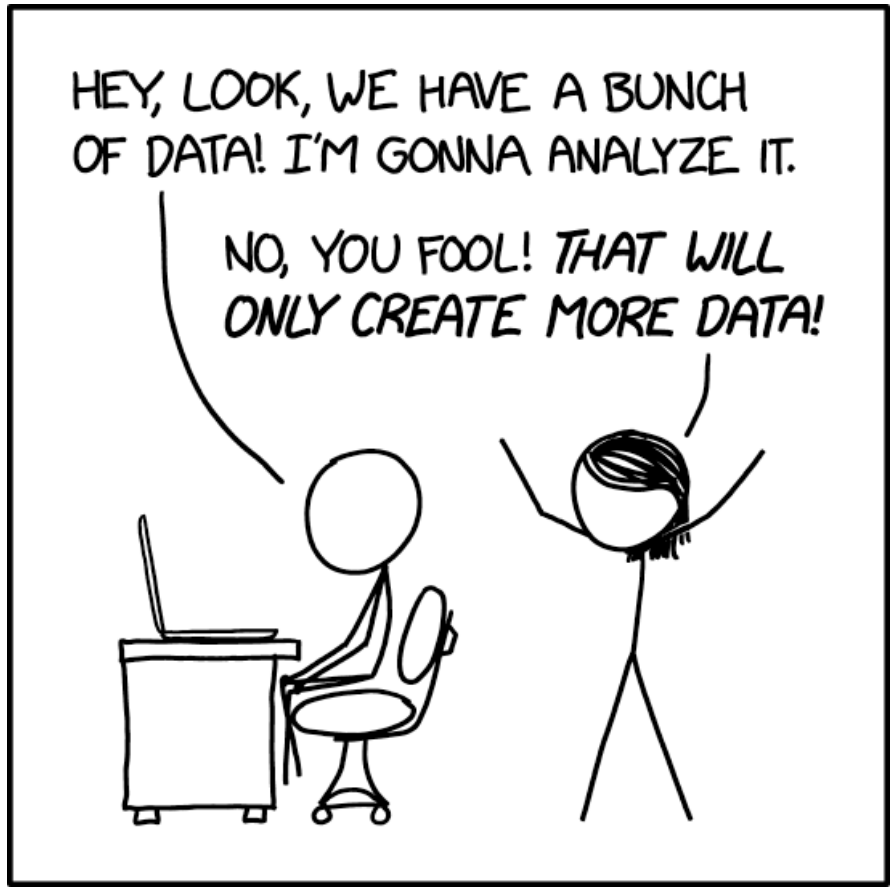
M1D5: Treat cells for CometChip assay

1. Comm Lab
2. Treat cells for CometChip experiment
3. Turn your data figure into a data slide
    1. Refine/edit data figure
    2. Write a Results and Interpretation section in bullet points

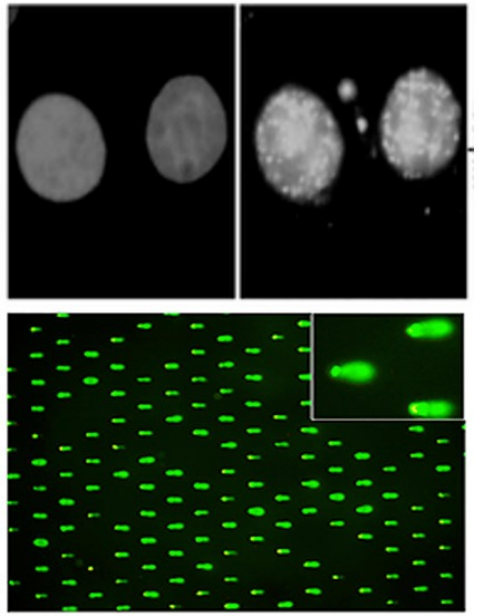


Mod1 Overview

Last lab:

This lab:

Next lab:



1. Use repair foci experiment to measure DNA breaks

- Examine effect of H_2O_2 +/- As on double strand DNA breaks by measuring γH2AX foci formation

2. Use high-throughput genome damage assay to measure DNA damage

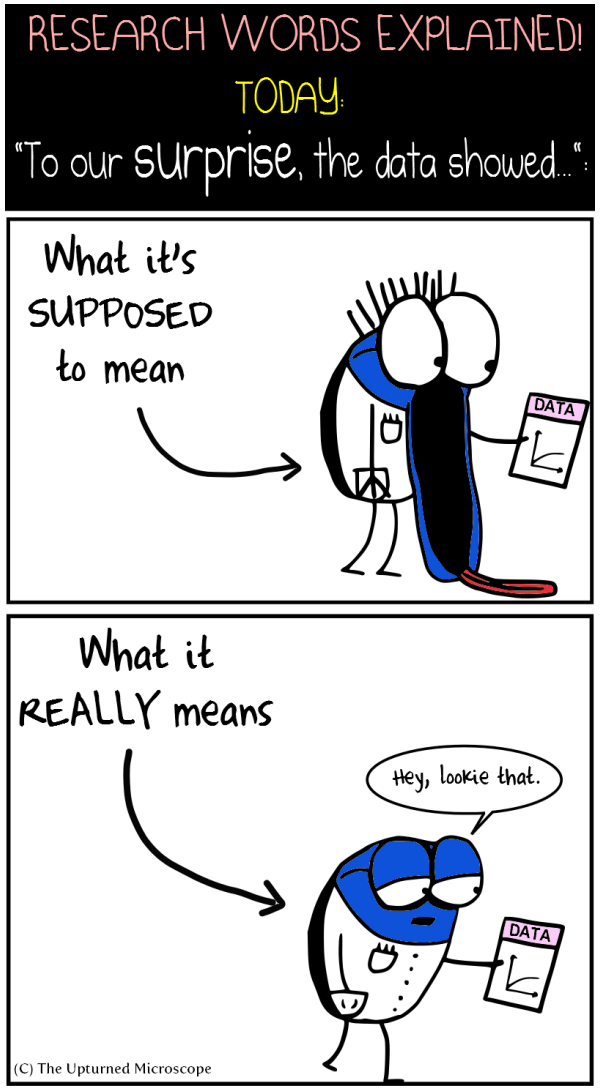
- Measure effects of H_2O_2 +/- As on DNA damage by measuring DNA migration in agarose matrix

What's up with this data?

- We've seen some confusing results for several groups
- Looks like there might have been a technical issue or reagent issue

- When encountering unexpected results, start with theoretical troubleshooting
  - Any technical issues when doing the experiment?
  - Any sensitive reagents that may be difficult to work with?
  - Any biology that may be more complicated than we thought?

Let's take the biology as an example…



Does model system play a role?

Model systems we've used in previous iterations of this experiment

- Immortalized MEF cell line
- CHO cells (Chinese Hamster Ovary) cell line
- TK6 (Human lymphoblast) cell line

Synergistic effect of Arsenic and DNA damaging agent

Magnitude varies (3-fold – 15-fold increase

Pilot data for this semester

- p4 Primary MEF cells established in culture

Foci numbers are significantly different between groups

Your experiment (and the tandem one):

- p1 Primary MEF cells coming out of a thaw

Why might model system matter?

How could you test it?

Now what?

- Your data set will be definitely be used!
  - Now that numbers are generated, take a second look at the more qualitative aspects of your data

- We have included the analysis of our pilot data set for you to graph and discuss in the Data Summary
  - Pilot images are also included

- Create a Panel A with your team's images and graph (just your 3 images/group)
- Create a Panel B with our pilot images and graph

- Briefly compare between data sets, but spend time discussing results of the pilot

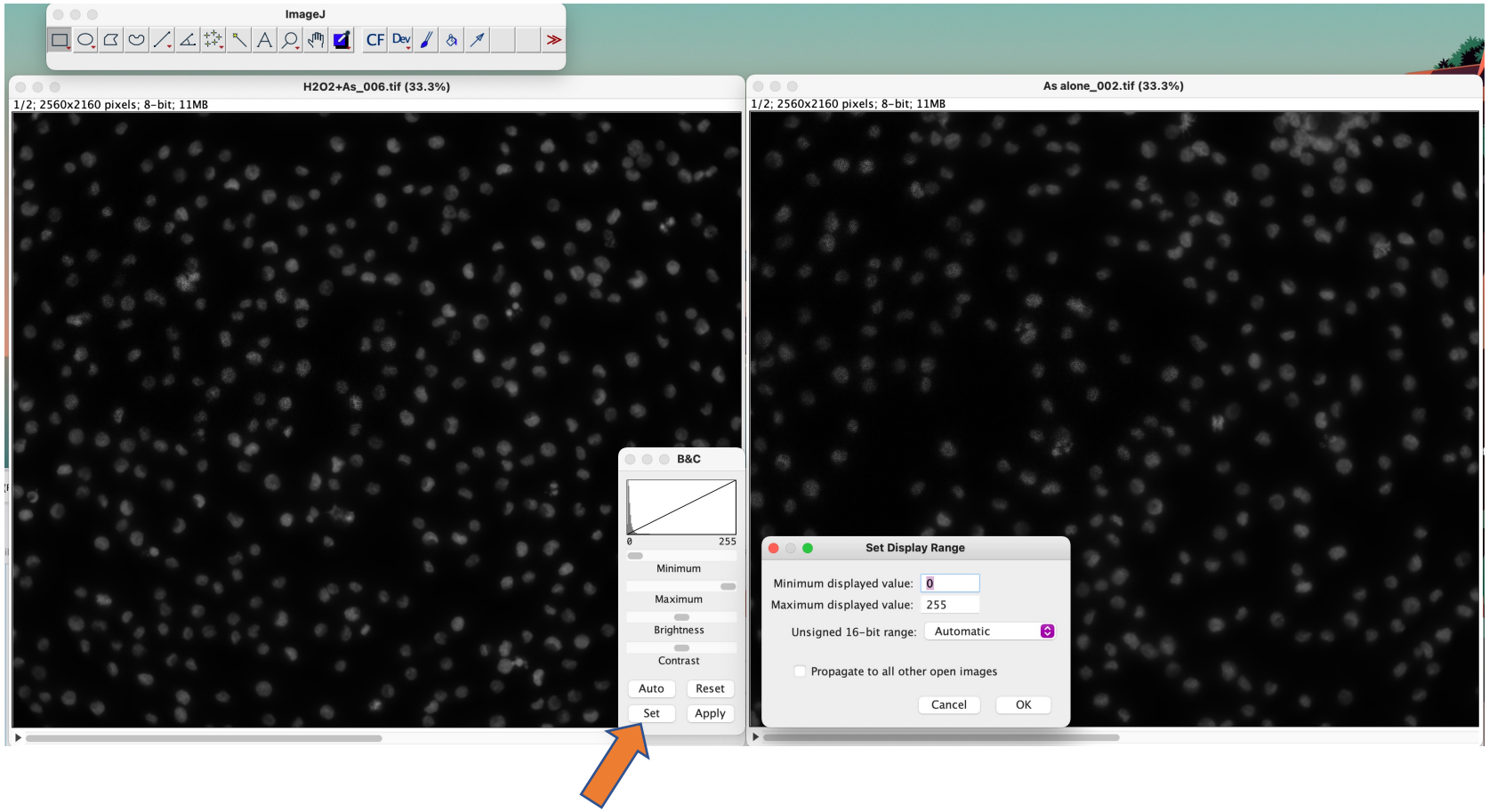
Adjusting images for “publication”

- Once images are analyzed, they are frequently enhanced to help visually convey the data more effectively

- Journals will accept images modified within certain parameters
  - To mitigate bias, adjust evenly across treatment groups
  - Do not modify images in a way that changes the overall conclusion of data

- You can do this with your raw images in ImageJ
  - Grayscale of gamma-H2AX is totally fine for the Data Summary
  - If you want to learn how to merge and pseudocolor, let me know.

Notes on adjusting ImageJ images evenly



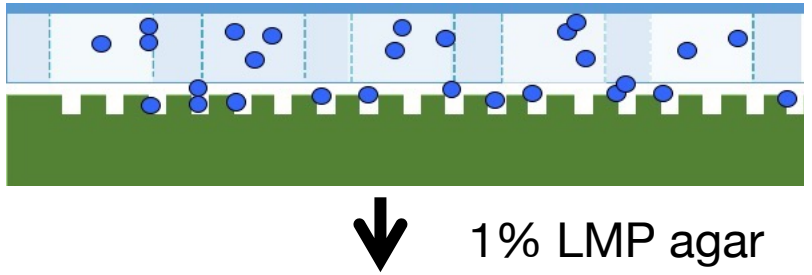
- Open all images you plan to adjust
- Adjust brightness and contrast levels as appropriate
- Set -> Propogate to all open images

- You are welcome to screenshot from open windows
- To export the grayscale image so that it's visible in ppt
- Image -> Type -> RGB color
- File -> Save as -> TIF

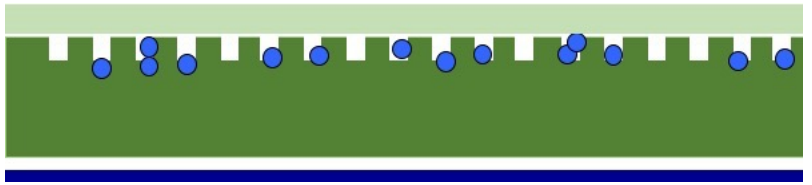
ImageJ adjustment with Jamie

Overview of the CometChip assay: treating cells

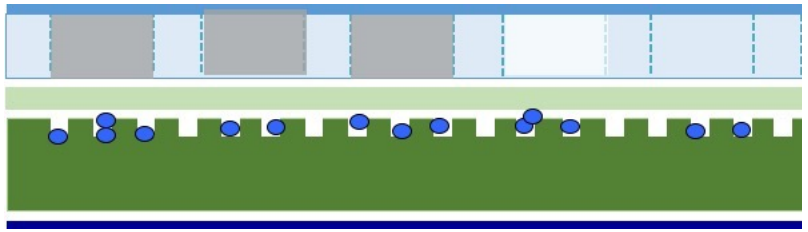
Treat with As for 2hrs



1% LMP agar



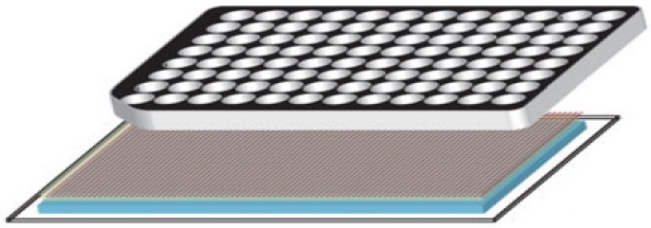
Treat with H_2O_2



Place in lysis buffer overnight

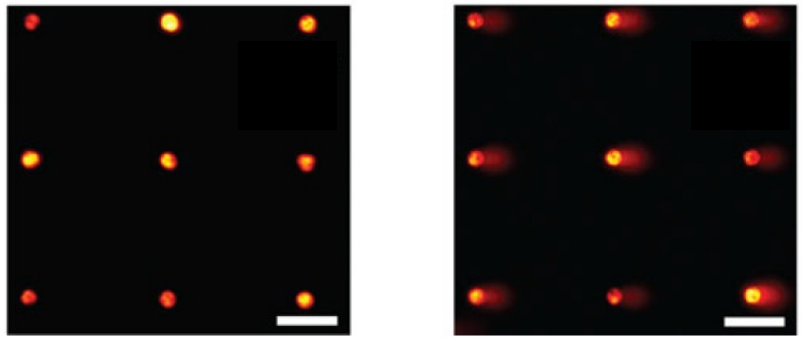
Overview of CometChip Assay: electrophoresis & visualization

Treat captured cells in comet chip with H_2O_2 and As

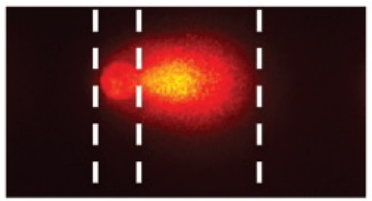


Lyse cells & unwind DNA (DNA still captured agarose in overlay)

Agarose Electrophoresis



Stain DNA and image via fluorescence microscopy



Analysis via Matlab

For Today

- Resuspend, count, and seed cells for CometChip assay
- Treat cells with H_2O_2
- Lyse cells overnight
- Work on data slide for Data Summary

For M1D6

- Data Summary slide (revised figure and completed bullet points)

Use wiki guidance!

Format powerpoint slides to 8.5” x 11” in portrait-mode

Work on figure arrangement so that figure and text are concise

- Because this is a complicated figure, it can be larger than ½ a page

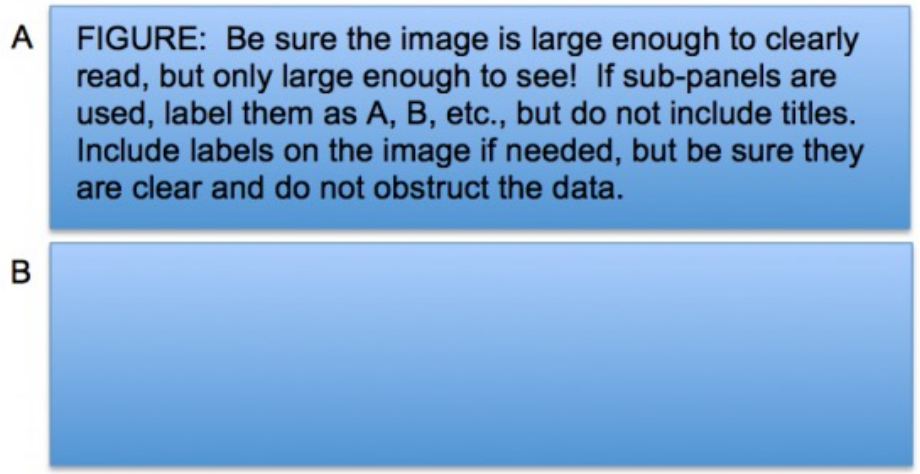


FIGURE TITLE: This should state the conclusion of the figure in very brief and precise language. CAPTION: Start with a topic sentence that introduces the figure or sub-panel. Provide all of the information that the reader needs to interpret the figure (define abbreviations, explain labeling scheme, differentiate between sub-panels A, B, etc.). You should not interpret the figure or give minor methods details.

RESULTS SECTION TITLE: This should state a conclusion concerning what you now know given the information provided on this slide…if there is more than one conclusion, consider separating the information into more than one slide.

RESULT(S)/INTERPRETATION(S): Use the questions below to guide the information you provide in your concise bullets.

- What is the overall goal of your experiment?
- What was your expected result according to your hypothesis?
- What evidence do you have that you result is ‘correct’ or ‘incorrect’?
  - What controls did you include and for what did these control?
  - Did the controls work as expected?
- What was the result?
  - Was the result expected?
- In sum, what do these data suggest or indicate?