Introduction to Microscopy in Biology

Viewing cells in 3D with the Allen Institute for Cell Science 3D Cell Viewer and FIJI

Overview

This lesson includes a presentation and an interactive, worksheet-guided exercise that introduces students to techniques in microscopy and 3D cell image data. Students will have the opportunity to learn about types of microscopy and how 3D image data is generated, and will interact with segmented single-cell image data from the Allen Institute for Cell Science. Two levels of interactive activity are provided: a basic level using the 3D Cell Viewer, an online visualization tool, and a more advanced activity using FIJI, a free open source software for image viewing and analysis.

Grade level

Introductory high school biology, grades 9-10 (basic activity)
Advanced high school biology, grades 9-12 (advanced activity)

Learning objectives

- Students will be able to describe the relationship between a single plane of data and a 3D volume.
- Students will understand the concepts of cells, organelles, and spatial localization of organelles.
- Students will be able to identify and describe the localization of two organelles, tubulin and mitochondria.

Outline and contents

- Introductory presentation: an introduction to fluorescence microscopy and interpreting cell image data
- Basic handout: detailed instructions for using the Allen Cell Explorer 3D Cell Viewer and analyzing data; activity also demonstrated in last section of presentation
- Advanced handout: detailed instructions for using FIJI, a free software tool, for advanced image viewing and analysis

Equipment

- Laptop or desktop computers
- Internet access
- For advanced FIJI activity: ability to install FIJI, a downloaded software program

Contents

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	
Constructing explanations and designing solutions	
Engaging in argument from evidence	X
Obtaining, evaluating, and communicating information	X

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

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-LS2: Ecosystems	
HS-LS3: Heredity	
HS-LS4: Evolution	

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. Launched in 2003 by founder Paul G. Allen, 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.

Cell Feature Explorer

- The 3D Cell Viewer (allencell.org/3d-cell-viewer) provides a multimodal view of tens of thousands of images of living human cells from the Allen Institute for Cell Science's advanced fluorescence microscopes.
- These cells are human induced pluripotent stem cells (allencell.org/hips-cell-biology-overview) (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. Some commonly used measurements have been precomputed, such as cellular and nuclear volumes.
- 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: pink).
- These cell images can also be opened and analyzed in FIJI, a free, industry-standard image analysis software. This software also enables quantitative analysis of cells. This more complex image analysis option is described in the "Advanced activity" handout.

Open research questions in cell biology

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 this article, Five things we still don't know about cells (alleninstitute.org/what-we-do/cell-science/news-press/articles/5-things we-still-dont-know-about-cells). Teachers may find additional background in the Allen Cell Explorer FAQ (allencell.org/faqs). Additional articles about recent discoveries, the process of research, and more are available on the news page (alleninstitute.org/news-press) of our website. These articles may be of interest for advanced students to pursue further reading.

Lesson material outline

This lesson includes three teaching materials:

- Introductory presentation: an introduction to interpreting image data and how fluorescence microscopy works.
- Basic handout: student handout that accompanies the 3D Cell Viewer demonstration in the second half of the introductory presentation.
- Advanced handout: a more advanced image analysis activity using FIJI. This software is free but must be downloaded and run locally.

Student guide: Analyzing cell images with the Allen Cell Explorer 3D Cell Viewer

Questions to consider

How do we produce microscope images?
What is a cell?
What is an organelle?

Step 1: Accessing the 3D Cell Viewer

Visit allencell.org/3d-cell-viewer. We will start by visualizing a cell that has fluorescent mitochondria.

Step 2: Visualize mitochondria

To the top right of the gallery of cells, you will see two drop down menus. From "Image type" select "Single cell," and from "Protein tag" select "Tom20." Tom20 is a protein found in mitochondria. Then select any one cell from the gallery to view in more detail.

Once you have selected the cell to view, adjust the viewing parameters. In the "Tools" panel to the right of the cell image, uncheck the boxes for DNA and membrane, and use the "Intensity level" slider to increase the image intensity, density, and brightness.

Step 3: Using the sliders, scroll through the Z axis

Use the slider below the image to scroll through it in the Z axis (up and down).

Cell observations

Describe what you are seeing in the cell image. What parts of the cell can you see? What can you *not* see?

How does the structure change as you move through the Z dimension? Observe the shape and position of the structure, and the image intensity.

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Step 4: Explore multiple channels

In the introductory presentation leading up to this activity, you learned about image channels. Each color in an image represents one channel. In these cells each color, and therefore each channel, represents one cell structure. In the default settings, the mitochondria are yellow, the DNA is blue, and the cell membrane is pink. Try turning different combinations of channels off and on. What additional observations can you make about the cell when you have multiple channels on?

Step 5: Select an alpha-tubulin cell

At the left side of your viewing screen, you should still see the Images panel that you used before to select a cell with labeled mitochondria. Using the drop down menu for "Protein tag" select "Alpha-tubulin," a component of microtubules. Then select any one cell from the gallery to view in more detail.

Once you have selected the cell to view, adjust the viewing parameters. In the "Tools" panel to the right of the cell image, uncheck the boxes for DNA and membrane, and use the "Intensity level" slider to increase the image intensity, density, and brightness.

Step 6: Using the sliders, scroll through the Z axis

Use the slider below the image to scroll through it in the Z axis. The Z axis lets us see higher and lower in the cell in 3D.

Cell observations

Describe what you are seeing in the cell image. What parts of the cell can you see? What can you *not* see?

Compare this cell to the cell you looked at before with labeled mitochondria. What is similar? What is different?

How does the structure change as you move through the Z dimension? Observe the shape and position of the structure, and the image intensity.

Step 7: Select the cell membrane channel only

In the "Tools" panel to the right of the cell image, check the box for cell membrane and uncheck the box for alpha-tubulin. Keep the DNA box unchecked.

Step 8: Using the sliders, scroll through the Z axis

Use the slider below the image to scroll through it in the Z axis.

Step 9: Select both alpha-tubulin and membrane

In the "Tools" panel to the right of the cell image, re-check the box for alpha-tubulin. Keep the cell membrane box checked and the DNA box unchecked.

Cell observations

What can you observe about this cell when you have two channels (labeled structure – alpha-tubulin, membrane) vs. when you only have one channel at a time?

Describe the orientation and shape of the alpha-tubulin, DNA, and membrane in relation to each other.

Step 10: Select the DNA channel only

In the "Tools" panel to the right of the cell image, check the box for DNA and uncheck the boxes for alpha-tubulin and cell membrane.

Step 11: Using the sliders, scroll through the Z axis

Use the slider below the image to scroll through it in the Z axis.

Step 12: Select both alpha-tubulin and membrane

In the "Tools" panel to the right of the cell image, re-check the box for alpha-tubulin. Keep the cell membrane box checked and the DNA box unchecked.

Cell observations

What can you observe about this cell when you have all three channels (labeled structure – alpha-tubulin, membrane, DNA) vs. when you only have one or two channels at a time?

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What are you still curious about related to this cell, this cell image, or the way the image was taken?

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For an added challenge, repeat this whole activity using a full field of cells instead of a single cell. In the Images panel to the left of your viewer, select "Full field" instead of "Single cell." These images are of the whole group of cells growing in the lab. In the single cell images, scientists have separated the field image into the individual cells.

Student guide: Advanced cell image analysis with FIJI

Questions to consider

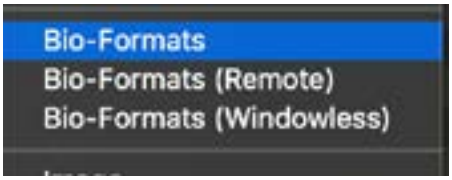
How do we produce microscope images?
What is a cell?
What is an organelle?

Installing FIJI

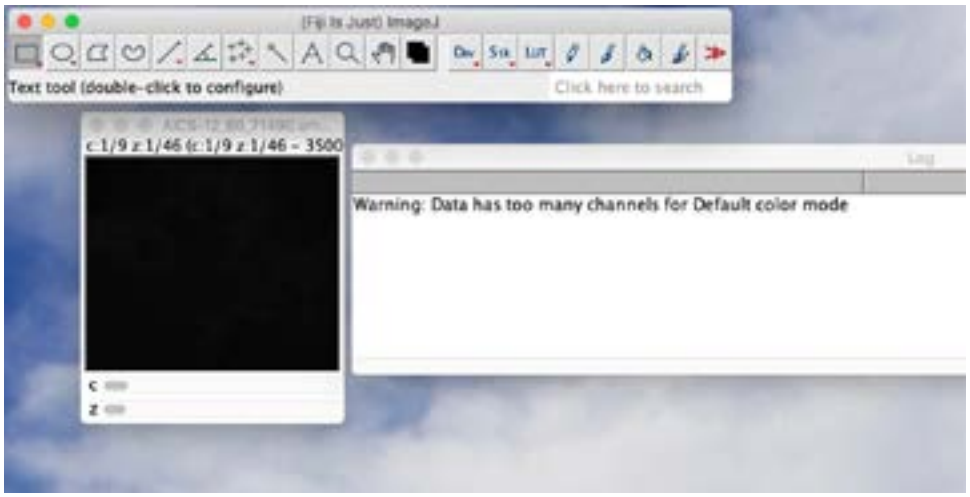
You may be provided with a flash drive that has FIJI pre-installed, or visit imagej.net/software/fiji.
You can double click on the FIJI icon to open the program.

Once you have opened FIJI, you should see a menu bar that looks like this:

In the FIJI menu, navigate to File > Import > Bioformats, and open one of the image files on your flash drive. The filename will end with the format ome.tif.



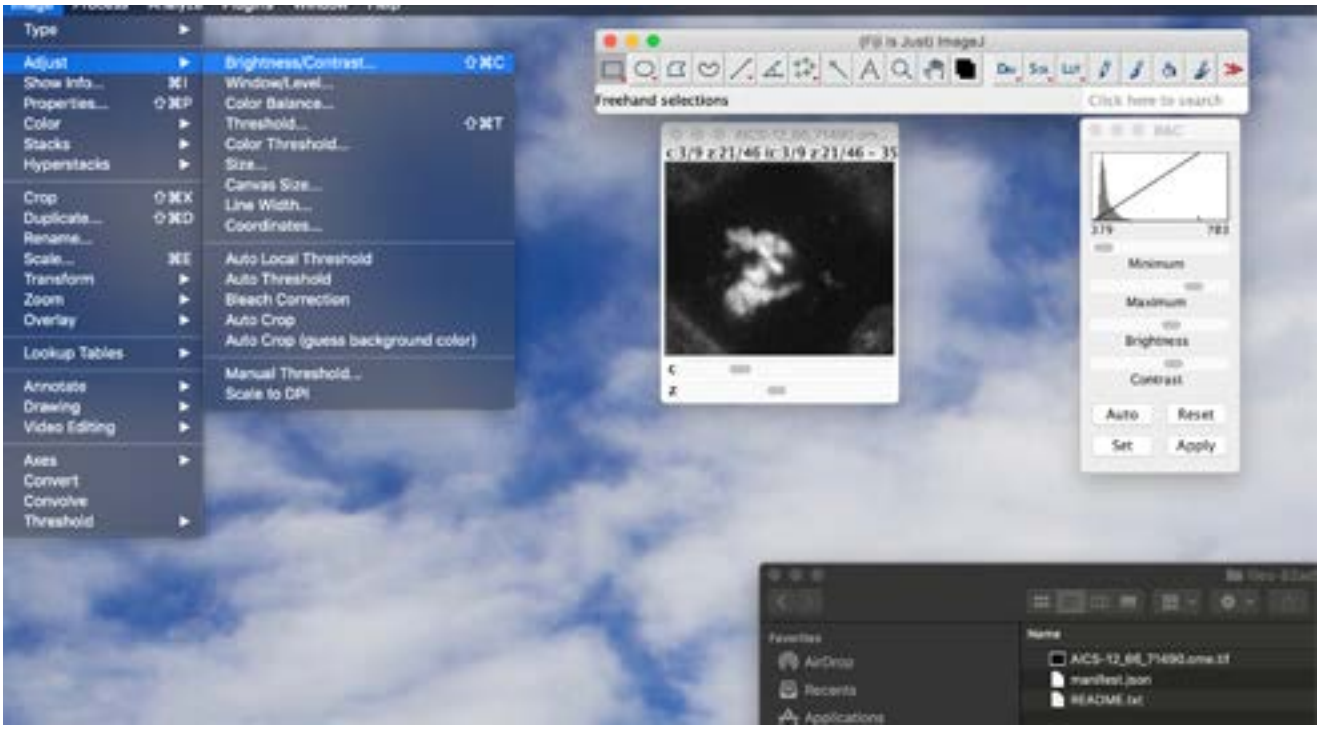
Once you've opened the image, you should see something like this:



The error message is just informational. You can close it.

Try scrolling through the 'c' and 'z' scroll bars. What do you see when you do that? Based on what you see, what do you think these labels correspond to in the image?

Important note: You will need to adjust the brightness of the image to see the details. To do that, go to Image > Adjust > Brightness and Contrast. Pressing the "Auto" button will help you see more details in the image.



Viewing cell data in FIJI

Describe what you are seeing in the cell image. What does the image look like?

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How are individual images related to the whole image stack?

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How many different structures can you see in these images? How do they look different from each other?

What is the relationship between these structures? For example, the nucleus is inside the cell membrane. What other relationships can you describe?

Open an image of mitochondria and microtubules. How do these structures differ from each other?

Where are these structures localized relative to the nucleus? To the top and bottom of the cell?

Describe each structure. How do they look different from each other? Are there any similarities between the structures?

How similar are the two cells you are looking at? What are their shapes? Is the nucleus in the same place? Are the cell shapes similar or different?

What kinds of cell shapes do you think are possible?

What kinds of nuclear shapes are possible? Try drawing a few examples of what you think you might see if you looked at more cells.

Using the Allen Cell Explorer to select a cell

We can use the Allen Cell Explorer to select a cell that has been gene edited to visualize tubulin and mitochondria.

Navigate to the Allen Cell Explorer: allencell.org.

Select Cell Feature Explorer from the drop down menu, or go directly to cfe.allencell.org.

Navigate to the gallery. Select the following cells by searching for their cell IDs:
- Mitochondria (TOM20) mitotic cell: Cell ID 12875
- Alpha tubulin mitotic cell: Cell ID 71490

For each cell, download the segmented cell and full field image.

Navigate to the Downloads folder on your computer. The images may be zipped (compressed) - you will need to unzip them, or your computer may automatically unzip the files and place them in a folder. Within this folder is a .tiff and that is the file that will be loaded into FIJI.

You previously downloaded FIJI. Open the program. In the plugins menu, navigate to the Bio-Formats category and select the Bio-Formats Importer. Navigate to the Downloads folder and find the .tiff and open it. It will take a little time to load because it is a big file.

A box will open with options to select. Select these view options:
- Select “View stack with: Hyperstack”
- Check the box for “Use virtual stack”
- Uncheck all other checkboxes

The image file will open. Again, this may take a little bit of time. Use the FIJI image analysis tools to explore your cell image. What do you observe about this cell? How is analyzing this cell different in FIJI compared to the online 3D Cell Viewer.