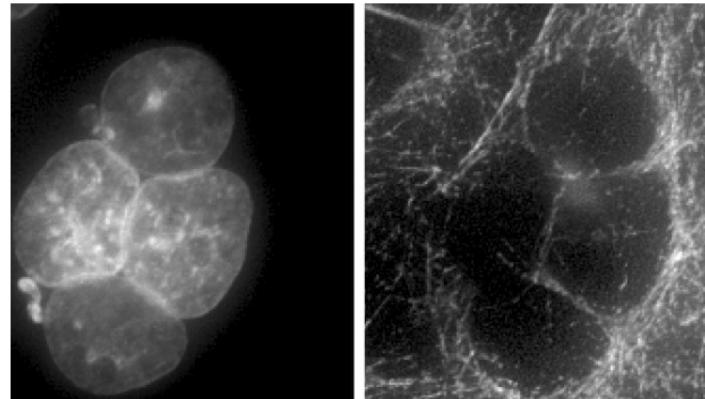


# Use cases: what to submit

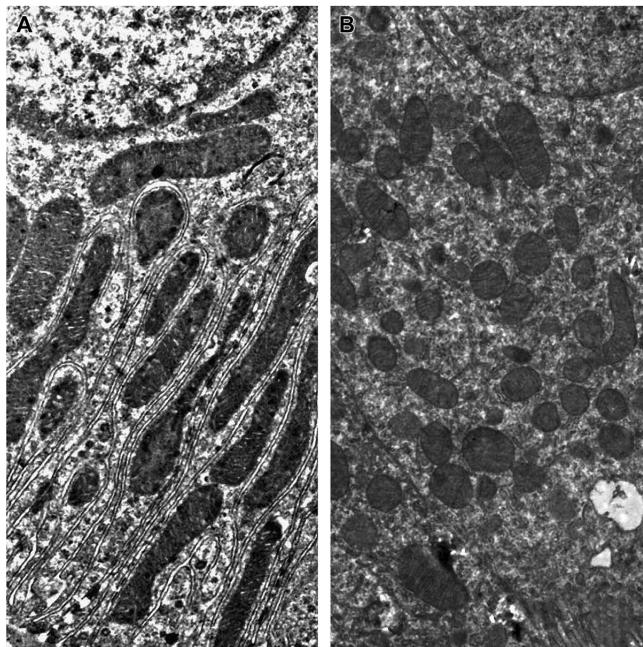
What is a question? A question includes an image (or set of images), some context information in text, and a question in text. Then you also provide the answer that you'd hope to get:

<b>Context:</b> I screened my cells with several new drugs, and one of them shows these results. The images show different channels of the same images. The first is nucleus, the second is cytoskeleton.
<b>Question:</b> What cellular pathways are affected?
<b>Answer:</b> The nuclei are clumped together in a single cell: they are multinucleated. This could be due to disrupted cell division, potentially cytokinesis.



You might have multiple questions that follow on from each other in a natural way:

<b>Context:</b> These are images are transmission electron microscopy of retinal ganglion neurons in culture at baseline (A, left) vs. treated with experimental compound BHO8651 (B, right).
<b>Question:</b> Describe what's in the two images.
<b>Answer:</b> Left: well-organized mitochondria of relatively uniform size and diameter Right: smaller, fragmented, and disorganized mitochondria
<b>Question:</b> What biological processes or organelles could be disrupted?



These kinds of 'multi-question' conversations are encouraged. This would count as "2 questions" when counting contributions for authorship.

## No need to repeat yourself in conversation

In the above figure, notice how the second question might rely on some information from the first question? So if that second question were a standalone question, it might make sense to add a bit more text in the question "what biological processes could be disrupted?".

But since it's a follow-up question, don't worry about it. The short question is fine, and you can assume that chatbot being asked this question would remember what it was told before.

### Let the model see for itself

The 'context' and the 'question' text should NOT describe the image features in detail. In this example, while it's okay to say "this is an EM image", it is NOT okay to say "the left image has mitochondria that is well-organized." Let the model see the image for itself.

### You don't need to DEFINITELY know the answer

It's about suggesting reasonable answers based on the image. For example, here are two reasonable but different answers to the same question:

#### Context:

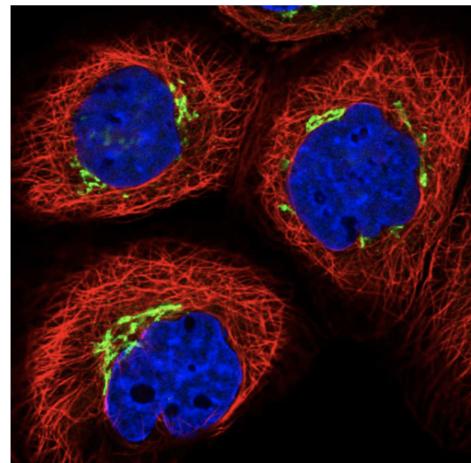
We have a newly discovered protein labeled with GFP in the green channel. The nucleus is the blue channel, and microtubules is red channel.

#### Question:

Why is the green protein concentrating around the nucleus like this?

#### Correct answer:

The protein could be targeted to the Golgi apparatus for post-translational modification



#### Context:

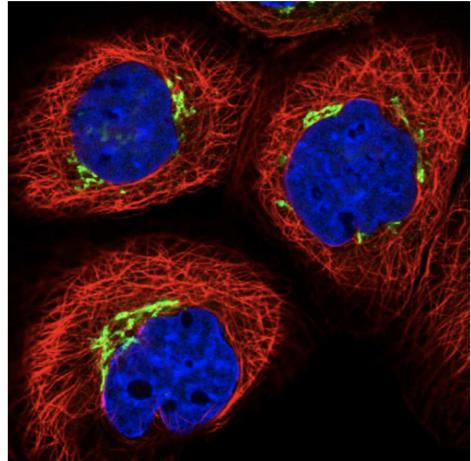
We have a newly discovered protein labeled with GFP in the green channel. The nucleus is the blue channel, and microtubules is red channel.

#### Question:

Why is the green protein concentrating around the nucleus like this?

#### Correct answer:

The new protein could be in the Golgi for sorting/modification and eventual secretion outside of the cell.



This is common because often you won't have enough information to make a final conclusion. The idea is that if you gave this image to an AI Assistant after collecting the image, then you want it to suggest hypotheses that an expert cell biologist might give. After getting these suggestions, you could then do more experiments to verify these hypotheses.

You can submit multiple answers to the same question if they're reasonable.

In the image above, there are 2 reasonable answers to the same question. When this happens, just submit them as two separate questions, where the 'question text' is the same, but the 'answer text' is different. This counts as 2 questions to your total count.

HOWEVER, two caveats:

- MAXIMUM 3 submissions (per question) that come from the same question.
- Do NOT submit answers that are technically possible but very unlikely. We only want reasonable h

## Use cases

The questions fall under 3 broad categories. They are:

- What is unusual or interesting in these images? And why is it unusual or interesting?
- Why am I seeing this? What are the possible cellular pathways causing it?
- How can I verify my hypothesis in an experiment?

We'll now give some examples of each.

### Use case 1: What is unusual or interesting in these images? And why is it unusual or interesting?

The questions are about identifying the important features of an image, and interpreting their significance.

#### Typical questions

- What is this structure and why is it significant in this context?
- Is this feature normal or abnormal in the context of this experiment on this cell line? Why is it abnormal, and how can you tell?
- Why are these fluorescent markers distributed in this pattern?
- (given 2 images) Why does this cell at the group of cells appear different from a typical cell under similar conditions?

#### 'Whats and whys'

In this category, questions can often be split into a 'what' followed by a 'why'. That's fine, and you can submit them as 2 separate questions (and that will be counted as 2 questions when counting total question contributions). However, IF you submit a what, it must be paired with a 'why' question as well. 'What' questions by themselves are not interesting enough.

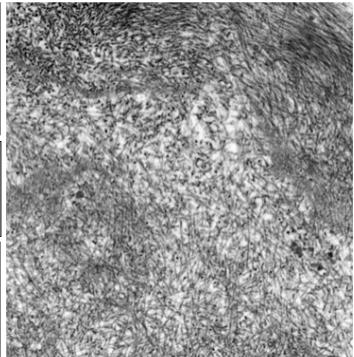
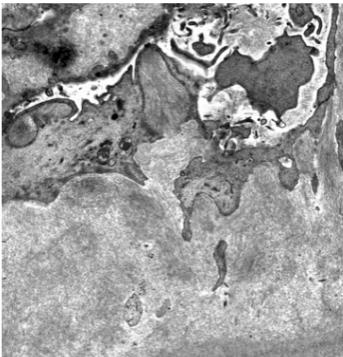
#### Examples

Example 1.1

**Context:**  
We have a mouse model of <disease>. We are performing electron microscopy to evaluate for defects in blood vessel. Image 2 is a low power view.

**Question:**  
What is in these images, focusing on what is unusual?  
If it is unusual, then why is it unusual?

**Answer:**  
There seems to be an accumulation of disorganized thin fibrils with uniform width/thickness. This is uncommon for blood vessels, which typically demonstrate ...

Ex1.2

**Context:**  
Transmission electron microscopy of Jurkat cell at high magnification.

**Question:**  
What is this structure and why is it important?

**Answer:**  
There are a pair of linear, darkly stained structures with less dense material inside. Depending on the cell and magnification, this could represent a centriole with a pair of dense bodies called centrioles. The centrioles are usually cylindrical and consist of a distinct pattern of nine triplets of microtubules arranged in a circle. However, this view could show an oblique section. The centrosome serves as the main microtubule organizing center in many eukaryotic cells.

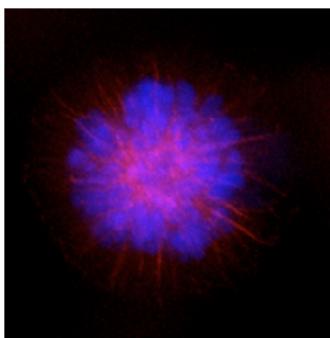


Ex 1.3

**Context:**  
Epifluorescence image of HeLa cells treated with micro-RNA 386 (miR-386).

**Question:**  
What is abnormal about this cell?

**Answer:**  
The miRNA treated cell shows a monopolar spindle compared to what a normal metaphase cell would show.



## Use case 2. Why am I seeing this? What are the possible mechanisms that could cause it?

Create questions that link what you see in the image to some underlying mechanism. E.g. the underlying biological processes, pathways, genes, or conditions. Understanding that mechanism requires good biological knowledge.

## Typical questions

- **What** cellular pathways might be disrupted based on the observed abnormalities in the image?
- **Why** is this cellular pathway or protein disrupted?
- **Why** do all cells not express this particular protein when treated with X?
- **Why** is the protein expressed in multiple subcellular locations?
- Comparative Question: Why are these two tissue samples showing different marker expression levels after similar treatments?

## Use case 2 questions might follow on from use case 1 questions

So for example 2.1 below, we ask about a cellular pathway causing multinucleated cells. But you could create a separate question asking "what is unusual about this image?".

Please do this! It's much more interesting to have multiple questions. When you do that submit them as separate questions.

## Examples

Ex 2.1

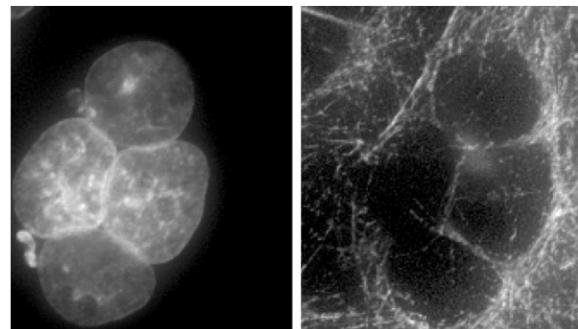
### Question:

Could you suggest an experiment to test your hypothesis about this image?

### Answer:

To test whether this is due to cytokinesis, you could do # to inhibit cytokinesis. You would expect to see #.

Proteins involved in cytokinesis include #, so your drug may alter their function...



Ex 2.2 is an example of "multiple valid answers", so you could submit these 2 answers (but no more than 3).

**Context:**  
Single plane of Cryo-Electron Tomography of iPSC-derived neuron expressing mutant Huntington.

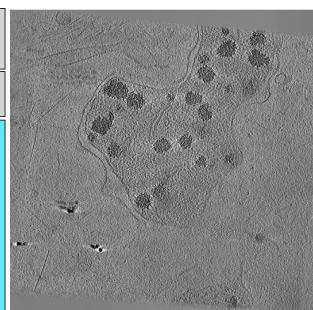
**Question:**  
Why are there dark spots inside the mitochondria?

**Answer:**  
Multiple possible answers. One is Mitochondrial RNA Granules (MRGs): MRGs are involved in the regulation of mitochondrial RNA processing and translation. They contain proteins and RNAs that help manage the synthesis of mitochondrial proteins essential for mitochondrial function. In the context of HD and other neurodegenerative diseases, MRGs might become more prominent or altered in size and number, reflecting changes in mitochondrial metabolic demands or stress responses.

**Context:**  
Single plane of Cryo-Electron Tomography of iPSC-derived neuron expressing mutant Huntington.

**Question:**  
Why are there dark spots inside the mitochondria?

**Answer:**  
Multiple possible answers. One is protein Aggregates: Proteins that are misfolded or damaged may aggregate inside mitochondria, especially under pathological conditions like HD where protein homeostasis is disrupted. This can result from the direct effects of mutant proteins or from cellular responses to stress. Such aggregates can appear as dark spots in electron microscopy due to their dense composition, which absorbs more electrons compared to the surrounding mitochondrial matrix.



Ex 2.3

**Context:**

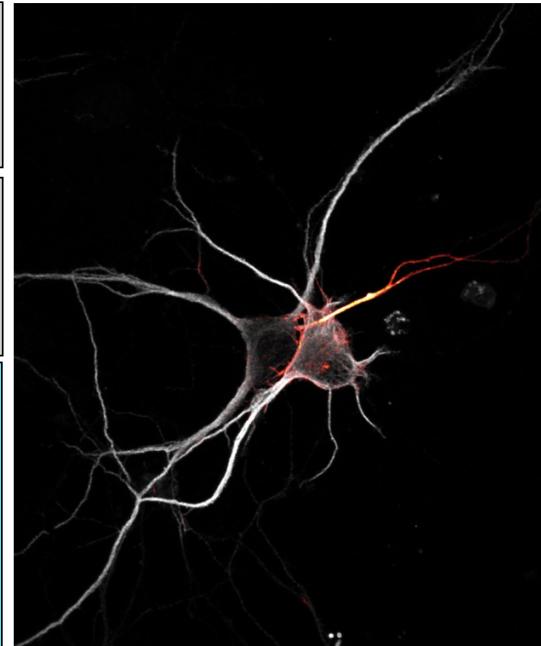
Fluorescence microscopy image of cultured mouse neurons. Neurons were stained with anti-MAP2 (gray) and anti-Ankyrin F antibodies (orange).

**Question:**

Based on the spatial distribution of MAP2 and Ankyrin, how might they interact to modulate the functional dynamics of neurons?

**Answer:**

Ankyrin F anchors integral membrane proteins to the cytoskeleton, and there is increased Ankyrin F (orange) staining in the axon initial segment (AIS) whereas the MAP2 (gray) staining is more prominent in the dendritic processes. In the AIS, Ankyrin F may stabilize voltage-gated ion channels which are concentrated there for action potential initiation.



Ex 2.4 is an example of a hard question where the answer has been demonstrated in only one prior paper (to our knowledge).

**Context:**

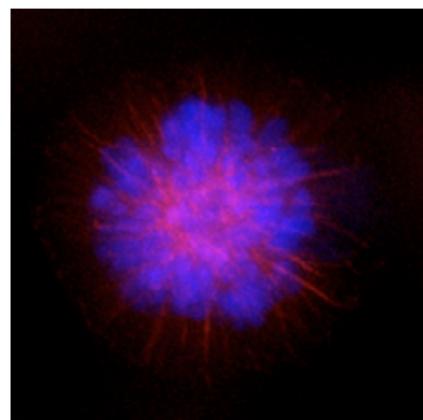
Epifluorescence image of HeLa cells treated with micro-RNA 386 (miR-386).

**Question:**

Why do the cells look like this?

**Answer:**

The miRNA treated cell shows a monopolar spindle compared to what a normal metaphase cell would show. Some prior works have shown that knockout of PLK4, Cep135, PCM1, or centriolar proteins results in a monopolar spindle phenotype as compared with other possible phenotypes such as a multipolar spindle, metaphase arrest, or apoptosis.



Ex 2.5

**Context:**  
We are studying a new open reading frame orf84331 that encodes a previously uncharacterized protein. We knocked down orf84331 in HeLa cells and see the findings in this image (WT vs. KD).

**Question:**  
Based on the image, how would you interpret the function of orf84331?

**Answer:**  
The knockdown image shows a multipolar spindle phenotype, indicating disrupted cell division. This could be caused by defects in spindle assembly or stability, centrosome duplication, previous failure in cytokinesis resulting in extra centrosomes and DNA.

Ex 2.6

**Context:**  
We have a newly discovered protein labeled with GFP in the green channel. The nucleus is the blue channel, and microtubules is red channel.

**Question:**  
Why is the green protein concentrating around the nucleus like this?

**Correct answer:**  
The protein could be targeted to the Golgi apparatus for post-translational modification

**Context:**  
We have a newly discovered protein labeled with GFP in the green channel. The nucleus is the blue channel, and microtubules is red channel.

**Question:**  
Why is the green protein concentrating around the nucleus like this?

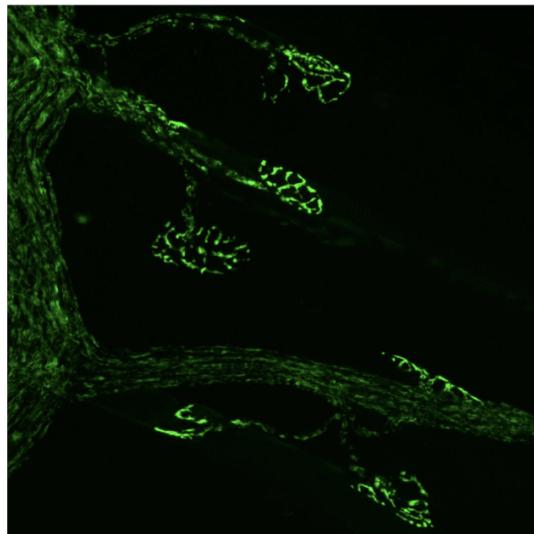
**Correct answer:**  
The new protein could be in the Golgi for sorting/modification and eventual secretion outside of the cell.

Ex 2.7

**Context:**  
Fluorescence microscopy image of a section of fixed tissue of mouse muscle in a ChAT-Cre x Mito-GFP mouse line.

**Question:**  
How does the arrangement of mitochondria in these cholinergic neurons affect the efficiency of synaptic transmission at the neuromuscular junctions?

**Answer:**  
The mitochondria appear to be densely clustered near the nerve terminals at the synaptic bouton with a slightly lower intensity along the axon shaft. Synaptic transmission requires significant energy to facilitate the rapid firing and recycling of synaptic vesicles. The clustering of mitochondria at the synaptic boutons ensures an immediate and localized supply of ATP, essential for the active transport mechanisms that maintain neurotransmitter release and uptake. The concentration of mitochondria at the presynaptic terminal also helps buffer calcium ions, which must be quickly removed from the synaptic environment after each neurotransmission event to prevent toxic effects and prepare the neuron for subsequent signals.



## Use case 3: What should we do next and why?

(Alt: evaluate hypotheses)

### Typical questions

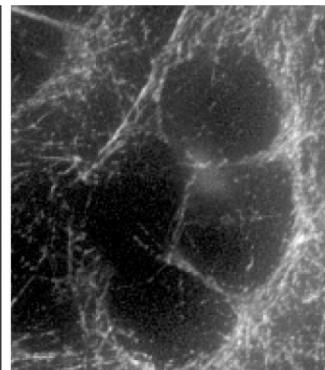
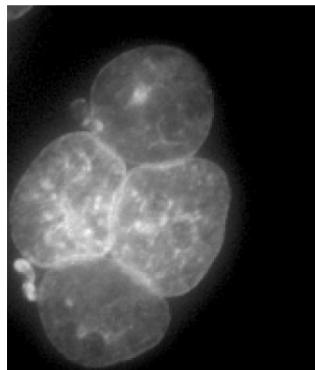
- Given the observed progression of cell behavior in these images, what might be the next step in this experiment?
- Why** would this be the best next step as opposed to an alternative next experiment?
- Why** might we expect different outcomes if we alter the current experimental conditions?
- Based on this imaging data, what hypotheses can we form about the cell cycle progression?
- Why** is the cell arrested/stopped?
- Comparative Question: Given the differences observed in these images over time, what can we infer about the treatment's effectiveness?
- Why** is the treatment effective/ or not effective?

### Examples

Ex 3.1 this question is a follow up to ex 2.1, so the question does NOT need to repeat the hypothesis that the multinucleated cells are due to "disrupted cell division, potentially cytokinesis", although the answer could say that.

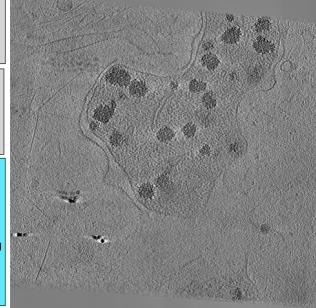
**Question:**  
Could you suggest an experiment to test your hypothesis about this image?

**Answer:**  
The multinucleated cells in the image could be due to disrupted cell division, potentially cytokinesis. To test this hypothesis, you could do # to inhibit cytokinesis. Proteins involved in cytokinesis include #, so your drug may alter their function...



Ex 3.2 is a follow up question to 3.3.

<b>Context:</b> Single plane of Cryo-Electron Tomography of iPSC-derived neuron expressing mutant Huntington.	<b>Context:</b> Single plane of Cryo-Electron Tomography of iPSC-derived neuron expressing mutant Huntington.
<b>Question:</b> I think the dark spots could be due to MRGs or protein aggregates. How can I determine the composition of the granules to distinguish them?	<b>Question:</b> I think the dark spots could be due to MRGs or protein aggregates. How can I determine the composition of the granules to distinguish them?
<b>Answer:</b> One approach is microscopy imaging, for example correlative light and electron microscopy (CLEM): This technique involves labelling specific molecules with fluorescent tags and then imaging the same region with electron microscopy to correlate the visible signals with ultrastructural details. This can confirm the presence of MRGs if the fluorescent tags are specific to mitochondrial RNA-binding proteins.	<b>Answer:</b> One approach is biochemical analysis, for example Mass Spectrometry: Analyze the protein content of isolated mitochondria to identify the molecular composition of the granules. This can help determine whether the granules are enriched in RNA-binding proteins (indicating MRGs) or in proteins commonly associated with aggregates.



## There are some use cases that we did NOT include

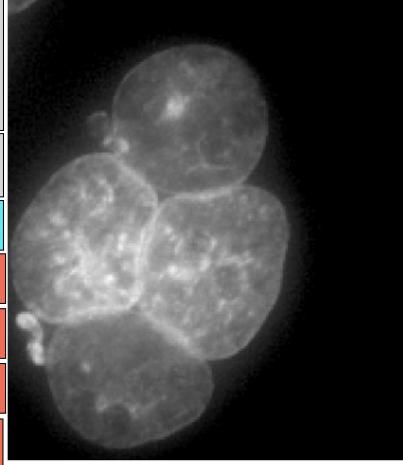
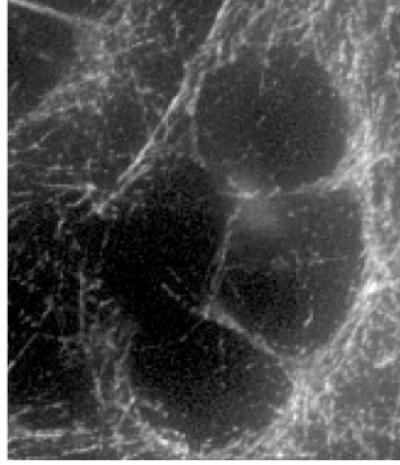
Our collaborators suggested a lot of very interesting use cases that we had to exclude for two reasons. First, we wanted to focus on questions that require image interpretation. Second, including more categories would make the scale too big for this first project. However we hope to explore these ideas in the future. So you should NOT submit questions under these use cases.

- Brainstorming new ideas without the use of images to guide the conversation.
- Experimental design, like "I have these microscopes and want to image X, what antibody stain should I use?"
- Assessing image quality, like "is this image in focus? If not, should I collect it again, or can I post-process it? If I can post-process it, can you provide code?"
- Interpreting scientific charts.
- Linking to relevant scientific articles.
- Recommending how to create scientific figures.

## Extra requirement: Recommend one "incorrect but reasonable" answer

For each question, we want you to provide one "wrong answer" to your question.

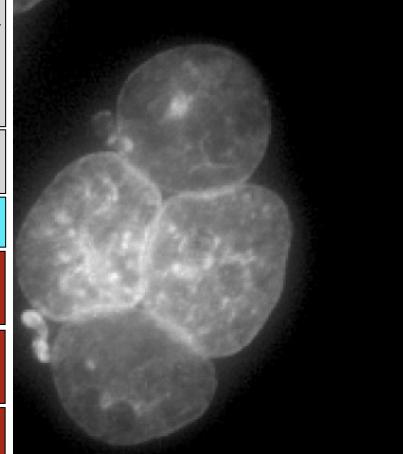
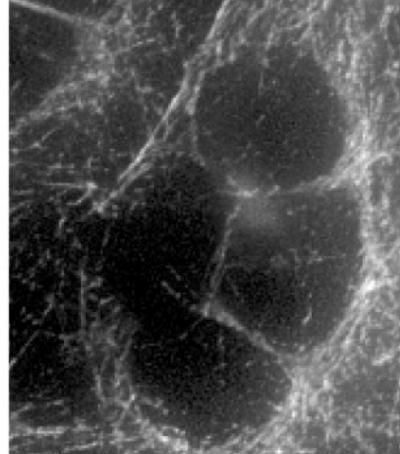
Why are we asking this? Because a popular way to test models like ChatGPT is to ask multiple-choice questions. The 'wrong' choices should seem reasonable. For example, on the left is the right answer above some reasonable wrong answers:

<b>Context:</b> I screened my cells with ..., and one drug consistently shows this phenotype. The image pair shows the same cell in different channels labeling the nucleus and the microtubules.		
<b>Question:</b> What's unusual about this image		
<b>Correct answer:</b> The cell is multinucleated		
<b>Incorrect but reasonable answer:</b> The microtubules are depolymerised		
<b>Incorrect but reasonable answer:</b> The cell is undergoing apoptosis		
<b>Incorrect but reasonable answer:</b> The cells are unusually large		
<b>Incorrect but reasonable answer:</b> The cell is arrested at the metaphase spindle stage.		
<b>Incorrect but reasonable answer:</b> The microtubules are depolymerized causing nuclear aggregation.		

What makes an answer reasonable?

- Suppose you knew the 'context' about the image — in this case, we have fluorescence microscopy stained with nucleus and cytoskeleton — but you couldn't actually see the image. What would be a reasonable guess to make?

On the other hand, you should NOT provide a completely silly answer, for example the red boxes below:

<b>Context:</b> I screened my cells with ..., and one drug consistently shows this phenotype. The image pair shows the same cell in different channels labeling the nucleus and the microtubules.		
<b>Question:</b> What's unusual about this image		
<b>Correct answer:</b> The cell is multinucleated		
<b>Incorrect and silly answer:</b> Golgi apparatus in this cell is directly connected to the central vacuole.		
<b>Incorrect and silly answer:</b> This cell appears to be undergoing mitosis and meiosis simultaneously		
<b>Incorrect and silly answer:</b> The microtubules are transporting the nucleus for exocytosis.		
<b>Incorrect and silly answer:</b> The nuclei shape looks like a giraffe playing ping pong.		

Without seeing the image — but reading the context+question, and knowing about biology — an expert would know that none of these are possible answers. For example the first answer is bad because we know from the 'context' that the Golgi isn't tagged.