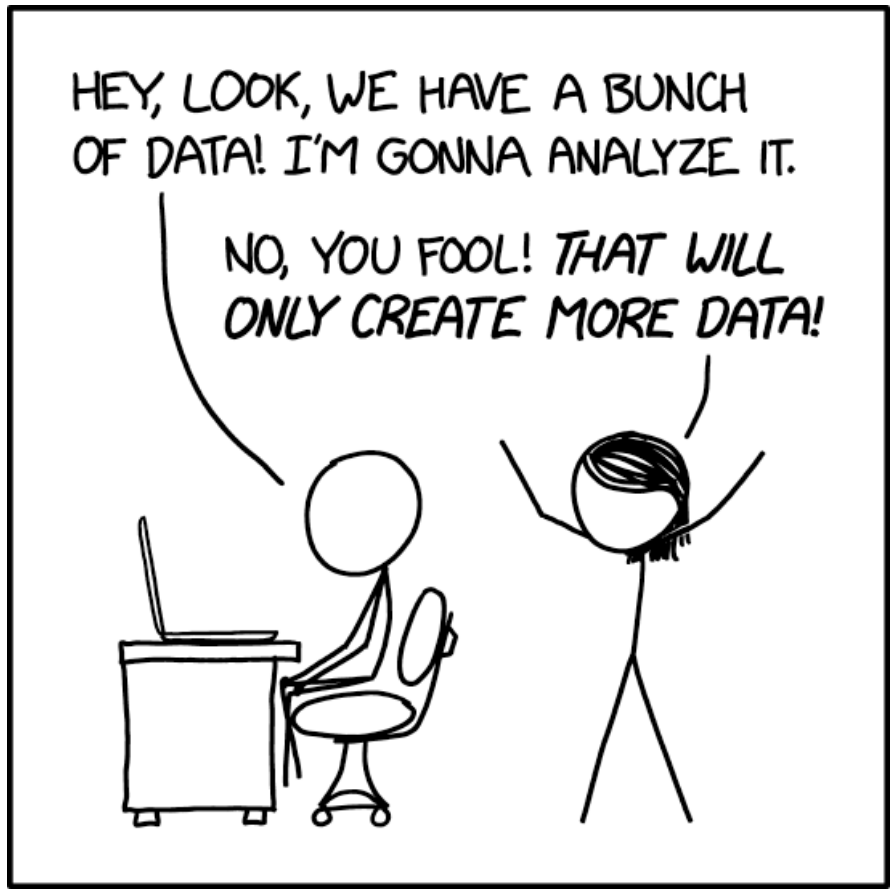
M1D5: Treat cells for CometChip assay

1. Comm Lab
2. Prelab
    1. What's up with data?
    2. Image beautification
3. Treat cells for CometChip experiment
4. 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:

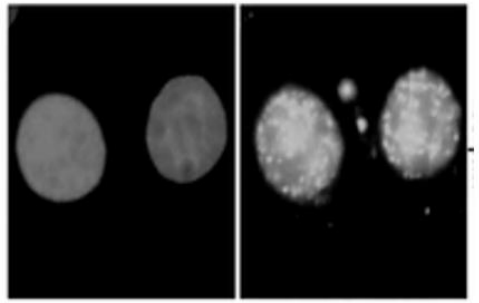
yH2AX data analysis
Pour CometChip

This lab:

Load & Treat CometChip cells w/ As & H202

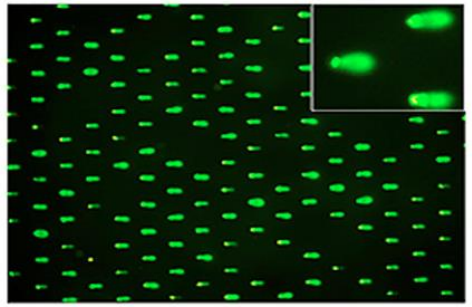
Next lab:

Analyze CometChip data



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 going on in our data?

We promise we had it working in the summer!

List as many things as you can that could have gone wrong (be specific)

Hypothesis	Predicted Outcome	Reality	Explanatory Power
Bad Arsenic	No change in any arsenic treatment	We correctly made a fresh batch of As solution prior to class	Low
Bad H202	No change in any H202 treatment	Brand new bottle of H202 from the same supplier for class	Low
Technical Issue with staining	Systemic difference between Instructors/Students	All our data are kinda janky	Low
Bad permeabilization	Low signal in DAPI	DAPI was very very bright	Low
Bad antibody	No/little nuclear foci staining	Some coverslips have a good amount of foci staining	Low
Something Biological?			

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

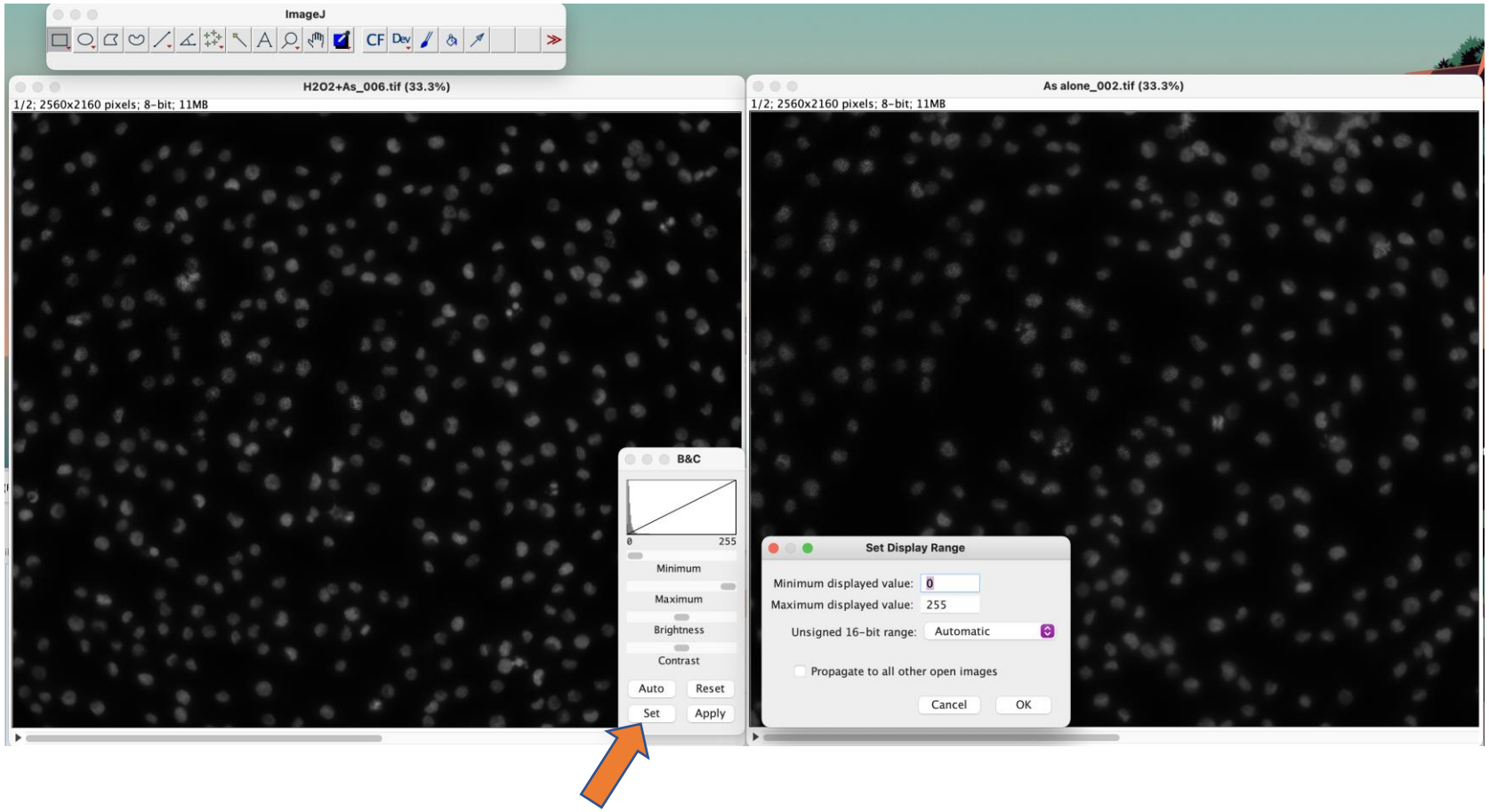
Adjusting images for “publication”

- 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

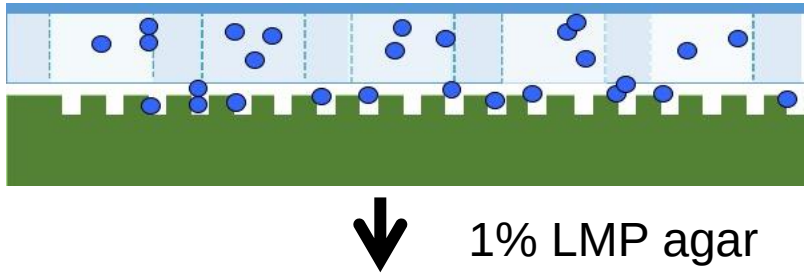


- Open all images you plan to adjust
- Adjust brightness and contrast levels as appropriate
- Set -> Propogate to all open images

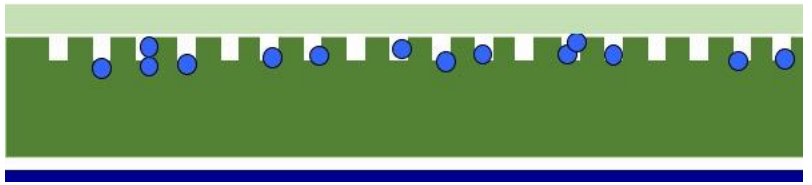
- You are welcome to screenshot from open windows
- To export the grayscale image so that it's visible in ppt
- Image -> Type -> RGB color
- File -> Save as -> TIF

Overview of the CometChip assay: treating cells

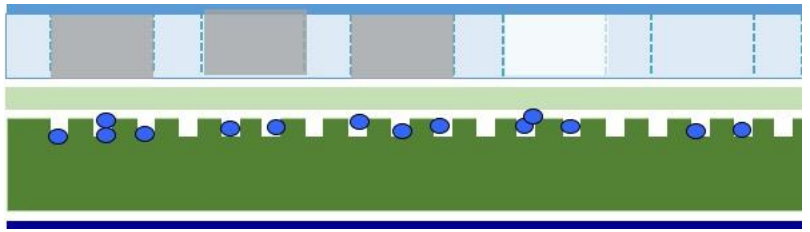
Treat with As for 2hrs



1% LMP agar



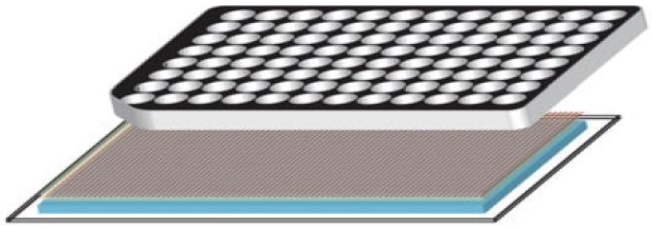
Treat with H_2O_2



Place in lysis buffer overnight

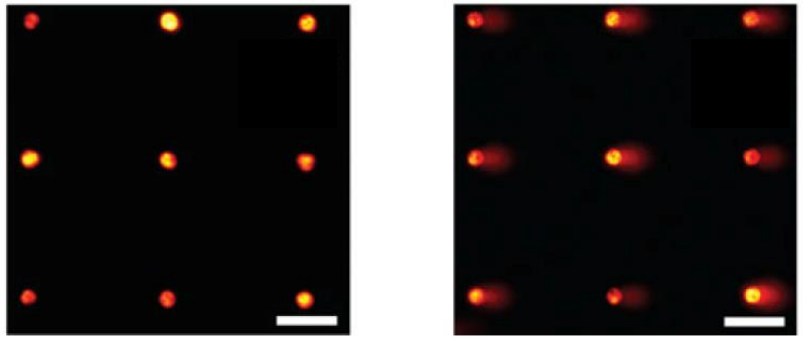
Overview of CometChip Assay: electrophoresis & visualization

Treat captured cells in comet chip with H_2O_2 and As

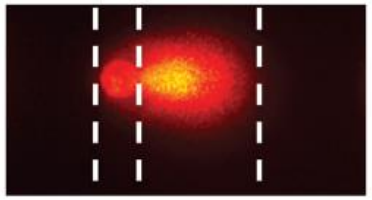


Lyse cells & unwind DNA
(DNA still captured agarose in overlay)

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
- Research Talk due Oct 1 10 pm

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

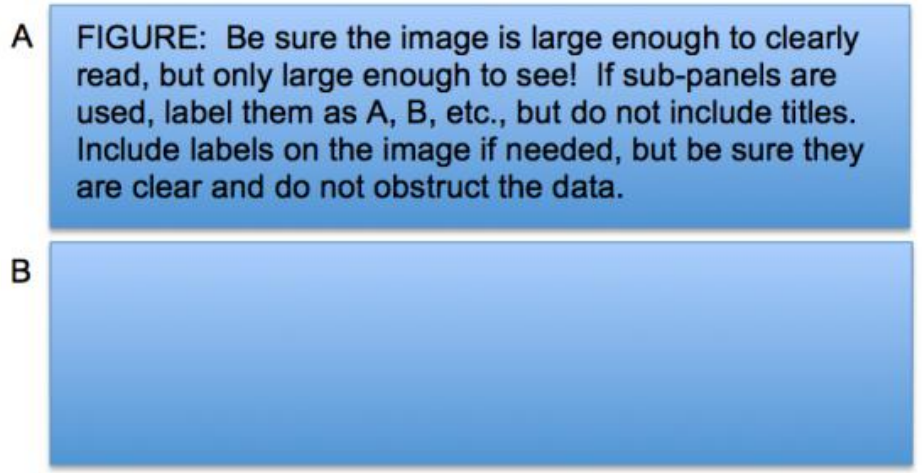


FIGURE TITLE: This should state the conclusion of the figure in very brief and precise language. CAPTION: Start with a topic sentence that introduces the figure or sub-panel. Provide all of the information that the reader needs to interpret the figure (define abbreviations, explain labeling scheme, differentiate 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?