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Conducting Authentic Collaborative Research in Undergraduate Biology Courses Using the Open Science Framework

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The Open Science Framework (OSF) is a free, online research management tool, currently in beta release from the nonprofit Center for Open Science. The OSF may soon interface with scientific journals to allow access beyond supplementary material to original raw data associated with a publication. Here, the OSF is used in an alternative way to manage authentic research projects in undergraduate lab curricula. A project from an upper level cell biology course is presented to demonstrate how the OSF 1) helps faculty manage many students working on an authentic research project, and 2) helps students practice rigorous, transparent scientific reporting.

Keywords: Open Science Framework/OSF, Authentic Research, Collaboration, Blind Overlapping Data Collection/Analysis, Research Workflow, Research Lifecycle, Best Scientific Practices

Introduction

Conducting authentic scientific research as an undergraduate is a transformational experience. For most students, investigating a real scientific question imparts a deep, firsthand understanding of the scientific process and solidifies an enduring scientific literacy (Russell et al., 2007). For some students, it is the defining event that leads to a scientific career.

Unfortunately, as a result of various institutional constraints and the classic research apprenticeship model, opportunities to conduct authentic scientific research remain limited to small numbers of undergraduates. Furthermore, these relatively rare research opportunities tend to attract the most proactive and competitive and science students. Contributions from a numerous and diverse range of people can thus be lost.

Many factors limit broad participation in authentic research during college (Spell et al., 2013). Some limitations derive from the type of institution. Traditionally, large research institutions focus on graduate students and postdoctoral fellows and may not have targeted programs for involving undergraduates in the research process. On the other hand, smaller teaching-focused institutions may lack necessary resources for providing authentic research opportunities. Regardless of these generalizations, most science educators lack the necessary time and support for making real research opportunities widely available to many students. Some professors pursue large research initiatives outside of their prescribed workload, but this model may not provide a long-term solution. The question remains, is there a workable way for faculty to involve many students in authentic scientific research?

This question is one of sustainability. Within the existing structure of higher education, the most direct way for faculty to sustain authentic research involving many students is to align that research with a laboratory course of record. This approach also benefits science students who often carry heavy and time-consuming course loads.

This question is also one of scale: how can a small number of faculty effectively manage an authentic research project involving 20, 40, or more undergraduate students doing real science perhaps for the first time? How will many students formulate a single focused hypothesis? How will they design and execute experiments? How will they analyze and interpret large data sets *en masse*?

There is an emerging research management tool called the Open Science Framework (OSF, https://osf.io/) that can help educators address these kinds of questions. At a glance, the OSF resembles an online lab notebook

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combined with a data repository and a citable project website. The OSF is a free online interface that facilitates every part of the scientific process from early-stage brainstorming to final interpretations. Through the OSF, researchers can communicate with collaborators and organize and store unlimited data. In the context of an academic course, the OSF facilitates remote collaboration and tracks revision history, important features for logistics, oversight, and grading of research projects in an educational setting. The OSF links with common existing tools such as Dropbox, Google Drive, figshare, Dataverse, GitHub, and Amazon Simple Storage, making implementation easy. It will likely be integrated with major scientific journals in the future. The OSF also offers detailed privacy controls. Projects (or portions of projects) can be shared privately with collaborators or made public, searchable, and citable online. The robust features of this emerging technology provide a centralized and powerful system for managing authentic research involving many students.

The OSF offers additional benefits from the faculty perspective. It facilitates longitudinal project management over time, allowing future students to move the project forward. The OSF also encourages best scientific practices. This promotes research integrity while teaching students specific ideals of the scientific process. For example, the case study presented herein will discuss how the OSF provides a structure for the following practices: including statistical methods in experimental design; preregistering hypotheses and methods before conducting experiments; collecting data blindly to avoid bias; preserving all documents, communication, and data indefinitely; and sharing raw data in a transparent way.

Through engaging in authentic research, students gain critical evaluative and analytical skills and learn to identify and create excellent work. They begin a meaningful scientific conversation that continues beyond educational walls. And because the answers in authentic research are not known, students and faculty get to share their love of learning and exploration. Whatever educators can do to make these experiences widely available, we should. In summary, the OSF can help undergraduate science educators involve more students in authentic research in a way that can be implemented and sustained.

The specific authentic research experience described in the following student outline was carried out in Cell Biology (Biology 206) at Piedmont Virginia Community College in the fall semester of 2014. The project may be viewed at https://osf.io/gsahd/?view_only=46d08cfae2b5434d99bbcc733dbbc61b as an example of managing authentic scientific research with many undergraduate students using the Open Science Framework.

Student Outline

Background

The cells you will use today were prepared by the laboratory specialist in our collaborator's lab in the Department of Cell Biology at the University of Virginia. They are cervical epithelial cells that were originally obtained in 1951 at Johns Hopkins hospital from a biopsy of a woman's cervical cancer. The patient's name was Henrietta Lacks, and the name of the resulting cell line is HeLa. HeLa cells have been cultured for over six decades. Research using HeLa cells, as well as other cell lines, has contributed greatly to our understanding of cell biology.

The HeLa cells we will use today were recently treated in three ways:

1. **Lentiviral-mediated knockdown of Arf6**. This treatment employs a viral construct to deliver small interfering RNAs to cells. These small interfering RNAs target Arf6 specifically by binding to and degrading Arf6 messenger RNA. As a result, there are very low amounts of Arf6 protein in these cells—Arf6 protein cannot be detected by Western blot in these cells. Nevertheless, the Arf6 gene remains present in the HeLa cell genome, and thus we use the term *knockdown* as opposed to knockout for this treatment.
2. **Lentiviral control**. This treatment employs a random, nonspecific small RNA sequence that should not alter any protein levels in the cell. This treatment controls for possible off-target effects of the lentiviral process.
3. **No treatment**. These cells were cultured under the same conditions as numbers 1 and 2 above, but they were not exposed to any lentiviral treatment. If there are no off-target effects of the lentiviral process, results from the lentiviral control should match results from untreated cells.

A note on best scientific practices: Despite good intentions, people can introduce bias into experiments unwittingly. To avoid this possibility, we will conduct both our experiment and our analysis without knowing which treatments were applied to which cells. After we collect and analyze our data, I will request the specific treatment groups from the lab specialist who prepared them.

Week 1 Protocol: Immunofluorescence Staining

A note on cell preparation: Just this morning, the lab specialist permeablized the cells using a short 10-minute treatment with a detergent called saponin. This step pokes tiny holes in cell membranes allowing antibodies and staining reagents to penetrate cells. Next, the lab specialist washed the detergent solution away and left the cells in PBS (phosphate buffered saline) and NGS (normal goat serum). NGS is a complex mixture of molecules that is used to block non-specific interactions of the antibodies we will be using today.

We will stain cells with a primary incubation solution: mouse-anti-gamma-tubulin monoclonal antibody (SIGMA T6557-100µl, clone GTU-88, ascites, lot #034M479V, used at 1:2,500 in PBS with 1% NGS).

Then we will stain cells with a secondary incubation solution consisting of a cocktail of several different reagents. There is a fluorescently tagged anti-mouse antibody that will recognize the primary mouse-anti-gamma-tubulin antibody. This reagent will allow us to see centrosomes. The secondary incubation mixture also contains a fluorescent dye called Hoechst that stains DNA and will allow us to see nuclei. Finally, there is a fluorescently tagged compound called phalloidin which binds actin filaments, and this will light up the actin cytoskeleton throughout each cell. Details for these reagents are as follows. Alexa Fluor 546 goat-anti-mouse IgG antibody (Invitrogen A11003, 2mg/ml, lot # 702338, used at 0.002mg/ml). Alexa Fluor 546 has a peak fluorescence emission in the orange range around 570nm. Hoechst is a dye that binds DNA, especially the minor groove and regions rich in adenine and thymine. Hoechst dye has a peak fluorescence emission in the blue range around 450nm. (Our supply of Hoechst dye is old. It still works well, but we no longer have the supplier information. We're using a dilution of 2µl/ml.) Texas Red phalloidin. Phalloidin is a compound that binds actin filaments. The Texas Red dye bonded to the phalloidin has a peak fluorescence emission in the orange/red range around 615nm. (Our supply of Texas Red phalloidin is old. Like the Hoechst dye, it still works well, but we no longer have the supplier information. We're using a dilution of 4µl/ml.)

Before you begin, please note: the cells are stuck (adhered) to the glass slide that comprises the bottom of the slide chambers. Today's procedure involves removing and adding many different solutions to and from these chambers. At every step, minimize the amount of time the cells are exposed to air. Before you dump liquid off, have the next solution ready to go.

1. Working over one of the lab sinks, pour the liquid out of the slide chambers. To each chamber, add 200µl of primary incubation solution. Tilt the slide gently from side to side to ensure even liquid distribution throughout each chamber—pretend you are tilting a frying pan to cover the bottom evenly with oil. Leave the slide on the benchtop for one hour.
2. After an hour, pour the primary antibody solution down the drain, and add 200µl PBS solution to each chamber. Wait 2-3 minutes. Repeat this washing step two more times for a total of three washes.

3. After dumping the last wash, add 200µl of secondary incubation solution. Ensure even coverage of the cells as before. Place the slide carefully in a dark place for 1 hour.
4. Dump the secondary antibody solution down the drain, and add 200µl PBS solution. Wait 2-3 minutes. Repeat this washing step two more times for a total of three washes.
5. Dump the last wash. Pop the chambers off the slides using the black and white plastic chamber removal tool.
6. Pipet 15µl Fluoromount-G mounting media on top of the cells where each chamber was. Delicately lower a glass coverslip onto the drop of mounting media. Once the coverslip is lowered, do not push on it or move it around—movement of the coverslip could disrupt the cells.
7. If necessary, use a task wipe (Kim wipe) to remove excess mounting media around the edges of the coverslip. Simply let the edge of the tissue barely touch the excess liquid such that the liquid is wicked away—do not blot or wipe the coverslip as this movement could disrupt the cells.
8. Seal the edges of the coverslip on all four sides with clear nail polish. Store the slides horizontally in a dark container at 4°C until you are ready to view your cells.

Week 2 Protocol: Viewing and Imaging Cells

The lab manager or instructor will turn on the Nikon Eclipse 50i epifluorescence microscope. This optical microscope shares many similarities with basic compound light microscopes and can be used without taking advantage of the fluorescence capabilities. Today you will use all of its capabilities to visualize your cells. Begin by cleaning anything that might need cleaning. Slides and other parts of the microscope (stage, oculars, etc.) may be cleaned with task wipes. Tips of objective lenses should be cleaned only with special lens paper.

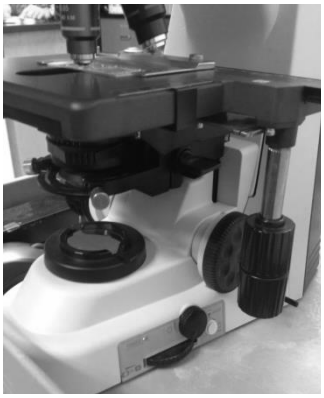

Figure 1. Getting started at epifluorescence microscope. Move the 4X objective lens (the smallest/shortest one) into position. Put your slide on the stage and center it using the stage control knobs that hang on a silver bar on the right side of the stage. The top knob moves the stage toward/away from you. The bottom knob moves the stage left and right. A small vertical black wheel at bottom right controls the white light located in the base of the scope. To visualize your cells with white light, use this small wheel to turn up the light. Then rotate the filter wheel (see Figure 2) to an empty position (position 5 or 6). When you are done viewing your cells with white light, turn the white light off. Otherwise it will interfere with your fluorescence viewing. The horizontal black wheel at bottom right controls the field diaphragm which controls the field of view. It is easiest to keep it fully open.

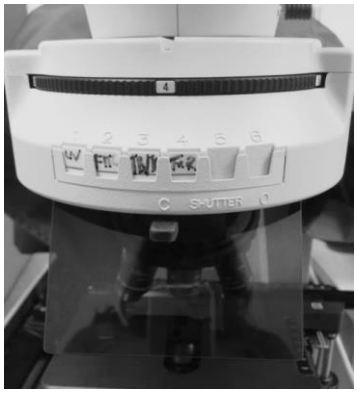

Figure 2. Using the shutter control and filter wheel. Keep the shutter closed (in the C position as shown) when you are not looking through the scope. This will prevent you and others from looking at the strong light on your slide, and it will prevent your sample from losing fluorescence via a light-induced process called photobleaching. When you want to look at your sample, open the shutter. Safety note: The source of light for this microscope is very intense. The orange shield is intended to protect your eyes, so look through it (not around it) when adjusting your slide or objective lenses. Make sure you adjust the large black filter wheel according to the wavelength of light you want to view. As indicated by the labels below the filter wheel, the filters are as follows: 1: UV (Hoechst, DAPI), 2: FITC (green), 3: TRITC (yellow/orange), 4: TxR (red), 5: blank (no filter), 6: blank (no filter). Use positions 5 or 6 to use the microscope without fluorescence.

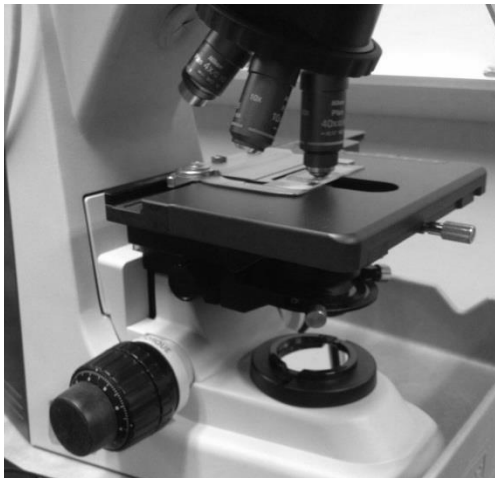

Figure 3. Focusing. As you would with any compound light microscope, begin your focusing sequence by lowering the stage and moving the 4X/scan objective lens into position. Start here every time you lose focus or change slides. The stage height (i.e. your focus) is controlled by the focusing wheel on the left side of the scope. The smaller/outer part of the wheel is the fine focus.

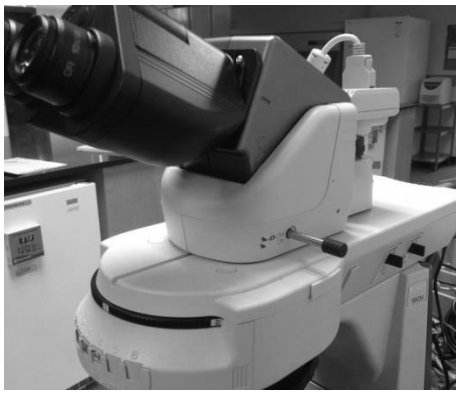

Figure 4. Directing light to the camera. When you wish to take a photograph, pull the silver rod at the lower right of the oculars to its outer position as shown.

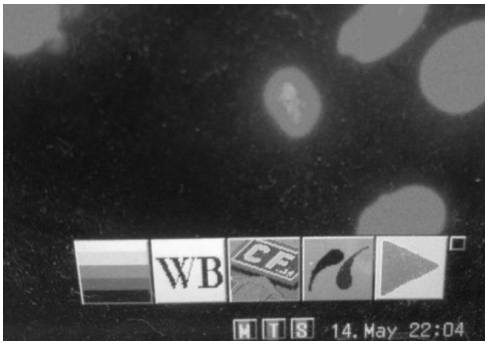

Figure 5. Digital display and image capture. View the image on the digital display next to the microscope. There is a slight difference between the focus through the oculars and the focus on the digital display. Looking directly at the digital display, use the fine focus knob to adjust the focus on the digital display. Your photograph will match the focus on the digital display (not the focus you see through the oculars). Look at the digital display. At any point, if you do not see menus on the digital display, use the mouse to right click. (Different menus appear depending where the cursor is on the screen. To see all the menus, right click when the mouse is centered on the digital display.) If the exposure is too bright or not bright enough, click on the icon with horizontal bars. That will reveal +/- buttons you can click to adjust the exposure. Insert a jump drive into an available USB port on the right side of the digital display. When you are satisfied with the way things look on the digital display, click this "capture" icon labeled "CF" to take a photograph.

Each student will take 20-30 unique, non-overlapping images of the Hoechst staining as follows:

1. Position your slide so that the tip of the objective lens is near the top left corner of your coverslip.
2. Using the white light, go through a focusing sequence, beginning with the 4X objective lens, followed by the 10X, followed by the 40X. Then shut the white light off and turn the filter wheel to position #1/UV.
3. Open the fluorescence shutter and look through the oculars. You should see bright blue nuclei on a black background.
4. Pull out the silver rod near the top right of the oculars to send light to the camera.
5. At this point, you can work entirely from the digital display.
6. Use the fine focus knob to bring the image into focus on the digital display.
7. Take a picture.
8. Using the bottom stage control, turn it about ¼ turn counterclockwise to advance your slide slightly more than a field of view. The key is to make sure that you are taking a picture of an entirely new field of cells. You are trying to

avoid overlapping images. Also note that you ought to take pictures in a systematic fashion that will give us a sense for the overall population of cells on the slide. Therefore, you are not searching until you find something interesting to image. Rather, you are moving the slide incrementally and taking pictures more or less at random as you move across the slide.

9. Before each picture, check to make sure you are in still focus on the digital display.
10. Take a total of 20-30 pictures. Unless there is something wrong with your slide in a particular area (say, a large bubble where the mounting media does not fill the space between the slide and the coverslip), you should only have to move across the slide as you take your 20-30 pictures. If for some reason you get to the edge of the coverslip before completing your imaging, use the stage controls to move down and back to the left, and then continue taking pictures systematically across a lower portion of your slide.
11. When you are done taking pictures, move the flash drive from the digital display.
12. Insert the flash drive into a lab computer and look at your image files. Make sure the images are in focus. Rename the image files using your last name followed by a number, beginning with 01. Example: Smith 01, Smith 02, Smith 03, etc. Save your images to a new folder on the desktop. (The name of this folder is not important, but it should contain only your images and no other files.)
13. Go to the Open Science Framework (OSF) at https://osf.io/login/ and log in. Go to our project titled 'Measuring Mitotic Index in HeLa Cells Depleted of Arf6 GTPase.' Click on the 'Files' tab. Drag your folder of images from the desktop into the 'Student Images' folder on the OSF.
14. Delete the images from the flash drive.
15. Clean up at the microscope: lower the stage, move the 4X objective lens into place, clean the objective lens with lens cleaner and lens paper, put your slide back into the class slide box.

Week 3 Protocol: Counting Nuclei

Log into the OSF (https://osf.io/login/) and go to your dashboard. To see all your projects, click on the + sign. To open a project, click on the little book icon to the left of the project name or the file/arrow icon to the right of the project name. Our immunofluorescence project is titled "Measuring mitotic index in HeLa cells depleted of Arf6GTPase." If you do not see it in your dashboard, let me know. To view images, click on the individual image files that correspond to either your name or to the name of the student whose pictures you are also counting. The table below lets you know which other person's images you are counting. Keep track of the image #. It is critically important that you record your data based on the image #. This record keeping ensures that we, or anyone else looking at our data, can know exactly where the measurements were obtained.

Record your data in the Google Docs spreadsheet entitled 'Hoechst nuclei counts.' Locate your sheet in the workbook (see the tabs along the bottom of the screen, and look for your last name). This is where you will enter your data.

Instructions for counting cells:

1. Enter data according to the image number.
2. Use the color handout of immunofluorescence examples as a guide. Let me know if you need another copy. This handout is also posted on Blackboard and copied below.
3. Use your best judgment. If your gut tells you a cell is in anaphase, then it's in anaphase!
4. Be as consistent as possible.
5. Don't count blurry cells. If an entire image is blurry, just write "blurry image" in the corresponding row in the spreadsheet.
6. Please enter zeros into the spreadsheet where applicable.
7. Pace yourself. Plan to do this work in at least 2 sittings.

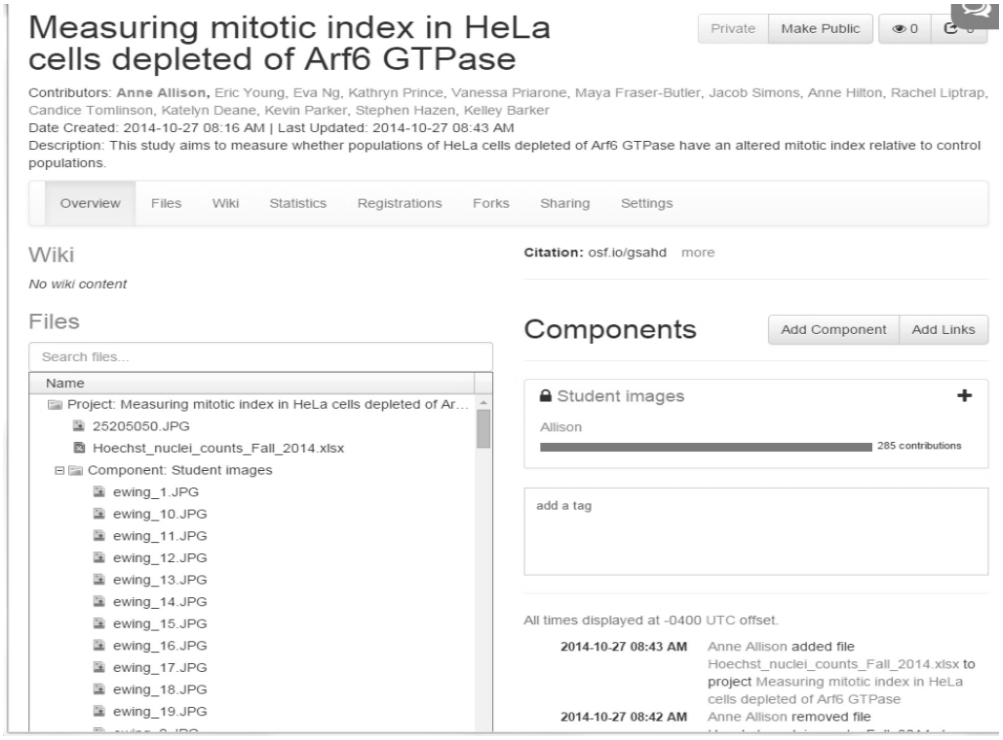

Figure 6. OSF project page. This screenshot exemplifies how research can be managed using the Open Science Framework.

Materials

1. Labtech sterile chamber slides (Thermo Scientific #177380)
2. Cells (note: cell culture for this experiment was done in the laboratory of a collaborator at the University of Virginia)
3. Phosphate buffered saline: dissolve the following in 800ml distilled H_2O: 80g of NaCl, 2.0g of KCl, 14.4g of Na_2HPO_4, 2.4g of KH_2PO_4, Adjust pH to 7.4, Adjust volume to 1L with additional distilled H_2O, Sterilize by autoclaving.
4. Hoechst dye
5. Mouse-anti-gamma-tubulin monoclonal antibody (SIGMA #T6557-100µl, clone GTU-88, ascites)
6. Alexa Fluor 546 goat-anti-mouse IgG antibody (Invitrogen #A11003, 2mg/ml)
7. Texas Red Phalloidin
8. Coverslips, task wipes, lens cleaner, lens paper
9. Fluoromount-G mounting media, Southern Biotechnology Associates, #0100-01, available via Fischer Scientific
10. Epifluorescence microscope, imaging capabilities

Notes for the Instructor

As cell culture requires considerable resources and time, this project was only possible in collaboration with the Casanova lab in the Department of Cell Biology at the University of Virginia.

From the secondary staining step onward, keep slides in a dark place to avoid photobleaching. When at the microscope, encourage students to close the shutter when they are not actively looking at or imaging cells. This will minimize unnecessary exposure to people's eyes by the strong light generated by the scope, and it will prevent photobleaching.

To give each student time at the microscope, have students sign up for 30-45 minute slots.

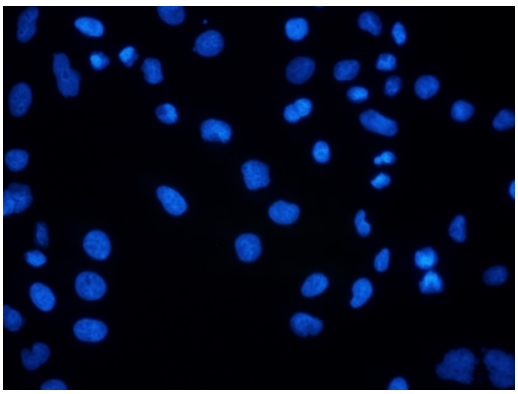

Figure 7. Hoechst staining of untreated HeLa cells. A representative image containing roughly 50 cells. Hoechst dye binds DNA, permitting visualization of nuclei and mitotic stages.

Literature Cited

Russell, S. H. M. P. Hancock, and J. McCullough. (2007). Benefits of undergraduate research experience. *Science, 316, 548-549*. doi: 10.1126/science.1140384

Spell, R. M. (2013). Redefining authentic research experiences in introductory biology laboratories and barriers to their implementation. *Life Sciences Education, 13, 102-110*. doi: 10.1187/cbe.13-08-0169

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About the Authors

Dr. Allison earned her bachelor's degree in biology from Harvard University and her master's and doctoral degrees in biochemistry and molecular genetics at the University of Virginia. She conducted postdoctoral research at the University of California, Berkeley and the University of Virginia before teaching biology for four years as an Assistant Professor at Mary Baldwin College. At Mary Baldwin, Dr. Allison held several major extramural grants and worked with undergraduate researchers investigating the relationship between Arf6 and B1-integrin isoforms. In 2013, Dr. Allison became an Associate Professor of Biology at Piedmont Virginia Community College where she teaches introductory biology, cell biology and genetics. She continues to involve large numbers of undergraduate students in authentic scientific research. Dr. Allison was Piedmont's 2015 nominee for the State Council for Higher Education in Virginia's Outstanding Faculty award.

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