Going Remote: An Online Adaptation to Using a Primary Cell Culture Model to Study the Neural Extracellular Matrix

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Abstract

In light of the COVID-19 pandemic, instructors have modified materials to transition to a remote learning platform. The challenge for science instructors has been to design lab exercises that incorporate both active and experiential learning. In this vein, in this Essay I describe how I modified a previously introduced Lesson using a primary cell culture model to study a specialized structure found within in the neural extracellular matrix, named the perineuronal net. When I taught this exercise originally, the students had completed the majority of the experiment in person, but to adapt to online learning, changes were made to Lab Sessions 3 and 4. Since students were not able to perform the analysis portion of the exercise, I provided images and the students were still tasked with completing a lab report. I made the handouts, information about the lab report, the rubric, and PowerPoints available through the learning management system. Herein, I also reflect on how an instructor could modify the entire four-week experiment to be conducted virtually. In the adapted exercise, students play a leading role in researching protocols, explaining why each step is carried out, establishing hypotheses, and drawing support for these statements from the literature. Therefore, it is possible to have students complete challenging and inquiry-based exercises even if they are not able to carry out the experiment directly. Moving forward, it will be particularly important to readily adapt exercises to fit into a remote learning environment, as described here.

INTRODUCTION

In the Spring 2020 semester, the COVID-19 pandemic led schools, colleges, and universities, to shutter, thereby canceling in person instruction. This quickly forced students to adapt to learning online and forced instructors to adjust their classes to a remote teaching platform. Due to the nature of the pandemic, both students and teachers alike had little time to prepare for such a transition. This was particularly difficult for large courses, discussion-based courses, and lab classes. In this Essay, I will discuss how the COVID-19 pandemic impacted the delivery of a previous Lesson studying the neural extracellular matrix that was published in *CourseSource* (1). Additionally, I will detail ways in which the entire exercise can be formatted for virtual instruction. It is my hope that adopting instructors will find this information useful as the pandemic has rendered the immediate future of in-person classes and laboratories uncertain.

In brief, the original Lesson was taught over four weeks with the overall goal of examining the formation and molecular composition of a substructure of the neural extracellular matrix called the perineuronal net. This structure enwraps the cell body and extensions emanating from populations of neurons within the nervous system and has been hypothesized to modulate synaptic plasticity. In terms of composition, perineuronal nets are highly heterogenous and are comprised of sugars, glycoproteins, and proteoglycans (2,3). The exercise described in both the original

Lesson and herein focuses on the role of the chondroitin sulfate proteoglycan, aggrecan, in the formation and organization of perineuronal nets.

This exercise is best suited for an upper-level undergraduate course in Cell Biology in which the students are Biology or Biochemistry majors. In Week 1, the instructor dissects the cerebral cortices of embryonic mice and the students use the tissue to isolate and then plate single cells. They then treat the cells with potassium chloride to globally enhance neural activity and with a glial cell inhibitor to eliminate glial cells from the cultures. These treatments are designed to allow students to answer two specific questions: (1) Is aggrecan expression modulated by activity? and (2) Is aggrecan likely made by neurons or glia? The cells are cultured for 14 days after which students fix, block, and stain the cells with primary antibodies aimed at identifying aggrecan and a glial cell specific marker. The instructor finishes the experiment by applying secondary antibodies and cover slipping the cells. The students then image the slides using a fluorescence microscope. During the time in which the cells are cultured, students generate hypotheses and predictions for the aforementioned questions by gathering support from the literature. There are two formative assessments after Lab Sessions 1 and 3, which allow for the instructor to provide meaningful feedback to the students in preparation for the summative assessment, the lab report (1).

LESSON ADAPTATIONS

How the Lesson was Adapted in Spring 2020

When my campus shut down in mid-March, my Cell Biology class had already completed Lab Session 1 of the exercise and the cultures had all been treated and were incubating for the requisite 14 days. All of my students had the opportunity to attend Lab Session 2, where we met in a computer lab to discuss the necessary background information about the experiment. Then students broke out into smaller groups to delve into the literature and state their own hypotheses. The timing of the school closure, allowed for only one of my two lab sections to complete Lab Session 3. This meant that only half of the class was able to fix, block and stain their cultures with primary antibodies. We also discussed the details of the lab report and assembled a list of reagents used in the experiment with associated company information.

To accommodate the switch to remote instruction, I made the pre-lab lectures for Lab Sessions 3 and 4 immediately available for students with notes, and used PowerPoint to record accompanying audio (see Supporting File S7. Studying the Neural Extracellular Matrix – Lab Session 3 Pre-Lab Lecture and Supporting File S9. Studying the Neural Extracellular Matrix – Lab Session 4 Pre-Lab Lecture in the original Lesson (1)). Additionally, I provided all students with a Word document detailing the names of the reagents that they needed to include in their lab report as well as corresponding company information with the intention that the students would find the location of each of the companies to cite in their lab reports (Supporting File S1. Going Remote: Studying the Neural Extracellular Matrix – Reagent Information).

Lab Session 4 also had to be modified. Given that we had invested a significant portion of the semester on this exercise, I still wanted my students to complete a lab report and thus, I opted to provide them with data to analyze. More specifically, I provided them with images of cultures stained with anti-aggrecan antibodies and anti-glial fibrillary acidic protein markers as well as Hoechst dye. To provide some context, I included written information as to what the students should be paying attention to when analyzing these images (Supporting File S2. Going Remote: Studying the Neural Extracellular Matrix – Lab Session 4 Modified Handout). Even though the students were not able to use the fluorescence microscopes firsthand, the lab handout still included instructions on how to operate the microscopes, and students took an online pre-lab quiz on this topic. The structure of the lab report remained the same even though the students could not analyze their own work. Instead, they used the provided data and then determined whether they could support their initial hypotheses. As explained in the original Lesson, the goal was not to have students generate a "correct" hypothesis, but rather to find support for their hypotheses and then discuss their own results in the context of a greater body of work (1).

Since I was not able to meet with the students face-to-face about the lab report, I continued to offer office hours remotely through Cisco WebEx. I also highly encouraged my students to email me with questions if they could not attend office hours as well as submit either complete or incomplete drafts of their lab report. Roughly 40% of my students submitted drafts of the lab report and those that took advantage of this opportunity reported being grateful for the feedback.

How the Entire Lesson Could be Adapted

In the following section, I will discuss how instructors wishing to adopt this lesson could modify the entire exercise to be delivered remotely. I also include information about how each session is carried out in a face-to-face versus a remote setting. Additionally, I provide tables comparing and contrasting these two ways of organizing the exercise.

Lab Session 1

In the face-to-face Lab Session 1, as detailed in the previously published Lesson, students were provided with dissected cerebral cortices from mice and they then carried out a series of steps to isolate and plate single cells from this material (1). Given the amount of time needed for the exercise, I typically do not give a pre-lab lecture, but I do administer a lab quiz to ensure that the students have read the lab handout (see Supporting File S4. Studying the Neural Extracellular Matrix – Lab Session 1 Handout in the original Lesson (1)). The questions on this lab quiz are confined to procedural questions instead of questions on the background material. After the students finish, they have to write the materials and methods in their own words and create a table that details their total cell counts, the calculated density, as well as how their cells were plated (i.e., how the cells were diluted to achieve the desired density). All of this information helps the students prepare their lab reports (Table 1).

In the remote Lab Session 1, the instructor could start by stating that the objective of the exercise is to study aggrecan, a molecular constituent of the perineuronal net found in the neural extracellular matrix. They could provide the part of the original lab handout that explains the background information, but not any procedural details (see Supporting File S4. Studying the Neural Extracellular Matrix – Lab Session 1 Handout in the original Lesson (1)). The students could be tasked with delving into the literature to find protocols that allow investigators to isolate single cells from cerebral cortices derived from embryonic mice. More specifically, the students could write up the protocol using proper citations since they are not able to complete the procedure in person (see Supporting File S1. Studying the Neural Extracellular Matrix – Lab Session 1 Assignment in the original Lesson (1)). In addition, the students could explain the purpose of each of the steps in the protocol (Table 1). There are a number of different resources that students can consult, some of which are included here (4-8). Some articles even have accompanying videos for students to view part or all of a procedure (5).

This open-ended assignment would lend itself well to a discussion, as students could meet in groups to outline the procedures they found. The instructor could hold these discussions through their preferred platform, using Breakout rooms to allow for smaller discussions. In the smaller groups, the students could share the details of the protocols and explain to their peers the purpose of each step. For example, one can use trypsin or papain along with manual trituration to isolate single cells from the dissected cortices. Students should be able to explain why such chemicals are used in addition to how trypsin or papain are inhibited prior to plating. Additionally, students should also determine the composition of the media that the cells should be incubated in, and this can provide another discussion point. To wrap up the session, the entire class should convene so that students can ask follow-up questions, or the instructor can pose questions to the students about the procedural details. Given that in this exercise students will likely

have varying steps in writing their own protocols, I would not recommend having a pre-lab quiz (Table 1).

Culture Treatments and care of cultures (in between Lab Sessions 1 and 3)

After the cultures are generated in the original Lesson, the cells are be broken up into experimental and control groups to manipulate overall neuronal activity with potassium chloride (KCl) and block glial cell growth with cytosine-β-D-arabinofuranoside (AraC). Students are responsible for treating the cultures on 1 day in vitro (DIV). Then the instructor removes these chemical treatments on 3 DIV by performing a full media exchange. Thereafter, the cultures are monitored, and half media exchanges are performed on 6, 9, and 12 DIV (1) (Table 2).

In the remote environment, the instructor has a few options. The instructor may wish to tell the students that the cultures will be treated with KCl and AraC. Then the students can come to Lab Session 2 having done some research into what these chemicals will do to the cells and how these treatments will help address the two main questions (Table 2). For example, there is an extensive body of literature detailing how the application of KCl increases bioactivity in neurons and this work delves into the underlying molecular mechanisms (9-16). The application of AraC has been widely used to limit glial cell proliferation (17-18) and students can be asked to provide support from the literature as to how both KCl and AraC have been used.

Alternatively, the instructor may wish to have the students propose ways to inhibit glial cell growth and increase neural activity by surveying relevant literature (Table 2). For example, investigators have reported that calcium permeable α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) glutamate receptors and L-type calcium-voltage gated channels mediate the activity-dependent formation of perineuronal nets (4,19). In addition to AraC being used as an anti-mitotic agent, others have employed the application of fluorouracil (20). If the instructor chooses to have the students research ways to inhibit glial cell growth and increase the levels of neural activity, the students should also be asked to explain the concentration and the duration at which they would use the various chemicals (Table 2).

Lab Session 2

Lab Session 2 will be very much the same in a remote setting as it would be in a face-to-face environment. Both sessions are focused on students stating their own hypotheses and predictions based on the two questions provided by the instructor. The students survey the literature to make such assertions. They can work in small groups as well as with the instructor during this process (Table 3).

The pre-lab lecture on the background material about the exercise, including how the neural extracellular matrix is structured, what perineuronal nets are, where they are found within the nervous system, and the proposed function of perineuronal nets will be the same in both settings, with a few minor modifications (consult Supporting Files S5 and S6. Studying the Neural Extracellular Matrix – Lab Session 2 Pre-Lab Lecture and Lab Session 2 handout, respectively in the original Lesson (1)). In the remote setting, the pre-lab lecture does not need to cover the experimental details for how to create primary cultures, as students have already researched and written up their protocols. Secondly, in the remote situation, the instructor

should inform the students that full media exchanges must occur to remove the treatments and then after that, every 3 DIV half of the media is exchanged for each culture well (Table 3).

If the adopting instructor wishes to perform this lab session remotely, then they should hold a live meeting so that students can meet in small groups to discuss their hypotheses and predictions and use the time to find relevant literature. The instructor should design time in the session to move between these small groups and then holder a larger discussion at the end. In a face-to-face setting, I would recommend that the students meet in a computer lab to facilitate discussions and group work (Table 3).

Since the pre-lab lecture is focused on the background information about the experiment, I do not recommend a lab quiz for this session, in either format. In my opinion, it is best to allow the students time to synthesize the information about the neural extracellular matrix and perineuronal nets before being tested (Table 3).

Lab Session 3

In the original Lesson, the instructor provided the students with a lab handout detailing how the cells would be fixed, blocked, stained, and coverslipped (see Supporting File S8. Studying the Neural Extracellular Matrix – Lab Session 3 handout (1)). Students also took a pre-lab quiz based on the information in the lab handout and then the instructor delivered a brief pre-lab lecture explaining why cells are fixed, the importance of blocking, and the details of indirect immunofluorescence (see Supporting File S7. Studying the Neural Extracellular Matrix – Lab Session 3 Pre-Lab Lecture (1)). The students carried out these procedures, and the instructor finished the exercise the next day by adding secondary antibodies, staining the cells with a DNA dye, and mounting the coverslips onto glass slides (see Supporting File S8. Studying the Neural Extracellular Matrix – Lab Session 3 handout (1)). In the corresponding assignment, the students were told to write the protocols employed in Lab Session 3 in their own words. In addition, they were asked to state their hypotheses and predictions for the two main questions that were being asked in this experiment and include support from the literature. Lastly, this assignment also tasked the students with gathering background information on what perineuronal nets are, where they are found in the nervous system, and which molecules comprise perineuronal nets (see Supporting File S2. Studying the Neural Extracellular Matrix – Lab Session 3 Assignment (1) (Table 4).

Since Lab Session 3 requires a number of steps and the protocols for all of these techniques can vary widely, it is a wonderful opportunity to have students do research and write up their own step-by-step procedures with appropriate citations. This can be easily executed in the remote environment. In advance of the exercise, the instructor can provide a pre-lab lecture and a lab handout similar to the ones explained above for the face-to-face experiment with slight modification. More specifically, the instructor should not provide experimental details, but instead allow for the students to write their own protocols. The instructor should let the students know that they need to stain their cultures using anti-aggrecan and anti-glial fibrillary acidic protein (GFAP) primary antibodies and that the main goal is to analyze the results using indirect immunofluorescence (meaning that a secondary antibody is conjugated to a fluorophore). First,

the students should determine which primary antibodies to use as there are different aggrecan and GFAP antibodies that are commercially available. To do this, the students will survey the literature to specifically find antibodies that have been used in primary cultures derived from embryonic mouse cortices rather than just searching more generally for anti-aggrecan and anti-GFAP antibodies. Next, the students need to identify appropriate secondary antibodies to use, as well as which dye to apply to stain DNA, and which cover slipping reagent to use. As part of their assignment for Lab Session 3, students should attach the articles that they found in which investigators used those primary antibodies. In doing so, the students will identify which fixative solution and blocking solutions to use. It is important for the students to understand that often times investigators try several different fixatives and blocking solution combinations to determine the best conditions. There are many resources for such staining procedures, both in the literature (21) and on the websites of companies that produce antibodies. The students should submit their complete protocols as part of their Lab Session 3 assignment in addition formalizing their hypotheses and predictions as well as answering specific questions about perineuronal nets as described above. In a remote setting, students would take a pre-lab quiz as to why the various steps enumerated above are performed (Table 4).

Lab Session 4

In the original Lesson in Lab Session 4, the students analyzed their results using a fluorescence microscope after taking a pre-lab quiz that covers the various components of the specific instrument that is being used in the experiment. The professor also delivered a pre-lab lecture (see Supporting File S9. Studying the Neural Extracellular Matrix – Lab Session 4 Pre-Lab Lecture (1)), which instructs students on how to use the microscopes, also detailed in the lab handout (see Supporting File S10. Studying the Neural Extracellular Matrix – Lab Session 4 Handout (1)). Each group worked together to interpret their results and then joined a larger discussion with the entire class so the instructor could determine how the students were answering the two central questions. The summative assessment is due two weeks after this exercise to give students time to prepare their formal lab reports (see Supporting File S12. Studying the Neural Extracellular Matrix – Lab Report and Rubric Information (1)) (Table 5).

In a remote setting the instructor will have to provide data for the class to examine since they cannot perform the microscopy work themselves. This can be accomplished by finding images from the literature and telling the students that these images are representative of what they would see under the microscope. Moreover, if the instructor wishes, the analysis could be more quantitative in nature, where the students are given a dataset to work with in addition to microscope panels. The students would then be tasked with analyzing the results in the context of the two questions that were posed (Supporting File S2. Going Remote: Studying the Neural Extracellular Matrix – Lab Session 4 Modified Handout). The instructor should also provide a brief pre-lab lecture that the students can access just as they would in a face-to-face setting. I would recommend that the instructor still includes information about the fluorescence microscopes as it would be beneficial for the students to understand how the equipment works. If the instructor opts to do this, then a pre-lab quiz could be administered about the components of a fluorescence microscope (Table 5). Many companies that manufacture and sell microscopes have videos that detail how

a fluorescence microscope works (ex: a video from Leica: https://bitesizebio.com/webinar/fundamentals-of-fluorescence-microscopy/). The adopting instructor might wish to hold an additional virtual session in which the students can talk in small groups or with the instructor about the provided images or dataset.

SCIENTIFIC TEACHING THEMES

Active Learning

Since the students will not be able to execute this experiment in a hands-on manner, it is particularly imperative for the instructor to engage with the class throughout the four-week exercise. For example, the instructor will provide feedback on the two formative assessments and can choose to hold one-on-one or small group meetings with students either through formal virtual office hours or by appointment. Lab Session 2 is an important exercise in that the students will work with their classmates as well as the instructor to establish hypotheses and find supportive evidence for those statements. For the analysis portion of the experiment, the instructor can provide notes or information on how to interpret the data in the lab handout, or again, could choose to meet with students individually or in small groups. Given the remote setting, the instructor should make themselves available for students to ask questions and should create breakout discussion groups to promote peer discussion. In the online adaptation of the exercise, the students have more opportunities to explore the literature, research protocols, and understand at a deeper level why these steps are carried out.

Assessment

I recommend two formative assessments after Lab Session 1 and 3 and the major lab report as the final assignment. This format could be modified if the instructor wishes to have students prepare posters or create oral presentations. The weekly quizzes allow for the instructor to monitor if the students have been reading the handout and preparing for the exercises.

Inclusive Teaching

Since this experiment will be carried out remotely, the instructor should make sure that all students have access to computers, the internet, and any other software or technology that is needed. Some campuses are able to provide such equipment to students and instructors alike given the need for virtual learning and teaching. As it possible that not everyone has a dedicated and private workspace, the instructor should reassure the class that they do not need to use video, but instead can rely or audio or even just typing in a chat box.

I would recommend that students form their own discussion groups, just as they would if the experiment was conducted in person. This will provide a sense of familiarity and comfort, which is likely to be appreciated given the fact that the experiment is being carried out virtually.

In addition, when designing videos for the pre-lab lectures, the instructor should keep in mind that they should provide notes, audio, as well as video to accommodate students with disabilities. I also recommend following up individually with students to ensure that the material has been presented suitably.

Lastly, the instructor needs to make sure that the individualized feedback that the students receive on both of the formative assessments is thoughtful, constructive, and well-explained. Because the students do not have traditional face-to-face time, they will rely on this feedback to prepare their lab reports. In addition, the instructor should be open to suggestions or modifications from the students given the unconventional way of completing this experiment.

SUMMARY

This Essay describes a remote adaptation of a previously published Lesson using a primary cell culture model to study the neural extracellular matrix to a remote or virtual learning environment. Although the students do not carry out the actual experiments, they can still accomplish the main objective of answering two questions about how the perineuronal net of the neural extracellular matrix forms. More specifically, the students are responsible for finding appropriate protocols, setting forth hypotheses, finding support for these hypotheses and completing both formative and summative assessments. Given the unique and unusual situation of the current pandemic, this adaptation can still provide students with a thought-provoking, challenging, and exciting Lesson.

SUPPORTING MATERIALS

• Supporting File S1. Going Remote: Studying the Neural Extracellular Matrix – Reagent Information. This Word document contains a list of reagents and equipment that were used in Lab Sessions 1 and 3. Some of the reagents and equipment are considered to be common use or standard items in which the students do not need to include the company and the location of the company. For the other items, the instructor has included the name of the company that makes the product and for the lab report, the students should add that information in along with the location of the company.
• Supporting File S2. Going Remote: Studying the Neural Extracellular Matrix – Lab Session 4 Modified Handout. This handout has been modified from the original Lesson to reflect the fact that the students were not able to analyze the slides they created. Instead, the instructor provided microscope images for the students to examine and analyze, which served as the basis of the lab report.
• Additional supporting files used in the original version of the lesson and referenced in this article are available at https://www.coursesource.org/courses/using-a-primary-cell-culture-model-to-study-the-neural-extracellular-matrix.

ACKNOWLEDGMENTS

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Table 1. This table outlines what material is covered in the lab handout, the particulars of the corresponding lab assignment as well as information about the lab quiz and lab lecture for Lab Session 1. In addition, this table compares and contrasts Lab Session 1 in a remote versus a face-to-face setting.

	Lab Session 1: Face-to-Face	Lab Session 1: Remote
Lab Handout	• Background details about the exercise (i.e., the neural extracellular matrix, aggrecan, perineuronal nets). • Steps detailing how to isolate single cells from dissected cerebral cortices.	• Background details about the exercise (i.e., the neural extracellular matrix, aggrecan, perineuronal nets).
Lab Assignment	Students will: • Write up the materials and methods section in their own words. • Organize their cell counts, calculated density, and how they plated their cells in a chart. • Submit the assignment at the start of Lab Session 2 .	Students will: • Research protocols for how to isolate and plate single cells derived from dissociated embryonic mouse brain cerebral cortices. • Write up the protocol in their own words and explain the purpose of each step. • Submit assignment before Lab Session 1 so that a discussion can follow with the entire class and small, breakout groups. • Discuss their protocols and the purpose of each steps. • The instructor will mediate the discussion and can show procedural videos, if desired.
Pre-Lab Quiz	• To be administered before students complete the exercise. • Should cover only the procedural details.	• A lab quiz would not be appropriate given the fact that student protocols might differ.
Pre-Lab Lecture	• Given the time constraints for this lab session, there is no time for a lab lecture.	• No lab lecture is needed as the handout explains the purpose of the exercise. • A thorough lab lecture will be given in Lab Session 2.

Table 2. In between Lab Session 1 and 3, cultures are treated with chemicals to increase bioactivity as well as inhibit glial cells. This table details how the cultures are treated and cared for in both a virtual, remote environment as well as in a face-to-face environment.

	Culture Treatments and care of cultures (in between Lab Sessions 1 and 3): Face-to-Face	Culture Treatments and care of cultures (in between Lab Sessions 1 and 3): Remote
Culture Treatments	• Students come to the lab on 1 day in vitro (DIV) to treat cultures with KCl (to manipulate neural activity) and/or cytosine-β-D-arabinofuranoside (to inhibit glial cells).	• Tell students that the cultures will be treated with KCl and AraC and then have the students determine how these treatments address the two main questions. • Alternatively: task the students with finding ways in the literature to increase neuronal activity in cultures as well as inhibit glial cells. In addition, the students should explain the amount of each treatment they would administer and the duration of the treatment.
Care of cultures (media changes)	• The instructor performs a full media exchange on 3 DIV. • The instructor then performs half media exchanges on 6, 9, and 12 DIV.	• The instructor should inform the students in Lab Session 2 that media changes are required, first to remove the treatments and thereafter, every 3 DIV, to replace half the media.

Table 3. In Lab Session 2, in both the remote and face-to-face circumstances, the instructor delivers a pre-lab lecture on all of the pertinent background information on the project. The students then work in groups to pose hypotheses and predictions to address the two main questions. This table explains how this would work remotely versus an in-person scenario.

	Lab Session 2: Face-to-Face	Lab Session 2: Remote
Lab Handout	• Include the main objective of today's session as well as a reminder of the overall purpose of the four-week experiment.	• Include the main objective of today's session as well as a reminder of the overall purpose of the four-week experiment.
Lab Assignment	• There is no formal assignment after Lab Session 2, other than to continue thinking about hypotheses and predictions.	• There is no formal assignment after Lab Session 2, other than to continue thinking about hypotheses and predictions.
Pre-Lab Quiz	• No lab quiz.	• No lab quiz.
Pre-Lab Lecture	• The lab lecture covers all of the background material needed to understand the two main questions that are being asked in the exercise (ex: molecular composition of the neural extracellular matrix, perineuronal nets, the role of aggrecan in perineuronal nets). • Additionally, the instructor should cover the experimental details from Lab Session 1. • After the lecture, students generate hypotheses and predictions and find support in the literature. • Students work independently and in groups to complete this work.	• The lab lecture covers all of the background material needed to understand the two main questions that are being asked in the exercise (ex: molecular composition of the neural extracellular matrix, perineuronal nets, the role of aggrecan in perineuronal nets). • After the lecture, students generate hypotheses and predictions and find support in the literature. • Students work independently and in groups to complete this work.

Table 4. In the third lab session, students perform portions of an indirect immunofluorescence protocol by fixing, blocking, and applying primary antibodies to the cultures. This table discusses how this can be adapted from a face-to-face exercise to a remote exercise.

	Lab Session 3: Face-to-Face	Lab Session 3: Remote
Lab Handout	Includes: • A step-by-step protocol for how students will fix, block, and apply primary antibodies • A detailed protocol for how the instructor applies secondary antibodies, adds a DNA stain, and coverslips the cells the next day • Why these steps are carried out in relation to the overall goal of the experiment • Information about indirect immunofluorescence	Includes: • Why the cells are fixed, blocked, and stained in addition to the application of a DNA dye and coverslipping, in relation to the overall goal of the experiment • Information about indirect immunofluorescence
Lab Assignment	• The lab assignment is due after the students execute the steps and involves the students writing up the protocol in their own words. • In addition, this assignment asks the students to state their hypotheses and predictions central to the two questions being asked in this exercise. Support from the literature for these hypotheses and predictions needs to be included as well. • Lastly, the students answer pointed questions about perineuronal nets in preparation for their lab report. This requires a survey of the relevant literature and inclusion of citations	• The lab assignment is due after the students watch the pre-lab lecture and take the pre-lab quiz. • Students have to write up their protocols for fixing, blocking, and staining their cultures through indirect immunofluorescence. • The instructor will provide information to narrow the focus as to which primary antibodies to use. Support from the literature is included in the submitted assignment. • Students identify specific fixation and blocking conditions based on the primary antibodies they select. Additionally, the students need to include the staining of DNA and coverslipping in their protocols. • In addition, this assignment asks the students to state their hypotheses and predictions central to the two questions being asked in this exercise. Support from the literature for these hypotheses and predictions needs to be included as well. • Lastly, the students answer pointed questions about perineuronal nets in preparation for their lab report. This requires a survey of the relevant literature and inclusion of citations
Pre-Lab Quiz	• The pre-lab quiz covers procedural details as well as the rationale as to why the various steps are executed.	• The pre-lab quiz covers the rationale as to why the various steps are executed. No specific procedural details are asked as the purpose of this exercise in a remote setting is for the students to find appropriate protocols.
Pre-Lab Lecture	• The pre-lab lecture provides the students with information as to how the steps are carried out and why.	• The pre-lab lecture provides the students with an explanation as to why the various steps that they need to research are carried out.

Table 5. In the last lab session, students analyze their results using a fluorescence microscope and gather observations to complete their formal lab report. In a remote situation, the instructor can provide a dataset for the students to analyze instead. This table summarizes how this lab session would run in these two situations.

	Lab Session 4: Face-to-Face	Lab Session 4: Remote
Lab Handout	• Reviews various components of the fluorescence microscopes that the students use in this session. • Provides space for students to organize their observations.	• Instructor should include a discussion about the workings of a fluorescence microscope. There are a number of videos and resources an adopting instructor could use. • In addition, the students are provided with representative images to analyze or if suitable, a quantitative dataset to examine. • The instructor could opt to provide some helpful hints to the class as to how to interpret the provided results. • Alternatively, the instructor could hold another virtual session in which the results are discussed with the class.
Lab Assignment	• The formal lab report requires an introduction, materials and methods section, results, and a discussion. • The lab report also includes references, images of their results, and any tables. • Students need to consult primary and secondary literature. • This assignment should be due at least 2 weeks after the students finish Lab Session 4.	• The formal lab report requires an introduction, materials and methods section, results, and a discussion. • The lab report also includes references, images of their results, and any tables. • Students need to consult primary and secondary literature. • This assignment should be due at least 2 weeks after the students finish Lab Session 4.
Pre-Lab Quiz	• The pre-lab quiz is administered before the students analyze their results and the questions should be based on the workings of the microscope and any other pertinent information in the lab handout.	• The pre-lab quiz is administered before the students analyze their results and the questions should be based on the workings of the microscope and any other pertinent information in the lab handout.
Pre-Lab Lecture	• The pre-lab lecture covers how to use the microscope and the function of the various components. • The instructor can also answer any other questions about the lab report.	• The pre-lab lecture covers how to use the microscope and the function of the various components. • The instructor can also answer any other questions about the lab report.