Does Organelle Shape Matter?

Exploring Cell Shape and Structure through the Allen Cell Explorer and High-content Microscopy Datasets

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Student Learning Outcomes

1. **Define** the function of the *endoplasmic reticulum* and *high-content microscopy*
2. **Explain** in your own words the process the Allen Institute used to create a label-free prediction method for determining the location of 3D structures inside the cell, directly from transmitted light images
3. **Access** data from high-throughput discovery experiments from public databases
4. **Use** the Cell Explorer 3D Cell Viewer tools to **analyze** image data to test your hypothesis
5. **Compare** cell structures in the Allen 3D Cell Viewer
6. **Evaluate** the validity of this high-content imaging approach
7. **Design** a future experiment to build on your findings

Part I. What has happened to these cells?

Dr. G and his summer students want to discover a drug that could be used as an antibiotic against *Delftia acidovorans*, an opportunistic pathogen that has been found in drains and faucets. They used high-throughput drug screening to test a library of cancer drugs from the National Cancer Institute (NCI) and were excited to find a promising hit, a compound with potentially useful drug properties. This compound is highly active at low doses against *Delftia acidovorans* and not cytotoxic when tested on mammalian cells. However, Dr. G's students were learning from a friend how to use a stain to label the endoplasmic reticulum (ER) in live Chinese Hamster Ovary (CHO) cells and noticed that cells treated with low doses of this compound have *wildly* variable ER sizes. They tell Dr. G., and together they search for information about ER structure. Dr. G. is a microbiologist and along with his students has limited cell biology experience.

Questions

1. Draw a eukaryotic cell and identify the endoplasmic reticulum (ER).

2. Explain to the average adult the function of the ER.

Find three or four peers and compare your drawings and answers.
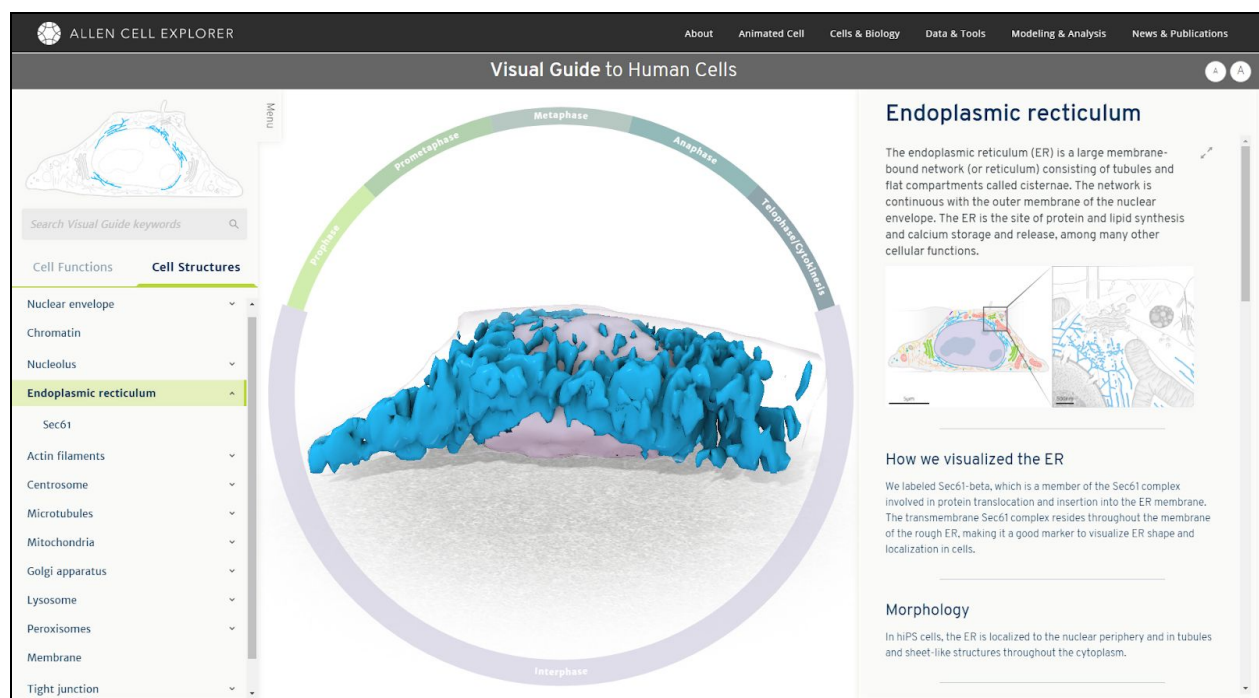Discuss your reasoning.

STOP HERE!

# Part II. This does *not* look like the textbook image!

Dr. G. and his students visit the *Allen Cell Explorer Visual Guide to Human Cells*:
https://www.allencell.org/visual-guide-to-human-cells.html
They read about the Endoplasmic Reticulum (ER) and how researchers visualized the ER by labeling Sec61-beta as seen below.

They then decide to view cells using the *3D Cell Viewer* and visualize cells with the Sec61-beta protein tagged as shown next. The *3D Cell Viewer* can be launched on any browser and found here: https://www.allencell.org/3d-cell-viewer.html
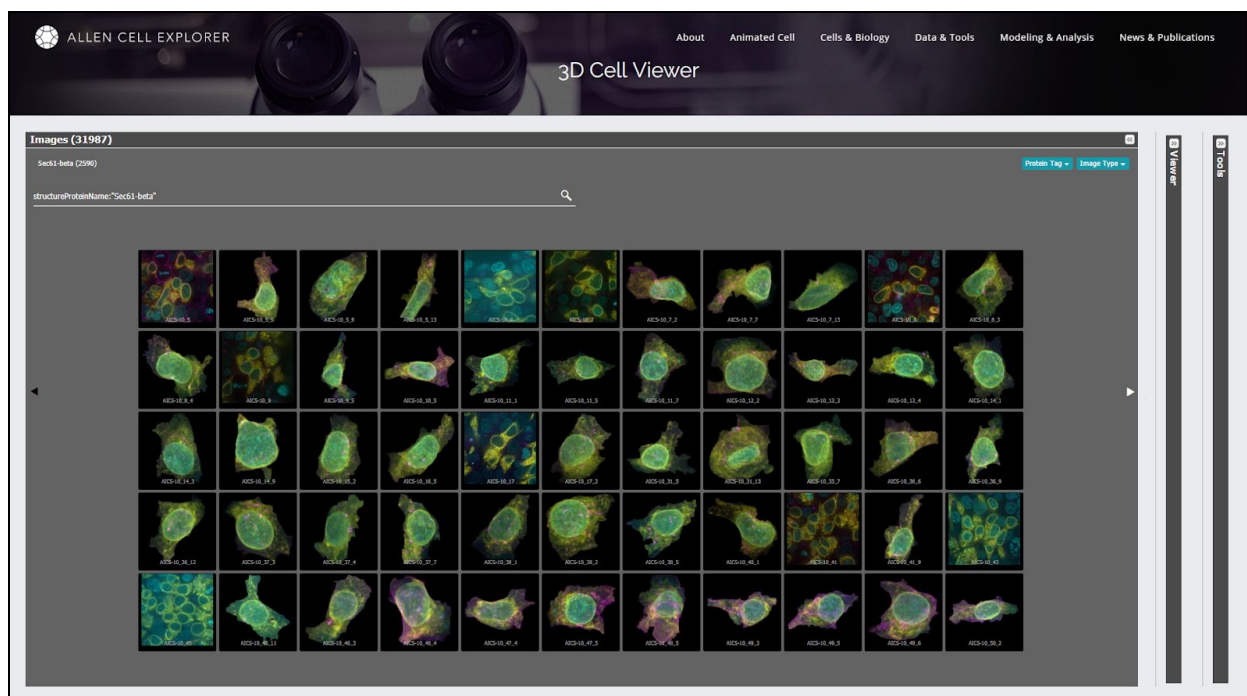
Questions

1. Using the *Visual Guide to Human Cells*, look at the shape of the ER during the different phases of the cell cycle. You can click on the name of the phase, and the visuals will change. *What changes do you notice?*

2. You notice under the Morphology section the following text: "In hiPS cells, the ER is localized to the nuclear periphery and in tubules and sheet-like structures throughout the cytoplasm". Dr. G's students saw staining using the ER-specific stain that varied in shape both within and between different cells. *What explanation do you have for this?*

3. You then navigate to the *3D Cell Viewer*: https://www.allencell.org/3d-cell-viewer.html and select Sec61-beta under Protein Tag from the turquoise drop down menu. The number of images decreased from 31,987 to 2,590. You click on a couple, keeping in mind that the yellow staining corresponds to the ER. *Do you notice any patterns?*

   STOP! Find two or three peers and compare your findings.

Part III. Why are there so many *different* looking cells?

You have been looking at cells for some time now and notice a wide variety of patterns for the Sec61-beta tagged cells. Furthermore, *it seems all cells have different shapes!* This wasn't what you are used to from your Cell Biology class in college...

You navigate to the *Deep Cell Zoom* under the **Data and Tools** menu on the top and see a beautiful wall of thousands of images of cells. Link here:
https://www.allencell.org/deep-cell-zoom.html

You start to wonder. *How are they visualizing the ER and other organelles?* You navigate to:
https://www.allencell.org/methods-for-microscopy.html
to learn more about the process used by the Allen Institute and begin to wonder...

Questions

1. Read the *Methods for Microscopy* page. Individually, summarize the methods used in no more than **three (3)** sentences.
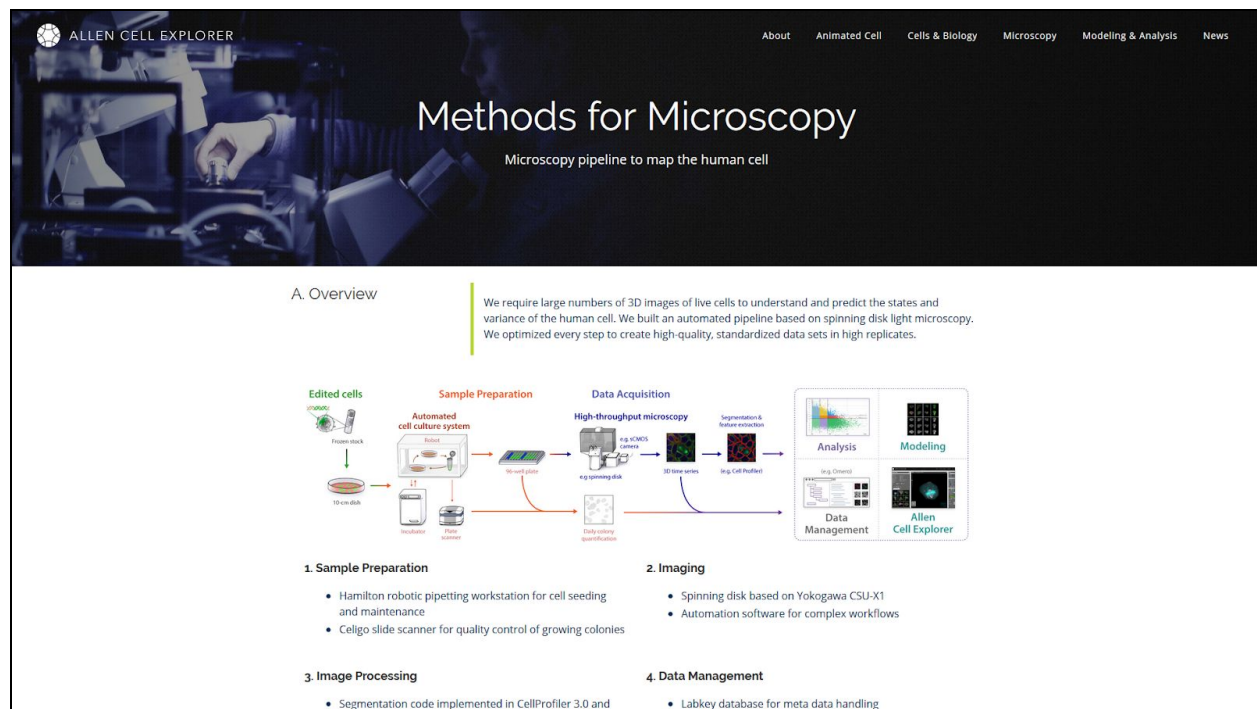
2. The microscopy methods help a little, but you are still curious about the label-free prediction method used to determine the location of structures and create these images. You visit: https://www.allencell.org/label-free-determination.html
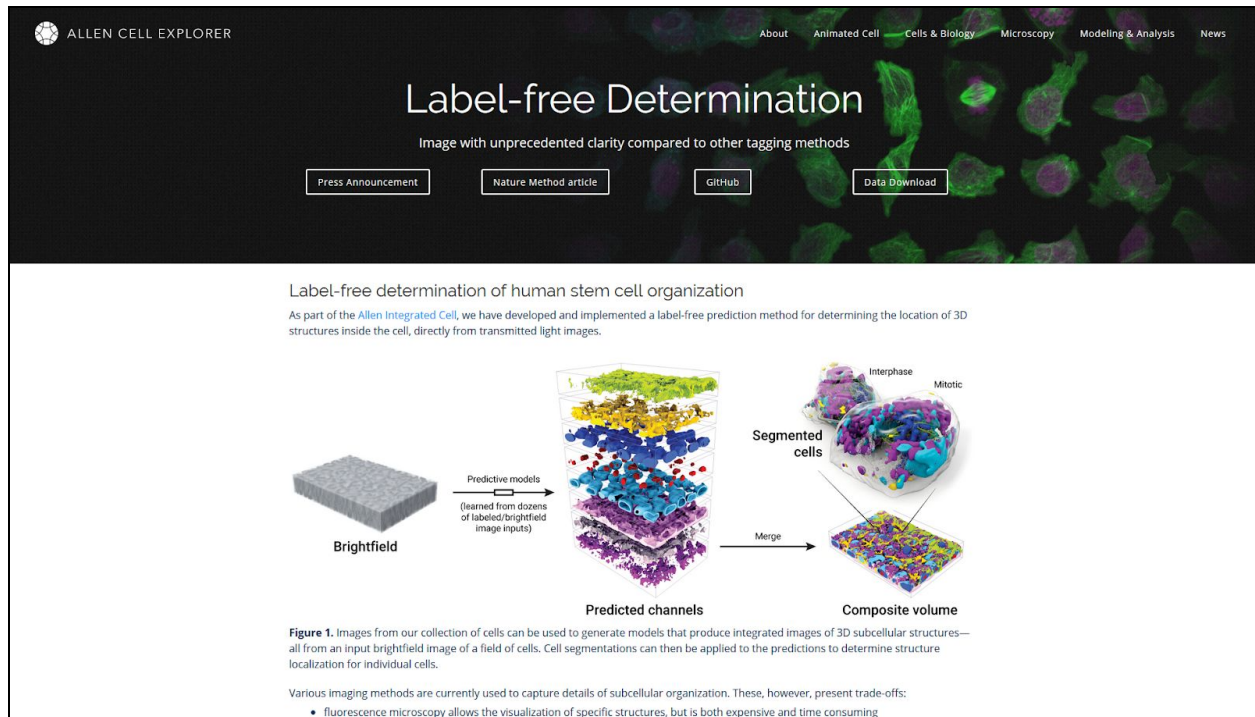
Explain *in your own words* the process the Allen Institute used to create a label-free prediction method for determining the location of 3D structures inside the cell, directly from transmitted light images.

STOP! Find two or three peers and compare your findings.

Part VI. Does this make sense Dr. G?

You have learned a lot from helping Dr. G's students and visiting the Allen Cell Explorer. However, you still have to report back to Dr. G.

Questions

1. Think about the cells you viewed, what you learned about the ER, and the *Delftia* spp. experiment. *Do you think this high-content imaging approach will help Dr. G's students? Why or why not? Think about the knowledge gained from this approach and the limitations of the dataset.*

2. If you could work with researchers at the Allen Institute, *what would you do next? Design a future experiment to build on your findings.*

Reflection

What was the most memorable concept or skill you learned from this case study?

What are you left wondering about?

Teaching Notes

Introduction & Background

This case was developed for upper-level undergraduates and first and second-year graduate students with one semester of molecular biology training. The course this case study was designed for is an 8-week module focusing on high-throughput approaches. Nevertheless, this case can be adapted for cell biology courses and upper-level cancer biology classes to emphasize the use of novel imaging and high-throughput methods to study cellular morphology and function. The scenario described in this case engages students in a realistic and relatable application for learning about the structure of the endoplasmic reticulum (ER). A scientist and his students are screening drug libraries to find novel anti-infectives against an emerging opportunistic pathogen, *Delftia acidovorans*. They identify a hit, a promising compound with drug-like properties, and test it on mammalian cells (Chinese Hamster Ovary, CHO) to determine if the drug is cytotoxic. The drug does not seem to be cytotoxic to CHO cells, but in the process, the students notice that a stain specific for the ER appears to indicate more and varied staining in cells treated with the drug. This leads the scientist and his students to learn about how the ER can be visualized and the tools the Allen Institute has developed to stain organelles using a high-throughput approach. In the end, we learn that the textbook visual of the intestine-like ER is not always what we *see*!

- The ER
  - History, shape, staining
- Methods for staining
- Allen Institute approach

Classroom Management

- Pre-activity preparation
- Steps in class
- Group work
- Individual reflection
- Computer considerations

Blocks of Analysis

References

Here

Acknowledgements

Guidelines I give graduate students in the High-throughput Discovery course for the case study design final project.

Introduction/Background. This section tells us what level of students you developed the case for and what other courses it might be appropriate for. It can also include a two- to three-sentence synopsis or summary of the storyline of your case. This section should also tell us what, if any, *prerequisite knowledge* or background the students should have before undertaking this case and where in the sequence of the course/class/subject area you would teach it. As part of this section, you should include a bulleted list of your **teaching objectives** for the case, being as specific as possible. *Background should be detailed enough to allow others to teach the cases!*

Classroom Management. Be as detailed as possible, telling us how you teach this class, including any variations that might occur for other audiences. What instructions are the students given, what are their tasks, in what sequential order? How long does each part of the case or case activity take, approximately? Do students get the entire case all at once or piecemeal? Do the students discuss the questions in groups? Is there a general class discussion as well? Give us timing for each activity and sequence. In this section, tell us also if you developed any written assignments that go along with and/or any follow-up assignments for the case and how you might *assess* the students' case work.

Blocks of Analysis. This is where you would give us some detail about the scientific concepts, principles, issues, topics, approaches, etc. of the case in blocks. Provide enough information for someone considering teaching the case to develop background knowledge in each major content area of the case. You must use evidence and data from *your references*.

References. Provide a formatted list of the sources you consulted in developing your cases as well as recommended further reading if appropriate. For our class this must include a minimum of **5 primary** literature references. You should include an annotated bibliography for each reference.

Image Sources

https://upload.wikimedia.org/wikipedia/commons/0/0d/0312_Animal_Cell_and_Components.jpg

http://labs.biology.ucsd.edu/hampton/PDF%27s/dynamicER.pdf
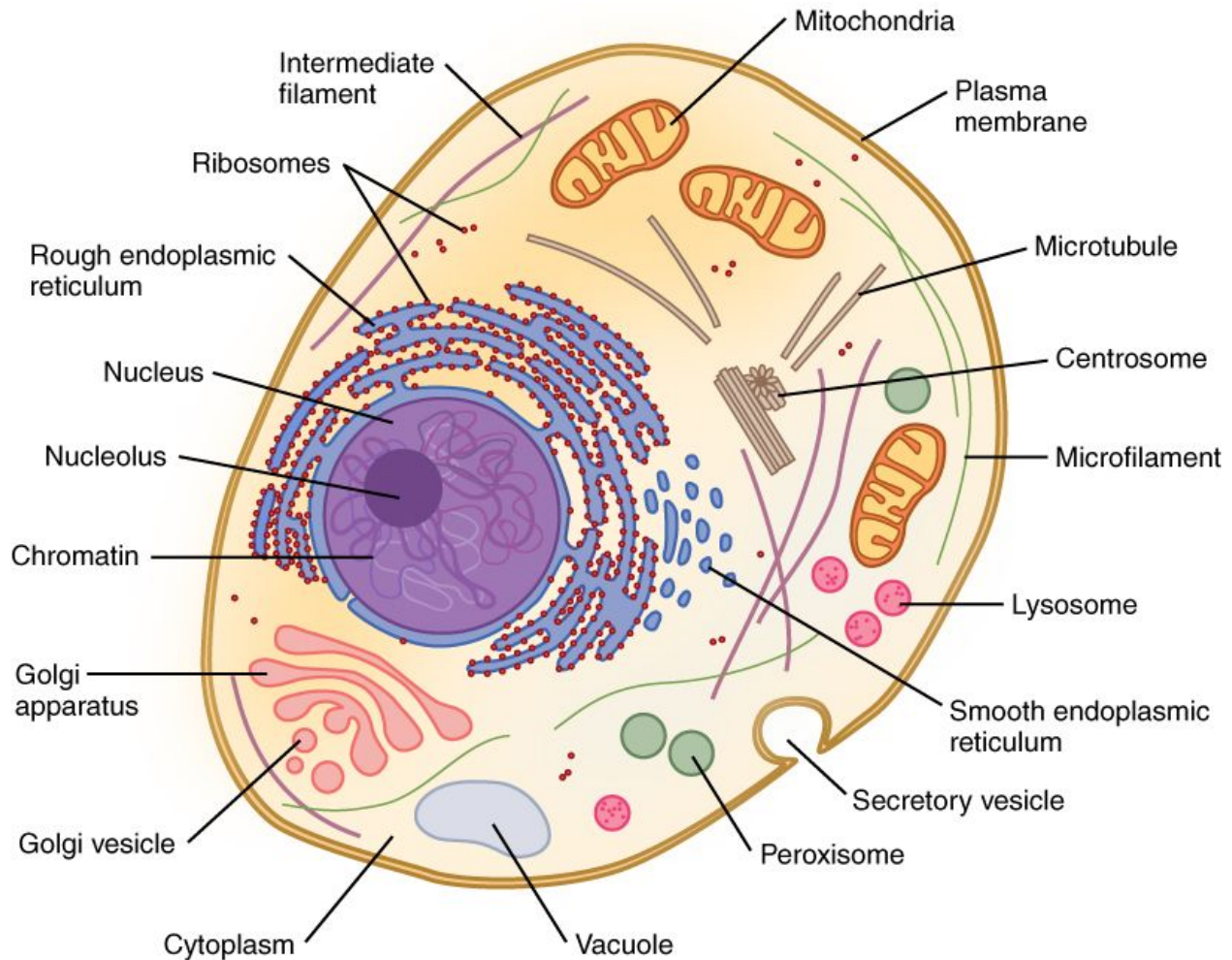
Tips from NCCSTS Conference:

- ✓ Personal
- ○ Not too many adverbs and adjectives
- ○ Must not be able to answer the questions without the storyline