# Animation Feedback

September 30, 2014

**Western blotting animation**

0-0:25 - the overview of the western blotting procedure is very fast. Highlighting just a few key places/spots in the procedure rather than a whole overview of the complete procedure would be more effective.

0:22 - Label or indicate which are the proteins and which are the organelles so that students know where to focus moving forward.

0:25 - Rather than starting with the cell and zooming in to see the proteins, it might be nice to start with the image of proteins you have and then zoom out to the cell since the narration moves in that direction as well.

0:49- Simplify this section (showing lysis) - both in the animation and script —> will talk about it.
**To simplify and provide Janet with more directive visuals.**

1:17 - Write out 'Endoplasmic Reticulum (ER)' in the label

1:38 - Centrifuge is missing the top (maybe dependent on how much time you have)

1:44 - Protein lysate supernatant is usually more clear than cloudy.

2:01-2:04 - Can we show the protein moving around rather than being static in its folded state?

2:05-2:14 - The message here is that enzymes can degrade proteins when the cellular environment is disrupted, but the animation shows unfolding. Perhaps we can show both at the same time?

2:14-2:23 - Show side by side how the room temp and cold temp scenarios. Refolding the protein after unfolding it is going to be confusing to the students.

2:29 - "Plasma membrane" is cut off on the left side"

2:30-2:45 - Show all of the lysates in the same color (mostly transparent), and then zoom in, using a circle for example, to indicate how the protein composition is different in the different lysates through the use of color. This way we communicate visually to the students that the 3 different lysate types appear the same but that they contain different types of proteins.

2:38 - Label the nucleus

2:39 - Label cytoplasm

3:06 - Label one of the primary antibodies

3:09 - Label one of the secondary antibodies

3:21 - Insert a 1 second pause that they students can absorb everything that has just been narrated/mentioned.

3:22 - Left indent the Title and parts of the western blot. This can be placed within the right portion of the animation where there is space. Take out the white band that separates the title from the rest of the animation.

3:26 - Show the bubbles coming up from the bottom when the charge starts up rather than have the bubbles appear everywhere within the buffer. Bubbles are usually finer/smaller.

3:28-3:31 - As the loading dye front begins to migrate through you no longer see a separation of the samples within the gel. This gel is also missing a ladder.

3:33 - It's hard to see the transfer sandwich with the semi-transparent banner at the top of the screen.

3:34-3:42 - I think we need a different type of visual here because the transfer process goes by too fast. Perhaps illustrating pulling apart the membrane from the gel once it has been transferred - students see the transfer of the ladder from the gel to the membrane and subsequently antibodies binding to sites within the membrane.

3:42 - Show the "1. Polyacrylamide Gel Electrophoresis (PAGE)" label to go along with the audio that this is showing only the first half now.

3:50 - Several points:

- The tip must go into the well when loading samples into a well (and should stay in the well while the entire sample is extracted from the tip).
- Would like to see the sample "fall" into the well in a more realistic manner. See one example here.
- Also, the color of the samples is a more vivid blue (like shown at 7:25). What does the whitish banner underneath the wells represent?

4:05 - Center “bis-acrymladie” label on the molecule.

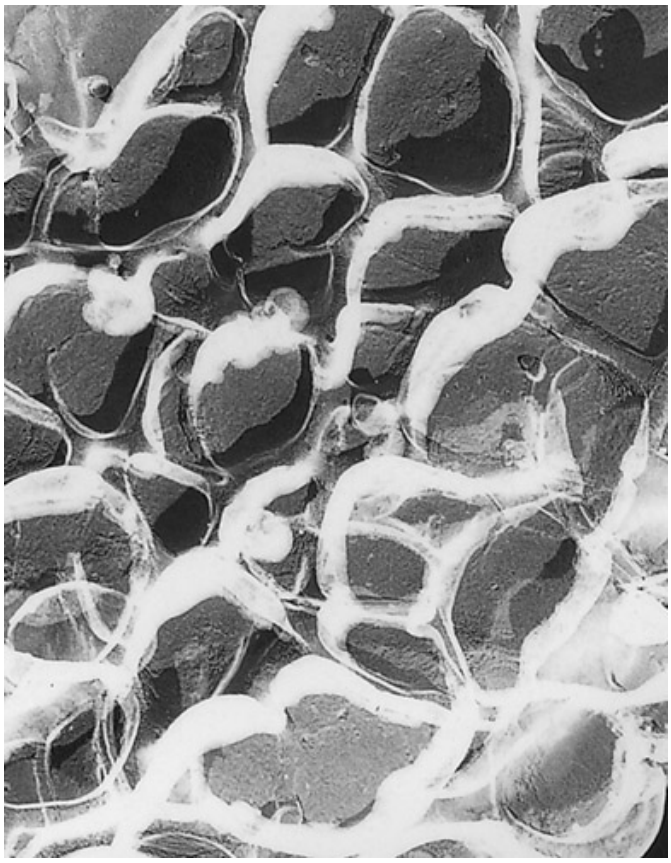
4:05-4:10 - Remove this animation and instead provide more time for the “pouring” of the gel into the cassette.

4:17 - The way that the gel is poured is not accurate - there will be a top gel layer (made of a different mixture) that will contain the wells. Perhaps to provide more time for all of this, show the cassette already assembled, rotate it and label the two gel plates and then rotate back to starting position to illiterate pouring.

4:21 - Add a pause.

4:24 - I think the long chains should look different - not are rigid as shown. I am in favor of showing them as more flexible chains.

4:27: Why are they long straight lines within the gel illustration? Also are the “wholes” similar in size? The whole are probably more heterogenous in size like in this electron micrograph of a polyacrylamide gel:

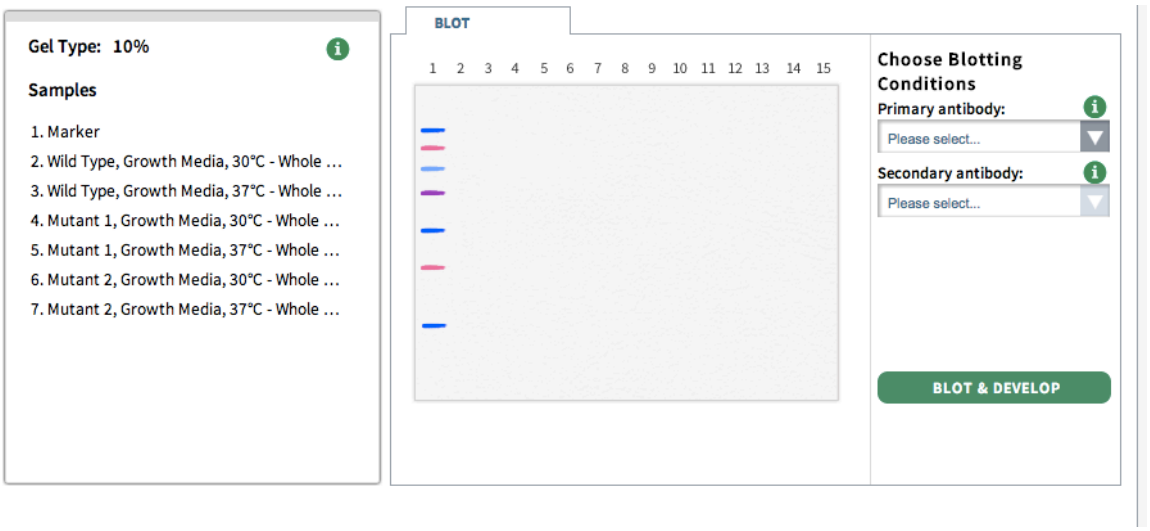


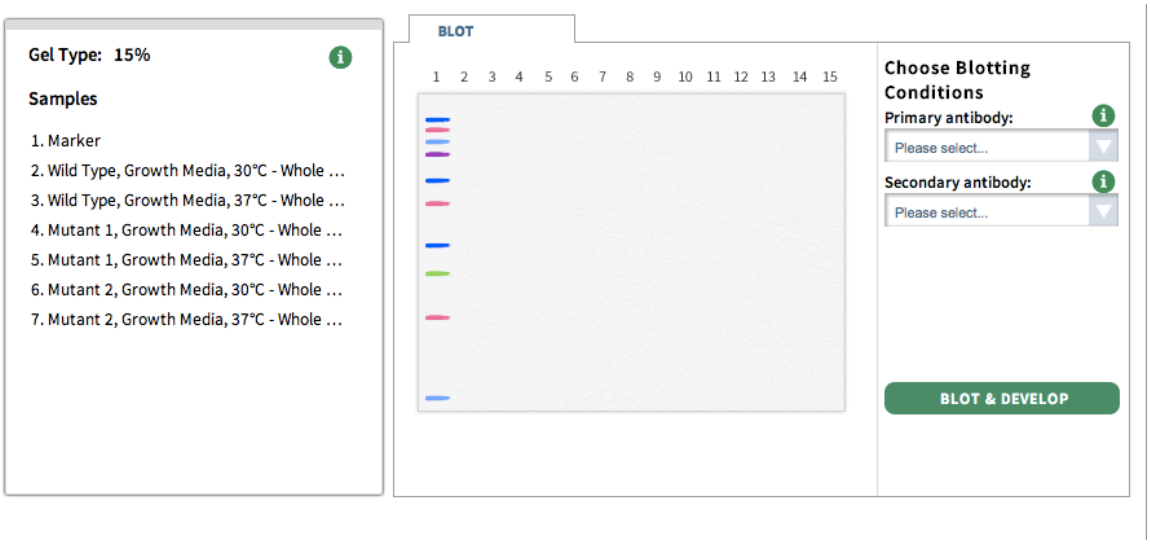
4:33-4:41 - It is really hard to see how the proteins are migrating through the gel. This could be because how the proteins are oriented within the animation and the scale of the whole vs. unfolded proteins. It might also help to just look at that within the animation and not show the actual gel.

4:41 - The gels should be shown as fairly clear, instead of having a purplish tinge to them. It would be nice if samples could be added to the wells prior to moving the colorful bands down the gel.

4:55 - See prior comment in 4:27.

5:05 - The high molecular weight proteins should not move as far through the 15% gel. Both the light and dark blue bands should be equal or above the light blue band in the 7.5% gel. Here are two examples from StarCellBio with 10% and 15%.




5:17 - The buffer inside the chamber looks like it has streaks. Is this intentional?

5:26-5:44 - Left indent “Common forms of PAGE” and subsequent types and place to the right within the animation window.

5:59 - I am confused about why the same protein will have different overall charges. Maybe drawing a very similar protein with a different charge might be better.

5:59-6:25 - The arrows within the diagram are confusing. Makes it seem like the proteins are migrating towards the other type of protein. Perhaps having slow and fast without the arrows will suffice with a line in the table to divide the two. The other option is to visually indicate a continuum  but with a different visual than an arrow.

6:25 - Would like to see the proteins move through the holes/pores in the gel more clearly - comment in the same vane as 4:33.

6:35-7:01 - I would like to see the proteins for a denatured gel represented in the same manner as the native gels, using the same color. Perhaps one thing would be two show the two proteins in the “folded” versus “unfolded” state, with examples of chemical modification in one of them and changes in protein expression

in the other. The unfolded animation can then perhaps be brought in when SDS begins to being mentioned in 6:48.

6:57 - Show "Mass" at the top in a similar fashion as done previously with the native gels to indicate that in denaturing gels, proteins are only separated by mass. Again, show that smaller molecular weight proteins will move through the gel more quickly.
**Look at the script for this part and add verbiage about migration.**

7:18 - Temperature should be 100 C.

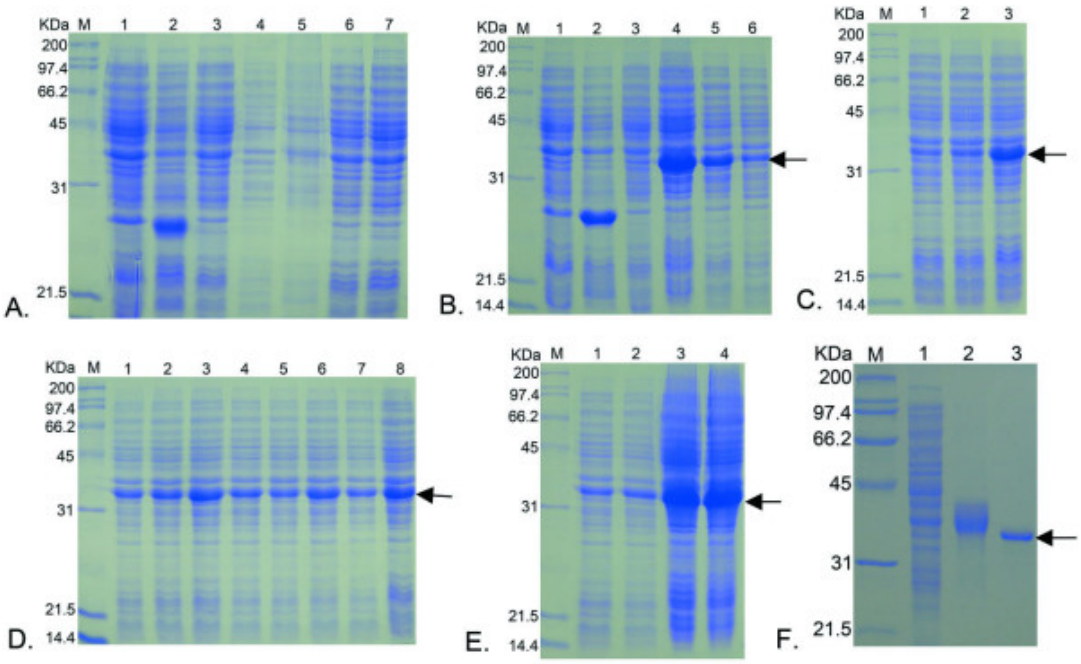
7:51 - Same comments about the samples and the sample loading in the gel as before.

8:06 - Show a protein ladder in one lane (far left lane). The protein ladder will have multiple colorful bands and these bands will separate as the gel is running. Also the tracking dye will appear as a single blue band that spans across the gel instead of looking like its in separate wells as it diffuses horizontally as the dye tracks down the gel vertically. Make bubbles more realistic (same comment as before).

8:18 - The ladder is shown now. I think it should be shown earlier as well (but can be explained now), pointing to it with a label.

8:51 - The staining should just show the gel being stained and the background within the animation should stay the same (no change in color). Show one gel stained with commassie and a film side by side.

9:00 - Make coomassie blue staining more realistic with lots more proteins shown, differences in lanes, etc. Like these:

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August 28, 2014

**Microscopy (basic) Animation**

2:40ish - media for cells growing in tissue culture should look more transparent. It looks opaque in the dish as the dish is moving onto the microscope stage, but then looks completely transparent once the dish is on the stage. The media should also be a more vivid orange/red color. - Janet will make the media color more vivid (red/pink) and look more transparent.

4:00ish - The color of the biotin on the secondary antibody should be a color other than blue so it is not confused with the hematoxylin staining in the image on the right. - Janet will change to white or gray/black

**Microscopy (fluorescence) Animation**
What color should the laser light beam be that is emitted from the source? Is yellow the correct color? A rainbow of light? or pick a specific wavelength of laser beam such as 488 or 595 nm? - Janet will change this to cyan/turquoise

**Western blotting Animation**
0:06 - We would prefer a more realistic representation of a protein, perhaps a more realistic cartoon version of proteins.

We will find structures for a good protein example w/ related modifications and protein partners.
We will find additional protein structures to show

0:06-0:13. Long period of voice recording without animation and it is not clear that the visual you see on the screen is a protein until 0:13. Perhaps stitching together images from the rest of the animation and placing them here, in particular those that demonstrate the mechanics of running a western, would be good. (LA)

0:24 - Make the current 2-D representation of the cell a little more realistic with some proteins on the cell membrane and possibly also adding in cytoskeleton - as in the image on the homepage of the Cell Image Library.

0:29 - The western gel should look more transparent in nature and the gel wells should appear more floppy and less rigid. We have attached the image that our graphic designer drew for us as a reference.

0:29 - We would also like to use a multi-color protein marker like the one we use in StarCellBio. We have attached an example of the marker.

0:30 - Should we show actual western blot film rather than a gel stained with Coomassie? If so, we can show the representative bands of the cartoon protein by outlining them, etc.

0:41 - As we discussed today, we decided that we would leave it as is for now, but consider this if we have time at a later point. Show the disruption of the lipid bilayer by detergents.

0:44 - We would like to show that proteins as well as the detergent are moving. But we discussed today that showing this is very complicated as the entire cell is dynamic. We either need to show movement for everything or nothing (including cell + detergent) with the easier solution being not moving anything since this is a lower priority than showing the western blotting apparatus and proteins, etc.

0:52 - Label plant cell (and animal cells?) instead of just using color. In the plant cell, label the plasma membrane and the cell wall.

0:59 - Would like to see a zoomed out version of the actual tool being used, followed by a zooming in to visualize what happens to the cell wall, otherwise it is very abstract for the students.

1:04 - Be more clear regarding the highlighting of the specific cellular structures as they are mentioned in the script.

1:38 - Show the lysate as more of a milky white color instead of a yellow color.

1:41 - Show a lid cover over the samples in the centrifuge? (only if time)

1:44 - Label the supernatant and pellet in the tube after centrifugation. Make the supernatant and pellet a more realistic color (milky white).

Starting at 2:29 - Label whole cell, nuclear, and cytoplasmic fractionations. It looks like there are pink/purple/orange proteins in the whole cell lysate that don't appear in the nuclear and cytoplasmic fractionations. Where are these proteins localized in the cell?

**Western protein migration test**
It's hard to see the pores in the gel matrix. As a result, it sometimes looks like the protein is moving on top of the gel and not through it. We like how DNA moves through the agarose gel in this animation, but we are unsure of what it would be like to implement an animation like this one.

**Western Gel Apparatus Image**
Update the power supply based on our conversation

- The overall shape of the box & lid look realistic.
- The gel box should be more transparent to see the gel & buffer a little better inside the box - especially the bottom of the gel / buffer so that the viewer can see the bubbles in the buffer when the gel is running. This will also make it easier to see the transfer apparatus inside the box as well at a later stage.

- power supply box - I feel like this normally has enough ports for 4 gels. The knobs are really intuitive but should we use a more updated power supply box and have up and down arrows? There's also usually a large "run" button on the box.

**Still Images**
Provide still images based on the more updated 'still image request' list.

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August 1, 2014

**Flow Cytometry Animation**
No comments :)

**Fluorescence Animation**
No comments :)

**Microscopy (basic) Animation**

1:55-1:58 - are the internal mirrors that re-direct the light supposed to be visible here? I would think that it would be best to start showing them when the screen becomes dark at 1:59

2:40ish - media for cells growing in tissue culture should look more transparent and should be more orange/red

3:34 - arrow should point toward a dark blue/purple nucleus for hematoxylin (just need to switch the direction of the arrow)

3:38 - Add an arrow for eosin to point toward the pink cytoplasm of a cell (right now, the label "eosin" is on several cell's cytoplasms)

3:40 - We need to find a new image. This image is not of good quality. Should also add arrows/lines to highlight where oil red o staining is to be consistent with the hematoxylin and eosin staining.

4:00ish - The color of the avidin on the secondary antibody should be something else than blue so it is not confused with the hematoxylin staining on the image on the right. Also make the antibody staining label more visible and perhaps label different places so that it is easier for students to visualize. Move the hematoxylin label to a less prominent place so that your eye goes to the antibody stain label first.

**Microscopy (fluorescence) Animation**

1:21 - when adjusting the excitation filter it looks like the whole laser beam between the laser source and excitation filter changes color. I thought that the laser beam being emitted from the source should stay the same color, but the light between the excitation filter and the sample would change to purple, and then the emitted light from the sample to the eye piece would change colors.

1:36 - add a "DAPI" label

1:42 - add a "Phalloidin" label

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July 23, 2014

**Timeline**

- Will send us the (hopefully) final edits for these four animations - by July 31st.

- Need to send her at least one of the western blotting and antibody scripts relatively soon so she can start on them soon

**Microscopy (basic) Animation**

Voice - do a complete read through with old and new parts of script (can hear the transition around 33 seconds)
Voice was all done together so no need to re-record it - think this was a problem with watching the animation online rather than the downloaded version

General concerns (LA):

- Lots of complex words are said - worried that they could be missed and wondering if there is a way to label certain things (the different components of the microscope, for example)
- Sometimes the images appear to be going in focus and out-of focus again - not sure if this is the case.
    - 
    - This may happen on the web, but shouldn't appear in downloaded version

0:38 - Put the microscopy type images side by side and label them. Highlight the ones we are going to focus on.
Will try quadrants and labeling the images

0:53 - Add labels for each part of the microscope as the voice mentions them. The label will appear and disappear.

1:33 - The light and focus controls are synchronized with the voice, but not the stage position. Re-position the worm in the viewfinder to be synchronized with the voice?
Ok, will do this.

3:32 - is it clear that this image has both hematoxylin and eosin (but the next image only has oil red o)? add in a label?
Add label to indicate where hematoxylin and eosin staining are strongest

3:41 - the image seems a bit out of focus (LA)
Need to find a better Oil Red O image that isn't copyrighted

3:41 - show one primary antibody, 2 secondary antibodies bind to primary, secondary antibodies bound to biotin, then have avidin-horseradish peroxidase step, then HRP catalyzes the reaction to brown, show other unbound proteins (square, circle), show nucleus in the schematic in blue

4:00 - add a label for antibody detection is in brown and hematoxylin counterstain is in blue

**Microscopy (fluorescence) Animation**
0:28 - Add a label for transmitted light microscope (left) and fluorescence microscope (right)
Janet will add labels

0:40 - Wait until the animated left microscope light path is finished before starting to illustrate the fluorescence microscope path (might need a pause on the voice recording too).
Ok, will fix the timing to match audio

1:46 - Show a cluster of 3 orange triangles, each bound by 1 primary antibody, each bound to 2 secondaries, and the secondary antibody is conjugated to the green fluorophores. Will flatten the image out so that it is clear that it is zoomed in. and make a green fluorescent "cloud" so it is clearer that it corresponds to a green dot in the image on the right.

**Flow Cytometry Animation**
3:27 - I don't see the changes that we discussed last time (slowing down the sheath fluid and adding 5 more cells

Janet will send us the updated flow cytometry animation
(there's also a flash where the middle detector still overlaps onto the other two detectors)

**Fluorescence Animation**
1:45 - Update image based on what is now in the fluorescence microscopy animation

Credits - add © Sierra Blakely to the appropriate line

- we decided we wouldn't add this in because then it will look like the animation is copyrighted to Sierra. Janet changed name to say Sierra Blakely

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July 3, 2014

**Flow Cytometry Animation**
1:46 - the middle detector still overlaps onto the other two detectors

2:14 - Individual lines or whole entire ray that is thick?

3:27 - It's a little slow for me. The cells are moving really slowly but the fluid appears to be moving at the same pace. Also, the cells should break the laser beam as they cross it.
Janet will slow down the sheath fluid and add in 5 more cells

**Fluorescence Animation**
1:21 - 3D rendering around DAPI molecule should stay for the remainder of the animation where it is shown?
Janet - add an electron cloud to indicate the base electron cloud

1:27 ish - (Janet) remove arrowheads, show dashed/dotted lines to more clearly indicate directionality

1:45 - This will be done after the microscopy animation is done

If we use this image, then:

- Label the laser
- Have purple light exciting and blue light emitting

Consider showing microscopy side by side with flow cytometry to show how fluorophores are excited and emit light in both situations - may be a little busy but could do still images side by side

Credits - add © Sierra Blakely to the appropriate line

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June 12, 2014

**Flow Cytometry Animation**
1:46 - parts of the components (laser/detector) are extending into other sections across the section boundary.

3:27 - I can tell that some of the cells are not emitting all four colors, but the fluorescence flashes so quickly that it appears that all the cells appear to have all four colors.

**Fluorescence Animation**
0:45 - DAPI image also shows two other colors. I think we should label that DAPI is in blue or only show an image with DAPI stain (which won't look as pretty).

0:48 - Ethidium bromide gel - tilt it up a little bit so it's not at an angle or put it on a darker background

0:57 - The molecule is DAPI. Should this be labeled? I wonder if we should show it in the same manner as we do on the previous slide so it looks the same?

- Flip orientation and use ball and stick model.

Show some movement in the electron cloud even when it hasn't reached the highest energy state

1:02 - Show arrowheads with direction of exciting and emitting light to/from the molecule

1:05 - different color of circle on energy diagram (not blue)

1:06 - show a faint line to indicate the past energy states of the molecule? Or alternatively, have an arrow to show the direction of movement?

1:45 - Label the laser
If we use this image, then have purple light exciting and blue light emitting
Consider showing microscopy side by side with flow cytometry to show how fluorophores are excited and emit light in both situations

End - Provide full references for all resources used in the video. Make sure that they are all not copyright protected.

**Overall To-Do**

- Provide credits at the end of the video
    - 
    - Provide Janet with the credits that we would like for the animations
- Send images to Janet - IHC? - wait to see if she needs images
- Microscopy animation storyboard - Janet will add in the actions and we will then edit the script as necessary

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May 13, 2014

**Flow Cytometry Animation**

Title Slide - would you like the StarCellBio logo file? Yes, send at high resolution.

0:10 - At the beginning, the image of the tube is hard to see next to the flow cytometer. What about if we place the tube in a tube rack?

0:51 - Add a computer screen next to the computer? remove the whole computer

1:23 - add sheath fluid around the cells

1:50 - I like this direct comparison with different sized cells and the different graphs. I would like to see the cell closer to the graph so it's easier to watch both things at the same time. Or perhaps we can slow it down slightly to allow the user to see what's going on. We may also show all three curves all at the same time with the different size cells - just like at 2:25

Width of forward scatter for smallest cell looks equal width of middle cell

2:11 - What is the illustration showing in terms of internal complexity? Neutrophils. These look accurate after more research.

2:33 - Maintain axes labels here - usually have FSC on X axis and SSC on y-axis. Will remove 2D Scatter plot.

3:10 - do need to show primary and secondary antibodies? will change to just GFP dots and can change it later to primary/secondary antibodies if desired.

4:03 - if detecting 18 colors - is this correct? I will need to do some research on the number of lasers and detectors in these machines. Show a cell with lots of fluorescence colors and have the inside of the machine with a laser with 8 detectors/colors instead.

Janet's To-Dos:

- Janet will record better audio
- replace some of the FACS data in the video

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Hour count - Janet Iwasa - as of May 13, 2014
Flow cytometry animation
total right now: 45 hours ($4500)

To-Do:
1. FACS data for Janet? - we will provide the first image 0:26), 2:00 ish - FSC vs cell count image, Janet will re-do the 2D scatter plots, we will provide the fluorescence histograms and 2D scatter plots (4:10 ish)

2. what is the diameter of laser beam? How do we want the laser beam represented?
The laser beam must be focused on the sample. This is achieved using a lens to focus the laser beam to the desired diameter.

Website with schematic of a 12-color FACS setup and also information about focusing a laser beam on the cell:
http://flowbook.denovosoftware.com/Flow_Book/Chapter_2%3A_The_Flow_Cytometer