outside of class time	Rubric found in Supporting File S1.
Prep for Week 5			
Passage HeLa Cells	Instructor passes HeLa cells using standard cell culture protocols.	approximately 2 hours of outside of class time	Students do not directly pass or plate cells in this lab. Prior mammalian cell culture experience is highly recommended for instructors to prep the cells for the students. Resources for instructors passing HeLa cells found at the CBEC YouTube Channel and other freely available resources (26).
Week 5			
Day 1: HeLa Cell Prep	Instructor plates 10,000 HeLa cells per well in an 8-well chamber slide.	approximately 2 hours of outside of class time	Protocol found in Supporting File S2. Resources for instructors plating HeLa cells found Resources for instructors passing HeLa cells found at the CBEC YouTube Channel and other freely available resources (26).
Day 1: Start Fatty Acid Prep	Instructor preps required solutions.	approximately 1 hour of outside of class time	Protocol found in Supporting File S2.
Day 1: Start Fatty Acid Prep	Students dissolve fatty acid in solution.	approximately 30 minutes of outside of class time	Protocol found in Supporting File S2.
Day 2: Finish Fatty Acid Prep	Instructor preps solutions for fatty acid prep.	approximately 2 hours of outside of class time	Protocol found in Supporting File S2.
Day 2: Finish Fatty Acid Prep	Students incubate cells with fatty acid.	approximately 30 minutes of outside of class time	Protocol found in Supporting File S2.
Day 3: Lipid Droplet Staining	Students stain lipid droplets, cell membranes, and nuclei and visualize on a fluorescence microscope.	2–3 hours	Protocol found in Supporting File S3.

Activity	Description	Estimated Time	Notes
Prep for Week 6			
Share Images with Students	Instructor provides students with data images.	approximately 1 hour of outside of class time	Instructor downloads image files from the microscope and shares them with the small groups.
Week 6			
Introduce Data Analysis	Instructor describes resources needed for students to complete data analysis.	15 minutes	Protocol found in Supporting File S4.
Watch Tutorials	Students watch video tutorials for data analysis with ImageJ and Excel.	15 minutes	Video links found in Supporting File S4.
Data Analysis	Students complete ImageJ data analysis and Excel statistical analysis.	1.5 hours + approximately 1 hour of outside of class time	Protocol and video links found in Supporting File S4.
Week 7			
Data Analysis	Students complete ImageJ data analysis and Excel statistical analysis.	2 hours + 1 hour of outside of class time	Protocol and video links found in Supporting File S4.
Week 8			
Students Submit Data Analysis Excel File	Student groups submit their data analysis as an Excel file prior to the lab session for instructor review and approval.	approximately 15 minutes of outside of class time	Protocol and video links found in Supporting File S4.
Website Design Introduction	Instructor introduces students to the Website Design Rubric.	30 minutes	Website Design Information and Rubric found in Supporting File S5.
Website Design	Students begin website design.	1.5 hours + approximately 1 hour outside of class time	Website Design Information and Rubric found in Supporting File S5.
Instructor Reviews Group Data Analysis Excel File	Instructor reviews group data analysis excel file.	approximately 2–3 hours of outside of class time	Protocol and video links found in Supporting File S4.
Week 9			
Discuss Small Group Data Analysis Excel File	Instructor meets with small groups separately for 30 minutes each to discuss data analysis and suggest revisions.	30 minutes	Protocol and video links found in Supporting File S4.
Website Design and Revise Data Analysis	Students design their websites and revise their data analysis as needed.	1.5 hours + approximately 1–2 hours outside of class time	Protocol and video links found in Supporting File S4. Website Design Information and Rubric found in Supporting File S5. At this point, some small groups will need to revise data analysis more than others, so some groups will be more focused on website design and other groups will be still on data analysis.
Week 10			
Website Design	Students finish designing their websites.	2 hours + approximately 1 hour outside of class	Website Design Information and Rubric found in Supporting File S5.
Week 11 (optional)			
Peer Review	Students perform a peer review of other group websites.	approximately 15 minutes	Option to include activity of peer-review of websites using the Rubric found in Supporting File S5.

Table 2. Research proposal sections. The instructor reviews the research proposal formatively and provides feedback based upon these guidelines. Then these same criteria are used to evaluate after revisions.

Section	Description	Notes
Title	Provides a descriptive title encompassing a summary of the specific aim.	Instructor checks that the title section for the proposal has the independent and dependent variables included.
Specific Aim	Provides a specific relationship between independent and dependent variables that is testable.	Instructor checks that the specific aim section of the proposal: (a) does not describe the experiments that are beyond the scope of the work; (b) does not add additional variables beyond those intending to study.
Rationale	Provides in-text citation evidence that builds towards the specific aim.	Instructor checks that the rationale section of the proposal includes citations, especially those of the peer-reviewed publications from Supporting File S1 that describes the previous work where long-chain fatty acids are added to cells including HeLa cells.
Experimental and Control Groups	Provides details on the conditions of the experiment.	Instructor checks that the experimental and control groups section of the proposal: (a) is described fully to include a solvent only condition as a negative control; (b) likely will need the long-chain fatty acid oleic acid condition as a positive control, as oleic acid has been previously published to effectively induce LD formation in HeLa cells (22); (c) provides details about the concentration of the fatty acid being added to the HeLa cells (400 μM) and the duration for which the fatty acid will be present prior to staining (24 hours). In this laboratory activity, we do ask that all groups use the same concentration and duration for their fatty acid incubation as it greatly helps the instructor preparation.
Data Collection Methodology	Provides the detailed methodology to be used and the data that will be collected.	Instructor checks that the data collection methodology section of the proposal: (a) contains the stains that will be used to collect data (green LD stain, red cell membrane stain, and blue nuclei stain); (b) contains the use of fluorescence microscopy and the ability to capture the images; (c) contains information about the use of ImageJ software to generate quantitative data on either area of LDs or number of LDs per cell. (d) contains the use of Excel software (or similar) to analyze the data generated from ImageJ for results interpretation.
Anticipated Results	Provides anticipated results that support the specific aim from the data collection using quantitative comparison and statistical terminology.	Instructor checks that the anticipated results section of the proposal contains a prediction if there will be a statistical difference between the experimental and control groups based upon the rationale section.
References	Provides a list of references that is referred to in-text earlier in the proposal.	Instructor checks that the references section of the proposal contains a list of references that are actually cited in text in the proposal itself and that the list is complete. In this course, it was not required that the students use a specific citation style.