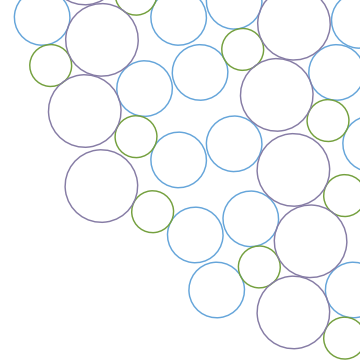




Lesson and experiment guide: Mitosis and Microscopy

Overview

This unit is structured as a two-lesson extension to curricula on the fundamentals of mitosis. It helps advance students' understanding of mitosis with real data and drives students to evaluate types of data, data interpretation, and experimental design.

The content of the lesson reinforces existing knowledge of mitosis, helps students transition from looking at drawn images or plant cells (such as in the classic onion cell mitosis experiment) to high-resolution microscopy data, and introduces open research questions in the field of cell biology. Students also have the opportunity to explore what happens to cell structures that are not directly related to chromosome reproduction during the process of mitosis and cytokinesis.

The data used in the virtual experiment portion of this unit come from the [Allen Cell Explorer](#), an open data set featuring tens of thousands of research-grade cell images. Students will learn what kind of data is collected in research settings and begin learning how it can be used.

Grade level

Grade 9-12

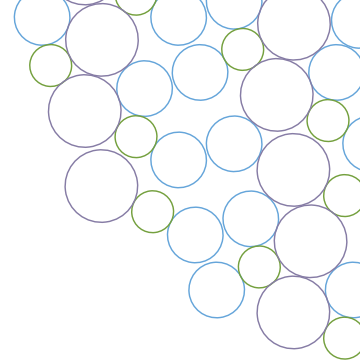
Existing student knowledge

Before starting this lesson, students should already have a basic understanding of:

- Why cells undergo mitosis
- The main stages of mitosis
- The major organelles and structures in mammalian cells
- How light microscopes work
- Why scientists use stains with microscopes
- How cell structures are composed of proteins with specialized functions

Students do not already need an understanding of many topics covered in this lesson, including:

- Fluorescence microscopy
- Basics of gene editing for research
- The specific roles of the highlighted proteins in mitosis
- The mitotic index



Learning objectives

- Students can describe key open questions in the field of cell biology.
- Students understand the basics of fluorescent tagging and microscopy, and the role of these techniques in creating the data in the experiment.
- Students are able to consistently identify the stages of mitosis in microscopy images. Students are able to calculate the mitotic index from this identification.
- Students are able to explain why studying cell structures that are not directly involved in mitosis is important in the study of mitosis.
- After completing the experiment, students are able to design their own experiment that uses the same type of data.

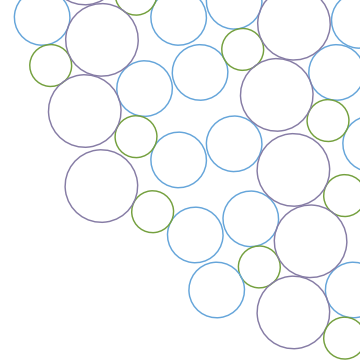
Curriculum outline

1. Assignment (in class or homework)
2. Class discussion
3. Pre-lab (in class or homework)
4. Virtual experiment
5. Lab report (in class or homework)

The experimental portion (pre-lab, virtual experiment, and lab report) can be used as a stand-alone unit.

Equipment

Computers, tablets, or other devices for students to work in groups of 2-4



Contents

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Teachers are welcome to adapt the lesson to suit their classes and curriculum, but may not share modified lessons. If you develop your own lesson plan using Allen Institute resources, we invite you to share your experience with us at info@alleninstitute.org.

Standards alignment

Next Generation Science Standards

Science and Engineering Practices	
Asking questions and defining problems	X
Developing and using models	
Planning and carrying out investigations	X
Analyzing and interpreting data	X
Using mathematics and computational thinking	X
Constructing explanations and designing solutions	X
Engaging in argument from evidence	
Obtaining, evaluating, and communicating information	X

Crosscutting Concepts	
Patterns	X
Cause and effect	
Scale, proportion, and quantity	X
Systems and system models	
Energy and matter	
Structure and function	X
Stability and change	X

Disciplinary Core Ideas - Life Science	
HS-LS1: From Molecules to Organisms	X
HS-LS1-1: Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.	X
HS-LS1-2: Develop and use a model to illustrate the hierarchical organization of interacting systems that provide specific functions within multicellular organisms.	X
HS-LS1-4: Use a model to illustrate the role of cellular division (mitosis) and differentiation in producing and maintaining complex organisms.	X
HS-LS2: Ecosystems	
HS-LS3: Heredity	
HS-LS4: Evolution	

Standards alignment

Advanced Placement Biology

Big Ideas	
The process of evolution drives the diversity and unity of life.	
Biological systems utilize free energy and molecular building blocks to grow, to reproduce, and to maintain dynamic homeostasis.	X
Living systems store, retrieve, transmit, and respond to information essential to life processes.	
Biological systems interact, and these systems and their interactions process complex properties.	

Science Practices	
The student can use representations and models to communicate scientific phenomena and solve scientific problems.	
The student can use mathematics appropriately.	X
The student can engage in scientific questioning to extend thinking or to guide investigations within the context of the AP course.	X
The student can plan and implement data collection strategies appropriate to a particular scientific question.	X
The student can perform data analysis and evaluation of evidence.	X
The student can work with scientific explanations and theories.	X
The student is able to connect and relate knowledge across various scales, concepts, and representations in and across domains.	



Teacher guide

The Allen Institute is a nonprofit biomedical research institute located in Seattle, Washington. Our four divisions – Allen Institute for Brain Science, Allen Institute for Cell Science, Allen Institute for Immunology, and The Paul G Allen Frontiers Group – are dedicated to answering some of the biggest questions in bioscience and accelerating research worldwide. We share all of our data and research findings with the scientific community and general public. The Allen Institute was launched in 2003 by founder Paul G. Allen as the Allen Institute for Brain Science; the Allen Institute for Cell Science launched in 2014. The Allen Institute is supported by government, foundation, and private funds to enable its projects.

The Allen Institute for Cell Science creates large-scale, open datasets that address fundamental questions about the states and functions of the human cell. These datasets and advanced data analysis tools are publicly available at allencell.org.

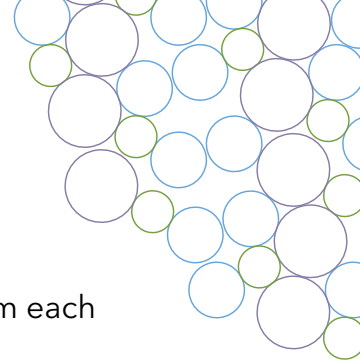
Key cell biology concepts underlying this lesson:

- [Mitosis](#)
- Microscopy
- Gene editing
- Image processing
- Fluorescence
- Human iPSCs

This curriculum uses two main resources from the Allen Cell Explorer at allencell.org: the Cell Feature Explorer and the Visual Guide to Human Cells.

Cell Feature Explorer

- The Cell Feature Explorer provides a multimodal view of over 32,000 images of living human cells from the Allen Institute for Cell Science's advanced fluorescence microscopes.
- These cells are [human induced pluripotent stem cells](#) (iPSCs) that have had one gene edited so the resulting protein will fluoresce.
 - This method allows scientists to track the normal functions, locations inside the cell, and quantity of that protein.
 - In each image, one protein has been tagged – each protein is associated with a specific cell structure or organelle.
- The three colors in each image represent the fluorescence from the edited protein (default: green), a dye that shows the location of DNA (default: blue), and a dye that shows the membrane of the cell (default: gray).
- Cells are labeled by stage of mitosis by experts.



Visual Guide to Human Cells

- The Allen Institute's scientific modelers and illustrators have used the data from each individually tagged protein and combined them in this model cell.
- The sample cell in the Visual Guide to Human Cells is based on real data from the Allen Integrated Cell and is representative of real human cells' shape and structure.
 - The Allen Integrated Cell is a model of cell structure and function.
 - Every time the Allen Institute for Cell Science fluorescently labels and studies another protein, its data is added to the model.
- Students can click on the cell or accompanying text to explore the cell.

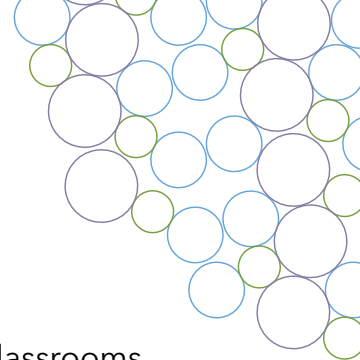
Open research questions and applications for resources

The data and analysis tools found in the [Allen Cell Explorer](#) portal can be used to address a wide variety of open questions in cell biology. Allen Institute staff and other scientists around the world conduct research using the data we collect and the cell lines we make available to other researchers. Some of the broad open questions are addressed in the students' homework reading, [Five things we still don't know about cells](#). Teachers may find additional background in the [Allen Cell Collection FAQ](#).

Additional articles about recent discoveries, the process of research, and more are available [here](#) and may be of interest for advanced students to pursue further reading. Core to the mission of the Allen Institute is our open sharing of data worldwide, and thousands of scientists use our resources in their research every day. Notable projects where other research teams have used these resources have included:

- [Cell Shorts: A new window into heart cells](#) shows research on cells from the [Allen Cell Collection](#) that have been differentiated into heart cells, and how the gene tagging technology enables new discoveries into heart function
- [Cell Shorts: Illuminating the kidney](#) shows research on cells from the [Allen Cell Collection](#) that have been differentiated into kidneys, and research that may lead to regenerative therapies for kidney disorders

The breadth and depth of the Allen Cell Explorer provide opportunities for advanced students to pursue additional projects, such as independent science fair projects, multi-week class experiments, and computer science-oriented projects. Teachers who use this curriculum or other Allen Cell Explorer resources in their classrooms, or who have students who pursue advanced independent projects, are welcome to [share their experiences](#) with us.



Expected time for lessons

This lesson is meant to help teachers integrate Allen Institute datasets into their classrooms. Teachers are welcome to modify the lessons to suit their classes. In particular, the experimental portion (pre-lab, virtual experiment, lab report) can be used separately from the other lessons and assignment.

Assignment #1: Introduction

- If you choose to use it, this assignment was designed to be completed before the second in-class discussion period. It takes approximately 30-45 minutes to complete. See section Copy Masters for worksheets for students and reading.
- Reading: [Five things we still don't know about cells](#)
- Exploration: [Visual Guide to Human Cells](#)
- Worksheet with short answer questions

Lesson #1: Class discussion on research methods, topics, and unknowns

- 60 minutes
- Video: [A common platform for human cell research](#) on cell biology data collection techniques
- Small-group class discussions using worksheet with guiding questions
- Introduction to experiment as a class

Assignment #2: Virtual experiment pre-lab

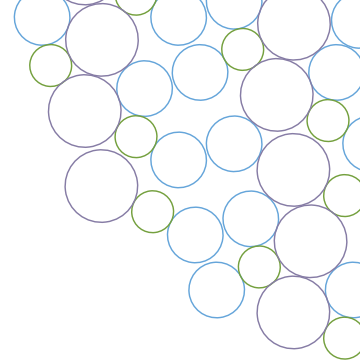
- 15-30 minutes
- Pre-lab review of experimental design
- Students read experimental design for virtual experiment and watch [Navigating the 3D Cell Viewer](#) video to prepare for class
- Pre-lab worksheet questions on the type of data involved in the virtual experiment and how it was collected

Lesson #2: Virtual experiment

- 60-90 minutes
- See next page for detailed outline of experiment

Assignment #3: Complete virtual experiment report

- 30-45 minutes
- Students complete the data analysis and additional questions from virtual experiment



Outline of virtual experiment for teachers

Equipment needs:

One computer or tablet per student or group of students. We recommend students work in groups of 2-3.

Whiteboard, form, or other method of collecting data from students

Part 1 (15 minutes):

Students access a dataset of cells labeled by stage of mitosis by an expert. via the [Cell Feature Explorer](#). They practice identifying cells by stage of mitosis, concentrating on cells with labeled alpha-tubulin. Worksheet questions address differences between drawn and microscope depictions of cells.

Teacher: Prepare collection method for students to enter their data (whiteboard, web form, etc.). The collection method should have the 5 main stages of mitosis and space for each student to record the number of cells they counted in that stage. (See Table 2 in student packet.)

Part 2 (30 minutes):

Students access the [3D Cell Viewer](#) and select alpha-tubulin tagged cells to view. They identify what stage of mitosis cells are in and record the counts on their worksheet. They calculate the mitotic index – all of mitosis vs. interphase cells only. They then report their individual counts on the whiteboard. (They will break down the amount of time in each stage of mitosis using the whole class's data as part of the lab report.)

Part 3 (15-30 minutes):

Students select a different tagged protein of their choice in the [3D Cell Viewer](#). They identify what stage of mitosis the cells are in and record those totals. They also note their observations on how the process compares to sorting based on microtubules..

Any remaining time:

Students work on analysis questions in packet.

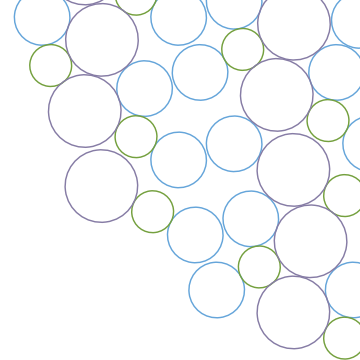
After class:

From whiteboard, online form, or other method of students sharing data: Teacher aggregates individual students' counts of cells in each stage of mitosis and shares totals with class for use in questions in the lab report.

Worksheets - copy masters

The following pages include masters of materials to be copied for students.

1. Pre-lesson assignment #1 worksheet for students
2. Discussion questions for lesson period #1
3. Pre-lab, including video, questions on experimental design, and questions on data used
4. Virtual experiment packet, including data collection instructions for students and worksheet (in class experiment periods), data analysis, and additional questions (assignment #3)



Mitosis and Microscopy: Introduction

Introduction and goals:

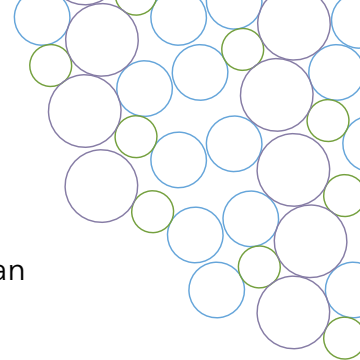
In this assignment, we are studying images of human induced pluripotent stem cells (iPSCs) taken by the Allen Institute for Cell Science. A healthy adult human donated a skin sample, which was put through a process that caused those cells to turn into stem cells. Stem cells can become any other type of cell in the body, which is called differentiation, but for this experiment we are studying them while they are still stem cells. These cells have also been specially modified so that specific proteins inside of them glow under a microscope, which helps scientists view their function more accurately.

Reading:

[Five things we \(still\) don't know about cells](#)

Questions:

There are a lot of open questions in cell biology. Which one of these questions are you most interested in knowing more about? Why?



What surprised you in the article? What do scientists know more about in cells than you expected? What do scientists know less about?

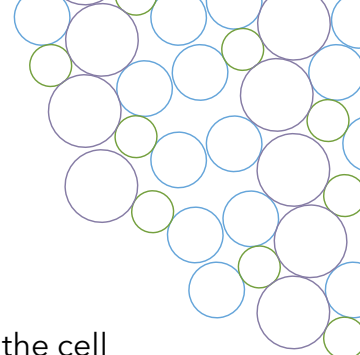
Exploration:

Visit the [Visual Guide to Human Cells](#).

These images come directly from real cell data. Biologists modify the cells to attach fluorescent tags called green fluorescent protein (GFP) to individual proteins inside of the cell. Under the microscope, scientists can see where that protein is and what it does in a lot of detail, but they can generally only do that one protein at a time using this method. A team of visualization scientists then took all the different microscope images showing one cell structure at a time and combined them in this model.

Question:

These cells have been grown specifically because the fluorescent proteins still function mostly normally. The data still only comes from only one fluorescent tagged protein at a time. Explain two ways in which that limitation might impact your experimental design.



Explore more:

In the left sidebar, click on "Cell Functions" and then "Divide." Click through all of the cell structures in the "Divide" category, read about the structure's function in the right sidebar, and click through the steps of mitosis using the wheel that surrounds the image of the cell. You can drag and scroll on the cell to rotate and zoom.

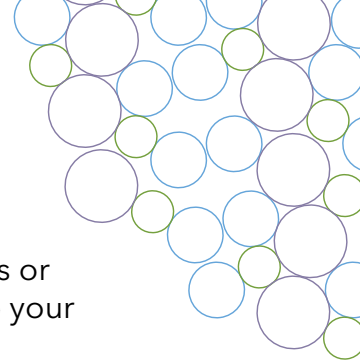
Question:

Compare the structures in the "Divide" category. How are these structures' roles in mitosis similar? How are they different?

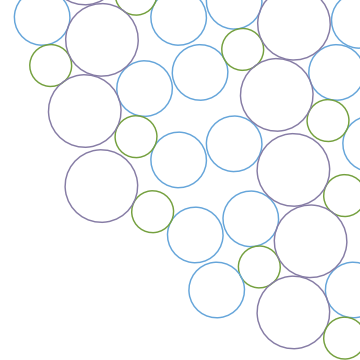
Pick a different cell function, such as respiration. Before you look at the Visual Guide, predict what a cell structure that is not involved in cell division is doing during mitosis. Explain why you think the structure would behave that way.

Cell structure: _____

Prediction: _____



Look at your structure in the Visual Guide. Did the model match your expectations or not? Describe what the model shows the cell structure is doing and compare it to your prediction.



Mitosis and Microscopy: Class Discussion Questions

Learning goals:

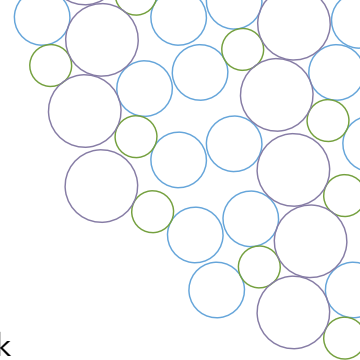
Understand how scientists measure cells during mitosis. Explain the applications and limitations of scientists' methods. Design an experiment to study mitosis.

In small groups, answer these discussion questions. Think about the homework reading and the video to help you with your answers.

As a team, read the Overview of the [Allen Institute for Cell Science workflow](#) that reviews how the data in the experiment you are about to conduct were collected. Think about this reading, the homework reading, the Visual Guide and how its models were made, and the video to help you with your answers.

Knowledge check:

Most of the cells in the database are in interphase and our experiment is on mitosis. Explain why we also need to study interphase if we are interested in mitosis.

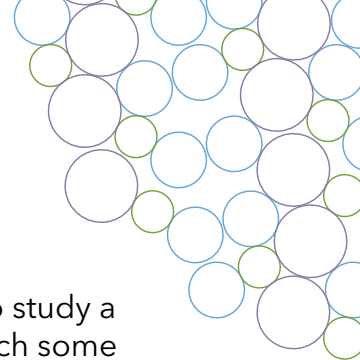


Questions:

Where does the fluorescence in fluorescence microscopy come from? (Hint: Think about the video.) What does the fluorescence let scientists track?

Imagine you work at a clinic and you want to diagnose a cellular disease in one of your patients. You can't use fluorescence microscopy. Why not? (Hint: Think about where the fluorescence comes from.)

Imagine you work at a lab like the one in the video and want to study a disease. Fluorescence microscopy is very useful! Why can you use it in your research but not the clinic?



Design your own experiment:

Outline an experiment using fluorescent tagging and fluorescence microscopy to study a disease. Answer these questions about your experiment. You may need to research some background information online about the disease you chose to complete your answer. Include your citations at the bottom of the page.

Example:

Disease: Alzheimer's disease

Cell function: Intracellular transport

Cell structure: Microtubules

Research question: How are healthy and diseased cells' microtubule positions in the cell different?

Data you would collect: The positions of the microtubules relative to the cell membrane in the healthy and diseased cells at different times and in multiple cells.

Prediction: The diseased cells' microtubules are more distributed around the cell and less organized than the healthy cells.

Disease: _____

Cell function: _____

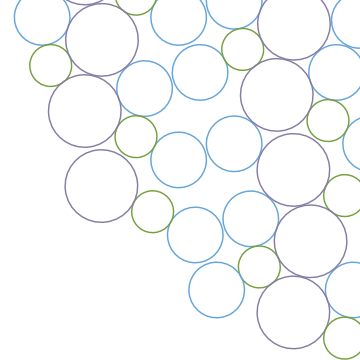
Cell structure: _____

Research question: _____

Data you would collect: _____

Prediction: _____

Citations:



Mitosis and Microscopy: Virtual Experiment Pre-Lab

Learning goals

The goal of this experiment is to calculate the mitotic index from real microscope images taken by scientists. By the end of this experiment, you should be able to recognize at least 2 cell structures in the microscope images, categorize microscope images of cells into stages of mitosis, and explain how the images were collected.

Experiment outline

The goal of this experiment is to identify features that can be used to identify cells by their stage of mitosis. For Part 1 and Part 2 of the experiment, the cells in the images have been tagged with a fluorescent protein for alpha-tubulin, which is part of the mitotic spindle. For Part 3, you will choose another protein to study.

Part 1: Practice with microtubules

- Access pre-labeled dataset of cells that have been identified by their stage of mitosis and view samples with fluorescent alpha-tubulin.
- Identify features of the cells that define the different stages of mitosis.
- You will use the Cell Feature Explorer to view sets of cells. This portal includes the user interface from the 3D Cell Viewer. Get familiar with the 3D Cell Viewer via this [tutorial](#).

Part 2: Sorting with microtubules

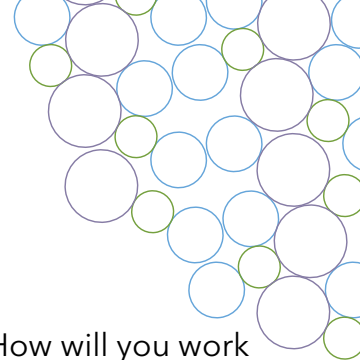
- Access an unsorted dataset of cells with fluorescent alpha-tubulin and sort the cells by stage of mitosis.
- Calculate the mitotic index from the class data

Part 3: Sorting with the protein of your choice

- Repeat Part 2 with another protein of your choice

Knowledge check:

Compare the drawn images of cells from your textbook and the microscope images of cells in the 3D Cell Viewer. Name some ways that the textbook images are realistic and some ways that they are too simplified.

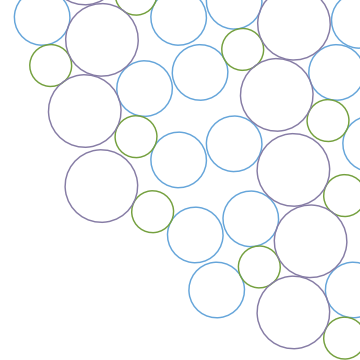


Questions:

What is a step in the experimental protocol that you expect to find challenging? How will you work through the challenge?

Analyzing mitosis data

Scientists still have a lot to learn about mitosis. Read this [story](#) by scientists on their current research to create the Integrated Mitotic Stem Cell, which uses advanced visualization techniques to study dividing cells. After learning about how the data was collected and analyzed, click through the Z-stack and 3D viewers to see cells at different stages of mitosis.



Mitosis and Microscopy: Virtual Experiment

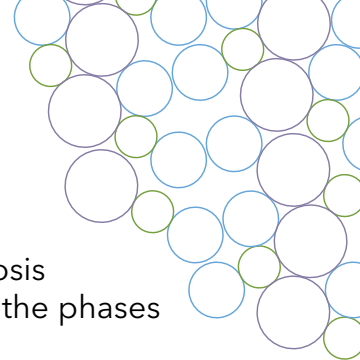
Part 1: Practice with microtubules

The Cell Feature Explorer includes cells that have been labeled by what stage of mitosis they are in. You will look at a selection of cells, concentrating on cells with fluorescently labeled alpha-tubulin.

Knowledge check:

These cells have been labeled so that alpha-tubulin, a protein associated with microtubules, will glow under the microscope. What is the primary role of microtubules in mitosis?

Think back to the reading for the last homework, the class discussion, and the video of the 3D Cell Viewer explaining the data access tools. In a sentence or two, summarize how the cells were grown and how the images were collected. When you look at the images, what do the colors mean?



Visit the [Cell Feature Explorer](#). Change the X axis variable to “Interphase and Mitosis Stages” and leave the Y axis as cellular volume. The X axis should show labels for the phases of the cell cycle.

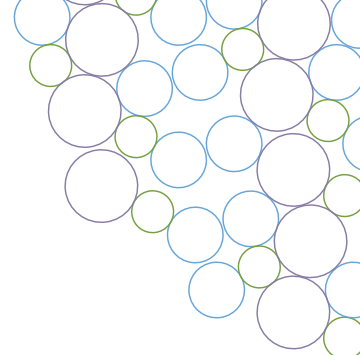
In the list on the left, select only alpha-tubulin from the list of proteins. Alpha-tubulin is a protein associated with microtubules and the mitotic spindle.

You can click on individual dots on the plot, each of which represents one cell. The cells will pop up in the 3D viewer below the graph.

Look through the labeled cells. Identify 1-2 features of the cell that appear consistently in the images of cells in each stage of mitosis. You will use these features to sort cells on your own in Part 2 of the experiment.

Table 1: your observations of pre-sorted cells

Stage of mitosis	Features of the cell at this stage of mitosis
Interphase	
Prophase	
Prometaphase	
Metaphase	
Anaphase	



Part 2: Sorting with microtubules

1. Now we will view cells without the labeling data in the [3D Cell Viewer](#).
2. From the "Protein" dropdown menu on the right, select alpha-tubulin. From the "Image type" menu, select "single cell".
3. Click on any image to see it in larger format.
4. There are a lot of settings in the viewer! The pre-lab video reviewed some of them, but the only thing you need right now is in the channel editor pane. The color key for the image is listed there.
5. Categorize the cell by its stage of mitosis. Record the cell ID (listed in the header of the cell viewer, or on the thumbnail image) in the table.
6. Try to sort at least 25 cells and record their cell IDs in the chart. If you're having trouble, try rotating the cell or changing the color balance.
7. View more cells by scrolling left and right through the thumbnails using the white triangles.

Table 2: your sorted cells

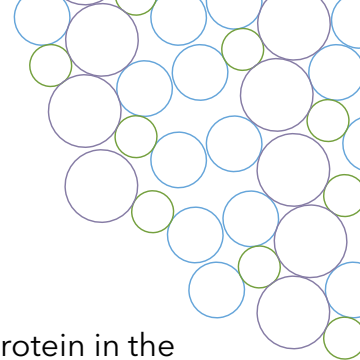
Stage of mitosis	Cell IDs	Total # of cells in stage
Interphase		
Prophase		
Prometaphase		
Metaphase		
Anaphase		

Record your data from the “total number of cells” column of Table 2 on the whiteboard.

Before you leave, record the class totals for how many cells are in each stage of mitosis in the alpha-tubulin dataset:

Table 3: class data

Stage of mitosis	Total # of cells in stage
Interphase	
Prophase	
Prometaphase	
Metaphase	
Anaphase	



Part 3: Sorting with the protein of your choice

Refresh the page to clear your search settings. Repeat Part 2, but with any other protein in the dropdown list besides alpha-tubulin.

Protein you picked: _____

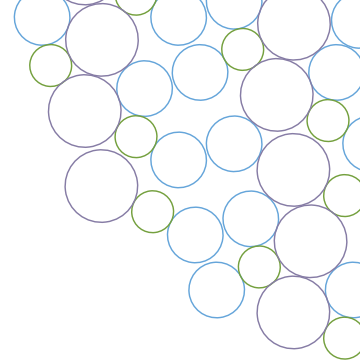
Cell structure that protein is associated with: _____

Cell function that structure is associated with: _____

Sort at least 25 cells. Record the cell IDs in the chart under what stage of mitosis they are in.

Table 3: your sorted cells, protein of your choice

Stage of mitosis	Cell IDs	Total # of cells in stage
Interphase		
Prophase		
Prometaphase		
Metaphase		
Anaphase		



Mitosis and Microscopy: Virtual Experiment Report and Analysis

Learning goals:

Analyze microscope images of cells with methods used by scientists. Explain how scientists and computers learn to recognize and categorize cells into stages of mitosis. Display your data in graphs and explain its biological meaning. Apply your knowledge to design experiments and interpret data.

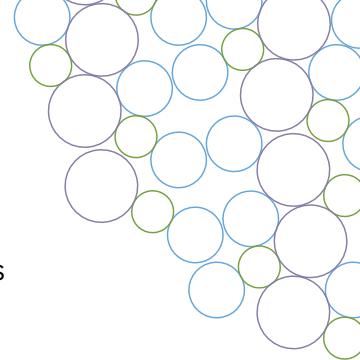
Question on Part 1 of experiment:

In Part 1 of the experiment, you identified some features of the cells that identify the stages of mitosis. Choose a stage of mitosis and sketch a cell with fluorescently labeled alpha-tubulin and dyed cell membrane and DNA below. Illustrate and label at least 3 features that you identified in Part 1 of the experiment as characteristic of this stage of mitosis and that you used to sort the cells yourself in Part 2 of the experiment.

Question on Part 2 of experiment:

Make a graph of **your** data in Table 2. Make sure you label the axes. (Hint: what kind of graph is used to show counts of data?)

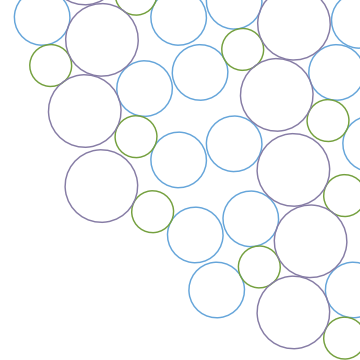




Make a graph of the **class** data in Table 3. Make sure you label the axes. (Hint: this will be the same type of graph as the previous one, but with the class data.)



Besides just having more data from the whole class, the patterns in the graphs are different. Why do you see a different pattern in your data and the class data?



Use the class data for the rest of the questions.

Calculate the mitotic index from the class data.

Mitotic index numerator = _____

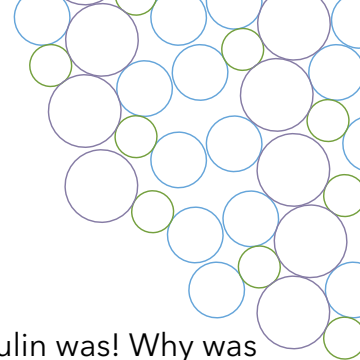
Mitotic index denominator = _____

Mitotic index = _____

Explain how we can use still images of cells to calculate the amount of time they spend in mitosis.

The mitotic index measures mitosis vs. interphase. Calculate the fraction of time the cell spends in each stage of mitosis. (Hint: Compare to the total amount of time, not time spent in mitosis.)

Stage of mitosis	Amount of time in stage
Interphase	
Prophase	
Metaphase	
Anaphase	



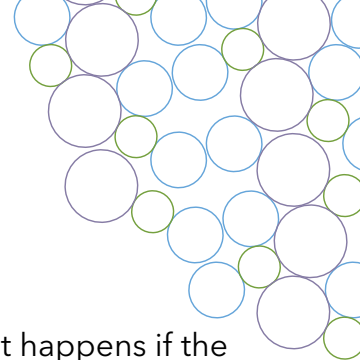
Questions on Part 3 of experiment:

Sorting with other organelles is much harder than sorting in Part 2 with alpha-tubulin was! Why was it harder? Relate your answer to the cell's biology and to the images.

Describe two ways sorting cell images from a microscope is different from looking at drawings of cells.

Difference 1: _____

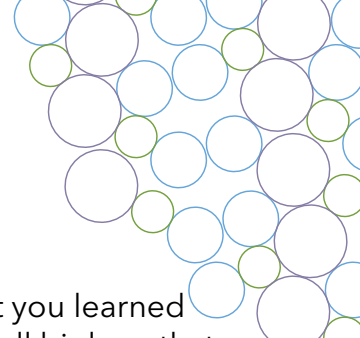
Difference 2: _____



Reflection questions:

What might happen to an organism if mitosis is not working correctly? (Hint: What happens if the division is not perfect? What if there are too many or not enough divisions?)

In this experiment, we were measuring healthy mitosis. Propose one way this knowledge informs our understanding of diseases related to mitosis.

**Design your own experiment:**

Think back to the article “Five Things We (Still) Don’t Know About Cells” and what you learned in this experiment. Describe a research project that investigates any question in cell biology that you are interested in. What kind of data do you want for your experiment? What would you expect to discover?

Research question: _____

Cell function: _____

Cell structure: _____

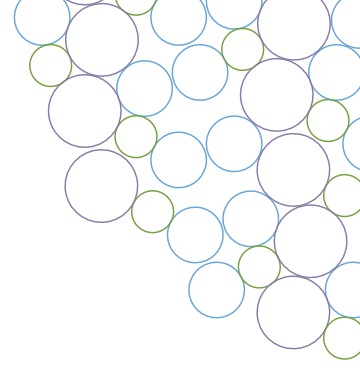
If applicable, disease: _____

Data you would collect: _____

How you would analyze or examine data: _____

Predicted results: _____

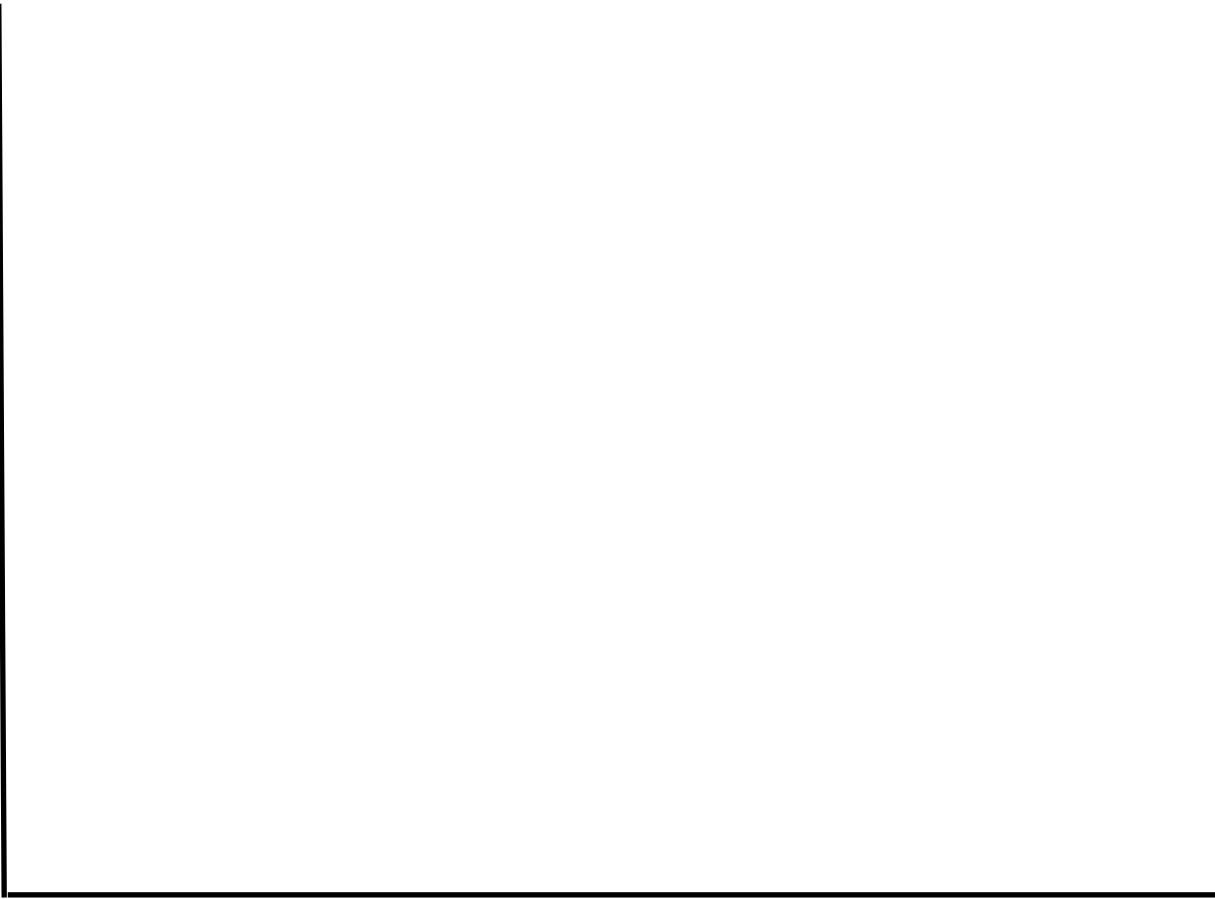
Citations:



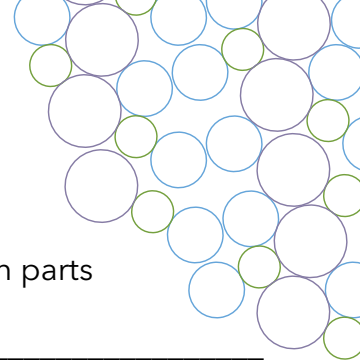
Mitosis and Microscopy: Above and beyond

Complete these questions for an added challenge.

Visit the Cell Feature Explorer. The default view shows the relationship between nuclear volume and cellular volume. Sketch the graph below. Don't forget to label the axes.



Now set the axes to "Interphase and Mitosis Stages" and "Cellular Volume." What relationship do you observe between cell division and cellular volume?



The whole cell acts as a system. What can we learn from studying what happens in parts of the cell other than the DNA during mitosis?

Using a labeled dataset to make rules for classifications is a common way that computers learn to sort things automatically. Pick two of the features you observed in Table 1 and describe them for a computer - that is, describe things that are happening in the image, rather than what the cell is doing.

Feature 1: _____

Feature 2: _____

Why would scientists find it useful to have a computer sort cell images automatically?
