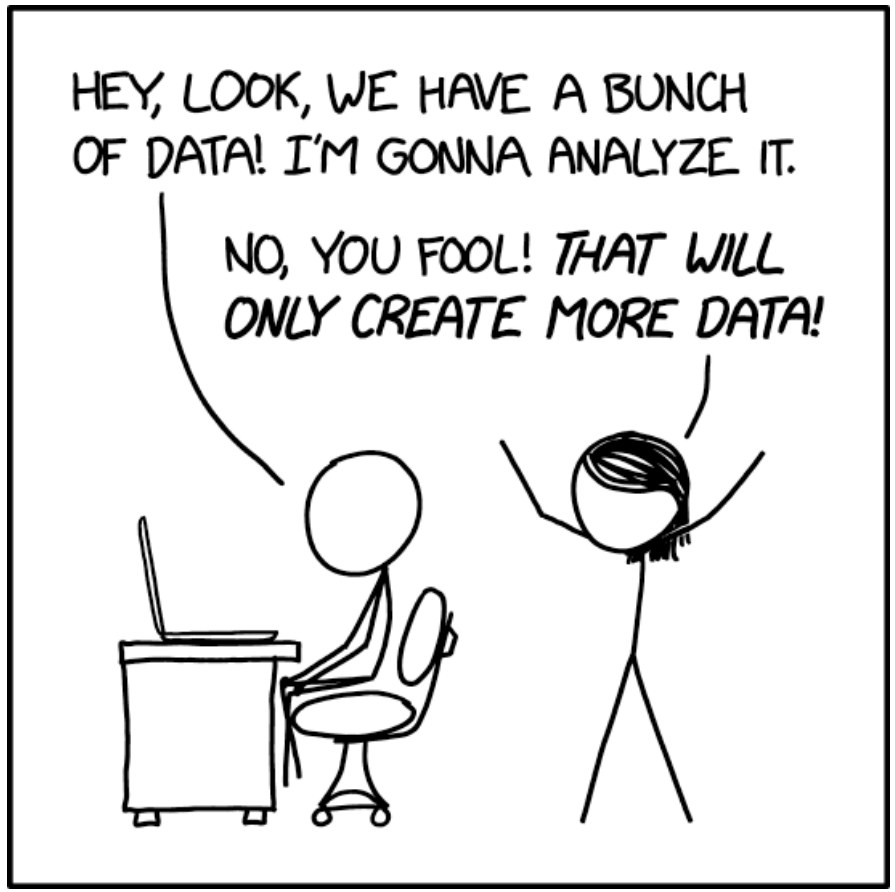
M1D5: Treat cells for CometChip assay

1. Comm Lab
2. Prelab
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



Homework

Data Summary slide

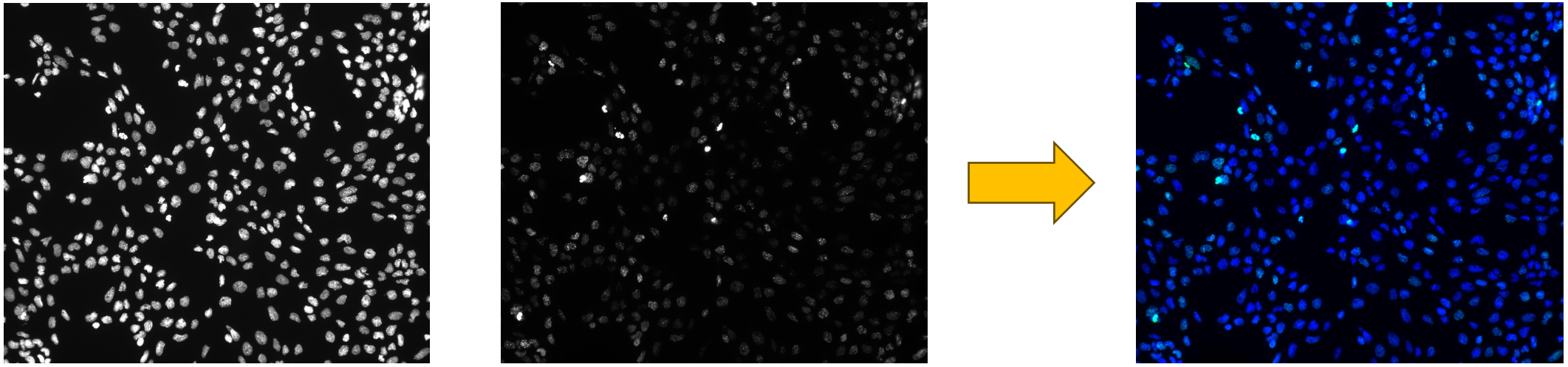
Examine your data to explore different ways of presenting analysis

- Bevin discussed this in lecture!

- Explore the data sets you've generated for your H2AX staining
  - Average foci per nuclei
  - Number of nuclei with certain numbers of foci

Examine your data to explore different ways of presenting your images

- Pseudocoloring can be helpful to show lots of data in a small space
  - Instructions for pseudocoloring on wiki
- Might sometimes be more informative to show the grayscale images
  - Can be difficult to see foci on the DAPI background



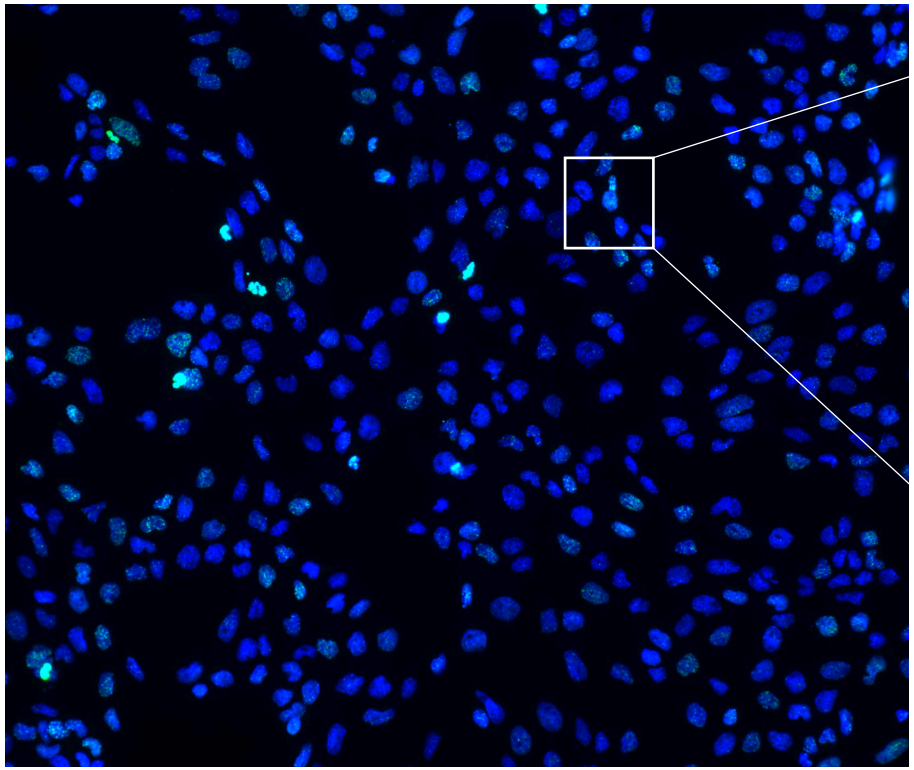
DAPI

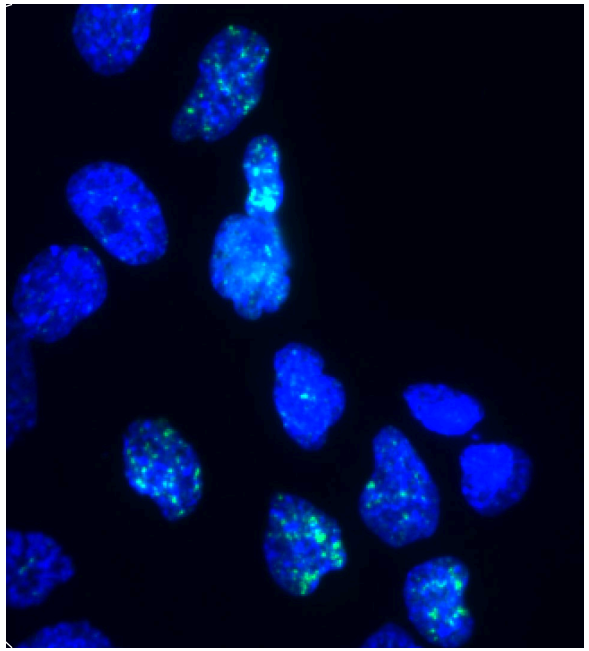
yH2AX

Blue= DAPI, Green= yH2AX

Examine your data to explore different ways of presenting your images

- Often helpful to show the entire field of view and then zoom in to a nuclei to show individual foci





Make data slide for your Data Summary

Include:

1. **Example images** from each of your treatment groups
2. Graph of **your group's data**
3. Graph of **pilot data**

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

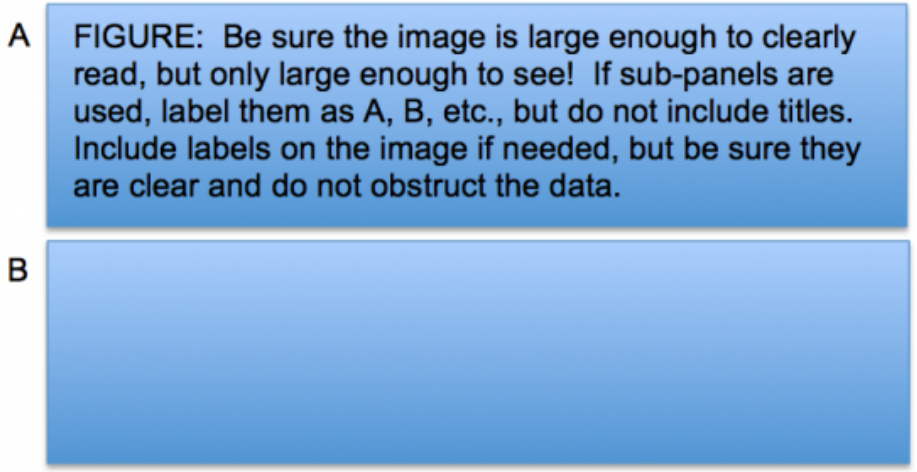


FIGURE TITLE: This should state the conclusion of the figure in very brief and precise language. CAPTION: Start with a topic sentence that introduces the figure or sub-panel. Provide all of the information that the reader needs to interpret the figure (define abbreviations, explain labeling scheme, differentiate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

Lab work

The CometChip assay

Mod1 Overview

Last lab:

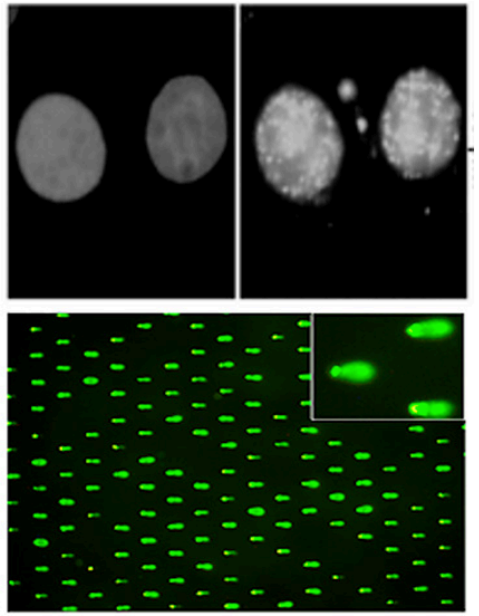
Analyze yH2AX data
Poured CometChip

This lab:

Perform CometChip

Next lab:

Analyze CometChip data



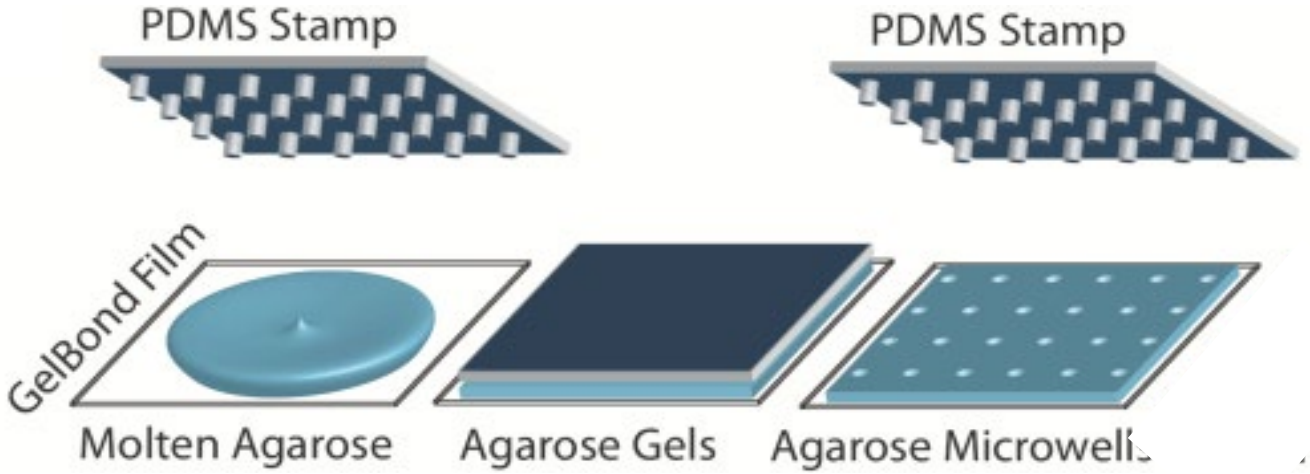
1. Use repair foci experiment to measure DNA breaks

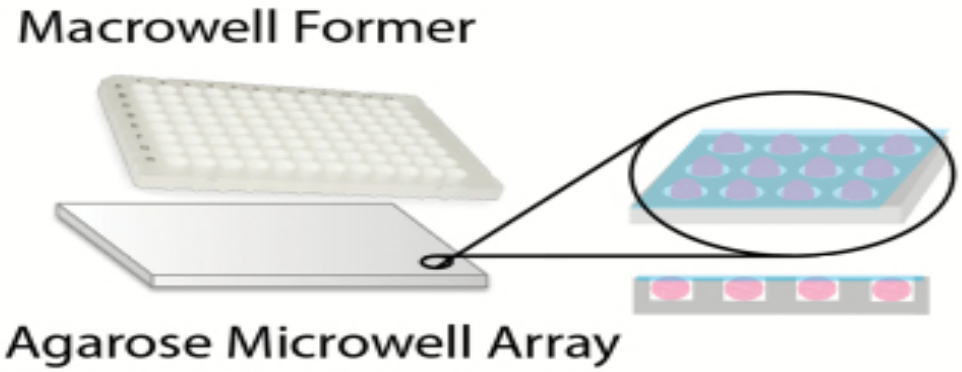
- Examine effect of H_2O_2 +/- As on double strand DNA breaks by measuring γH2AX foci formation

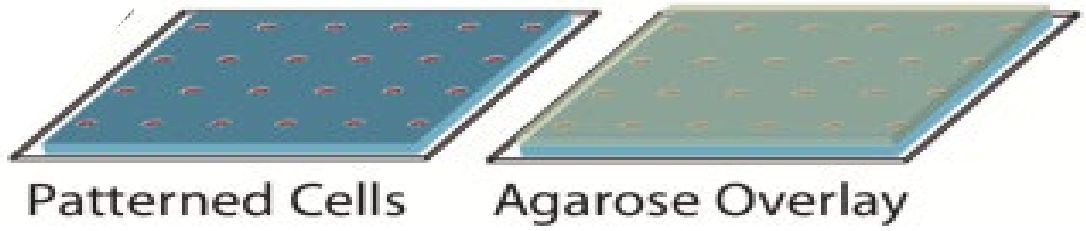
2. Use high-throughput genome damage assay to measure DNA damage

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

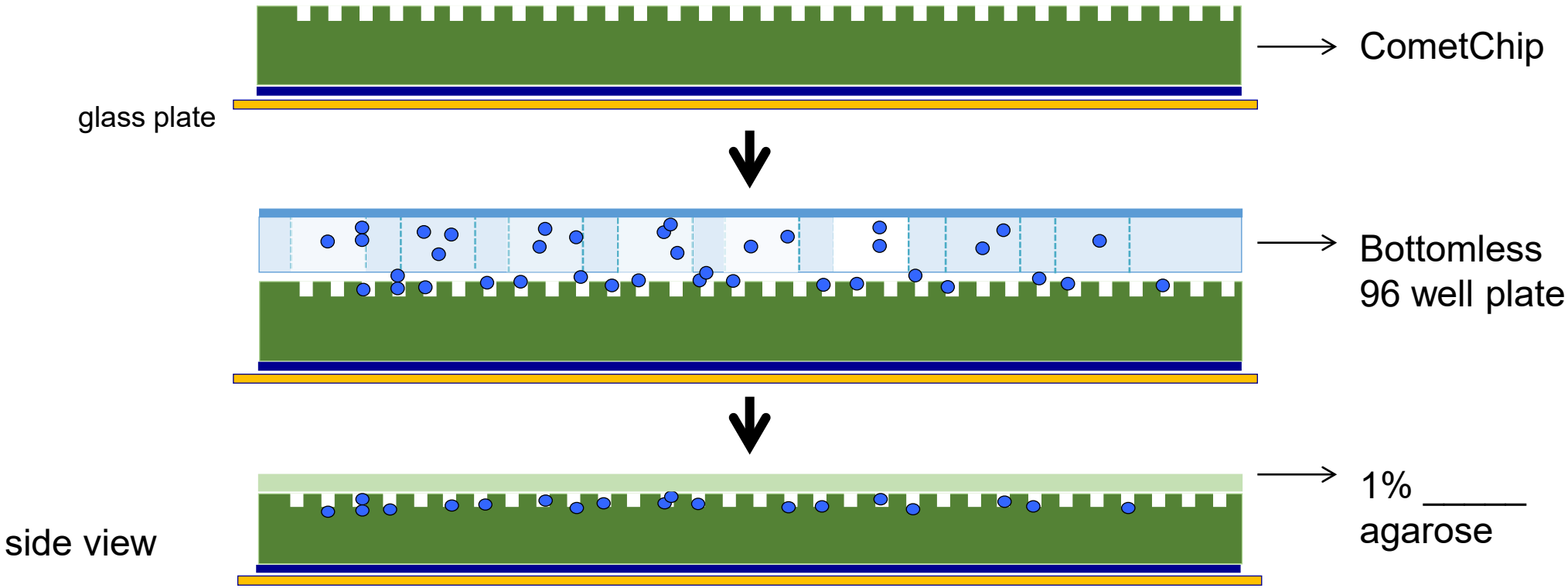
Review: CometChip creation and loading cells into wells





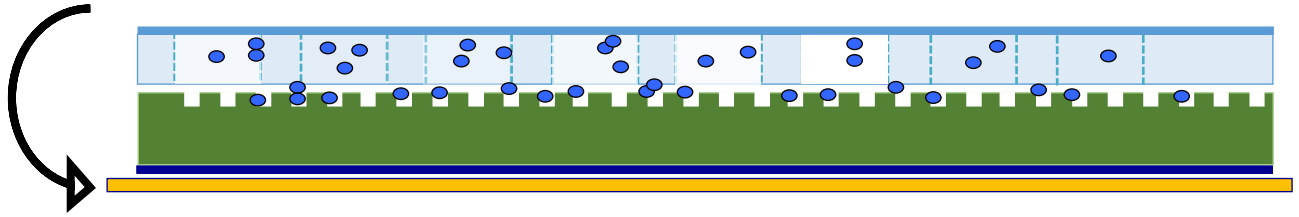


Load cells onto the CometChip



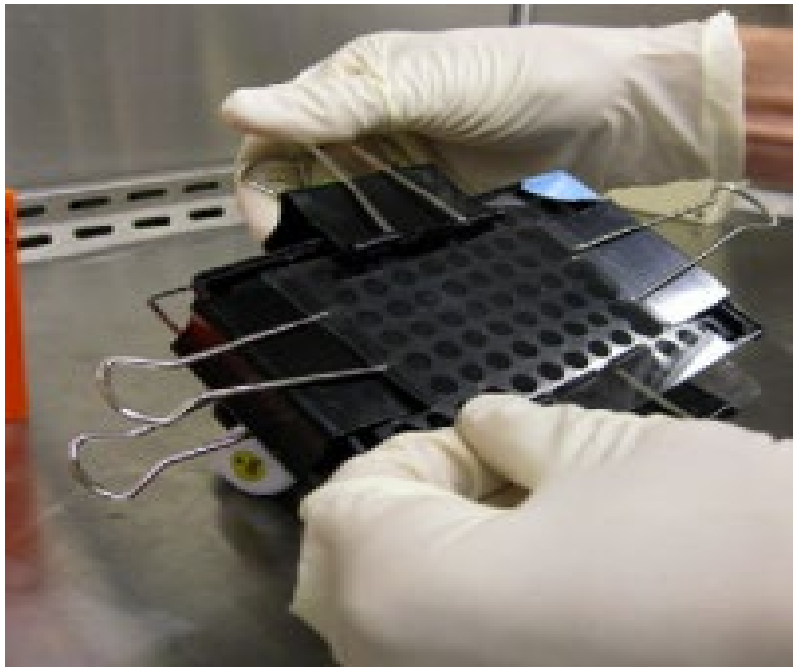
What this looks like IRL

Clamp with binder clips

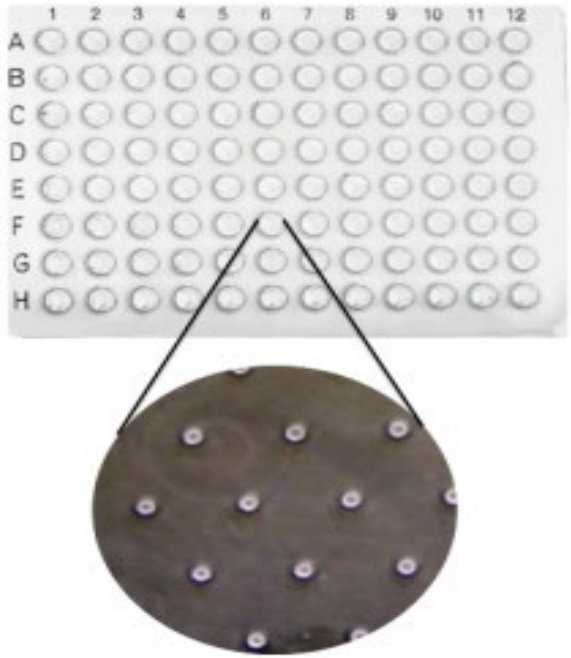


Side view

- Glass plate
- Bottomless 96- well plate
- 4 binder clips
- 37°C incubator (in main lab)

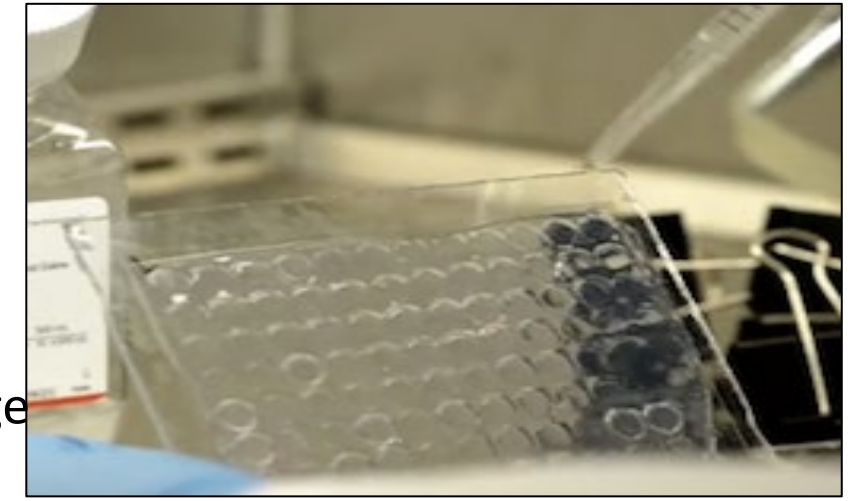


Top view

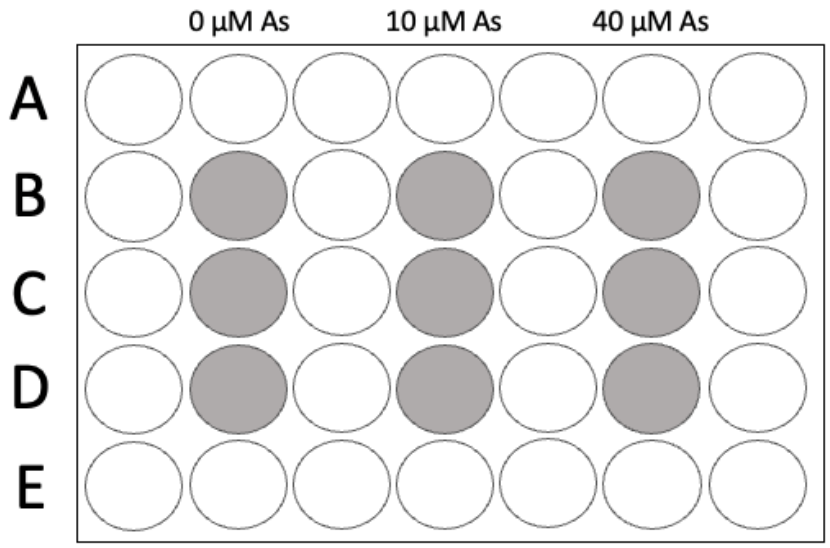


Major critical steps

- Cell loading
  - Line up macrowells carefully so they are straight across gel
  - Load cells and place in 37deg incubator
  - Might not have the space for all the spacing as shown in image
  - Leave for 15 minutes (why?)



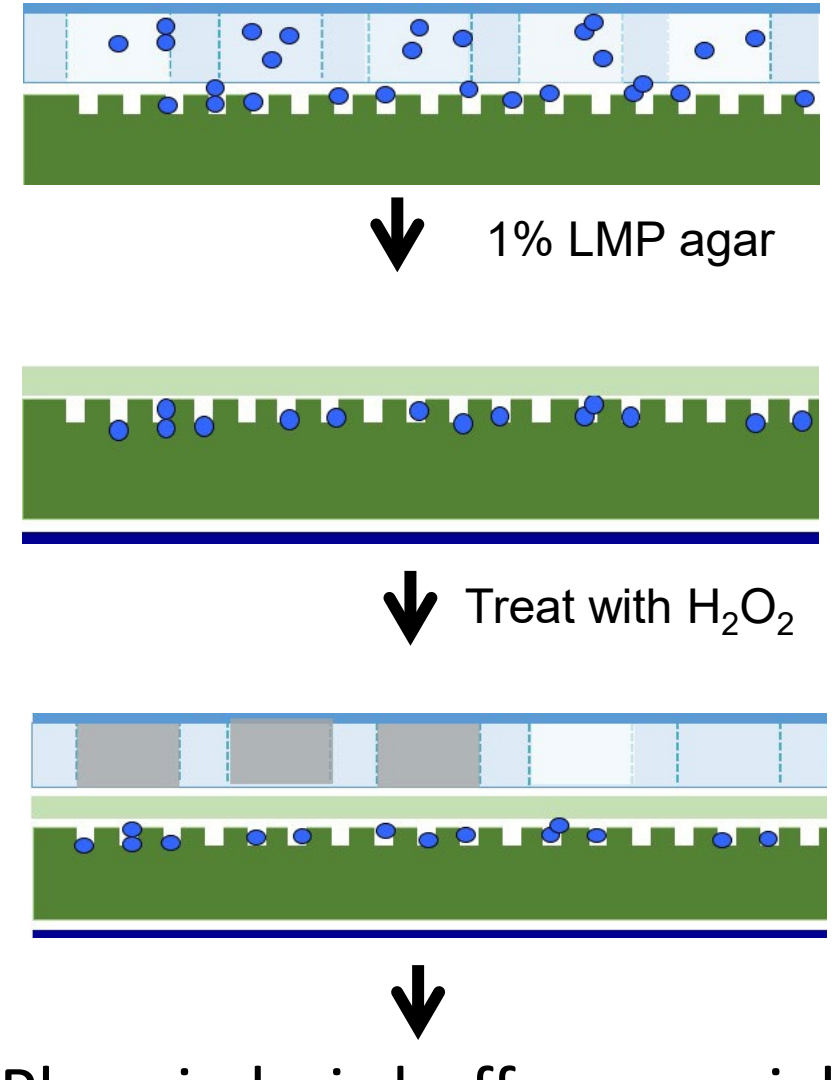
- Washing
  - Not too much!
  - Across the top of the glass plate
  - Wash from low to high concentration



- Cover the cells
  - Dispense 1ml of 1% LMP agarose over the gel with a P1000
  - 1% LMP agarose gels *quickly*
  - Leave it undisturbed for 3 min then move to 4°C for 3 min

Overview of the CometChip assay: treating cells

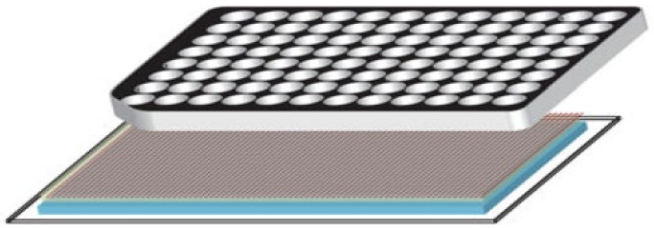
Treat with As for 4hrs



Place in lysis buffer overnight

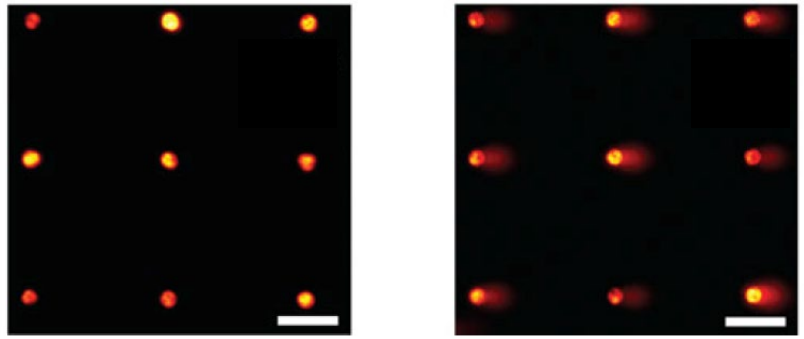
Overview of CometChip Assay: electrophoresis & visualization

Treat captured cells in comet chip with H_2O_2 and As

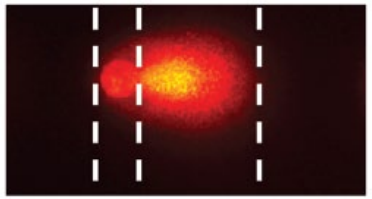


Lyse cells & unwind DNA (DNA still captured agarose in overlay)

Agarose Electrophoresis



Stain DNA and image via fluorescence microscopy



Analysis via Python

For Today

- Seed cells for CometChip assay
- Treat cells with H_2O_2
- Lyse cells overnight
- Continue H2AX analysis
- Work on data slide for Data Summary

For M1D6

- Data Summary slide (revised figure and completed bullet points)
  - Complete as a team