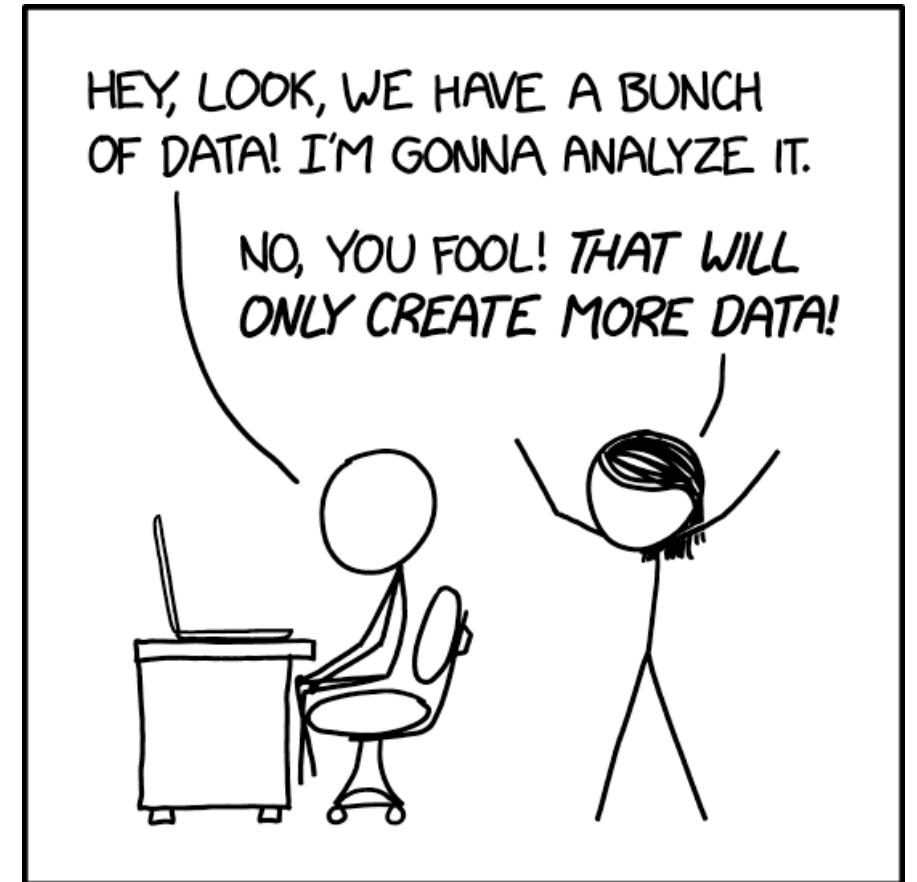


M1D5: Treat cells for CometChip assay

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

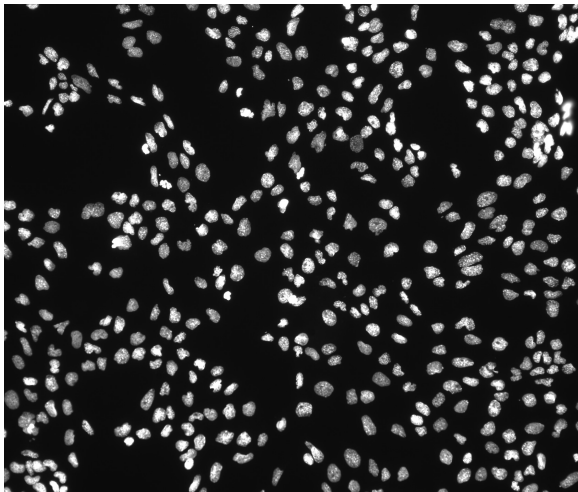
Data Summary slide

Examine your data to explore different ways of presenting analysis

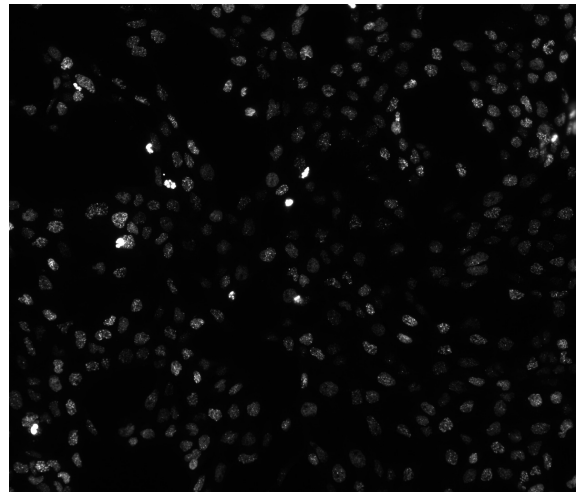
- Bevin discussed this in lecture!
- Explore the data sets you've generated for your H2AX staining
 - Average foci per nuclei
 - Number of nuclei with certain numbers of foci

Examine your data to explore different ways of presenting your images

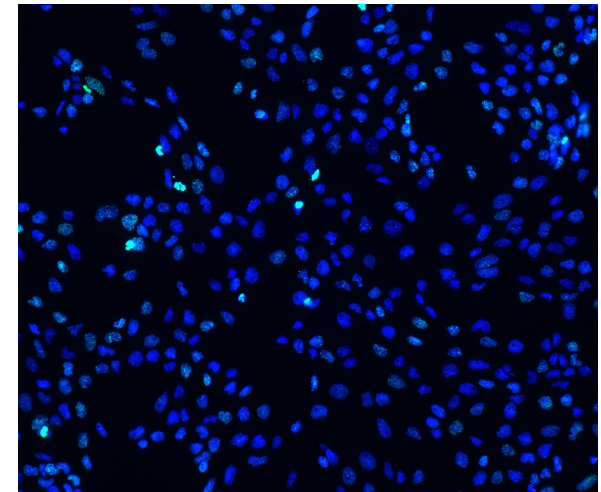
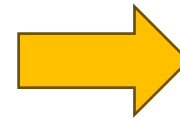
- Pseudocoloring can be helpful to show lots of data in a small space
 - Instructions for pseudocoloring on wiki
- Might sometimes be more informative to show the grayscale images
 - Can be difficult to see foci on the DAPI background



DAPI



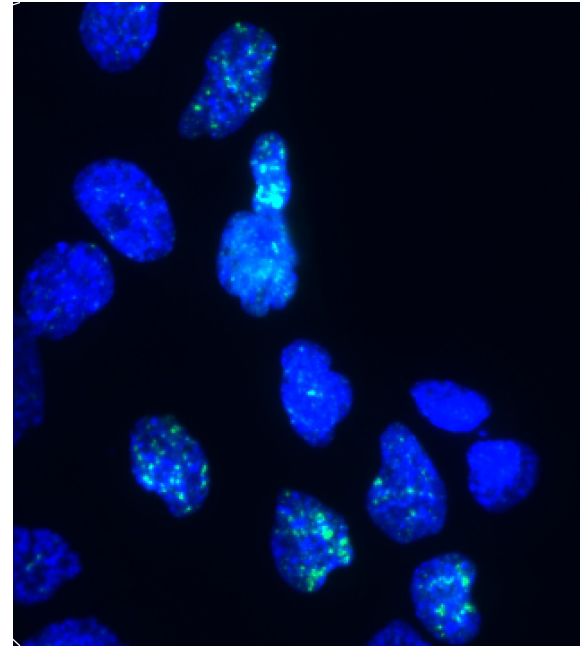
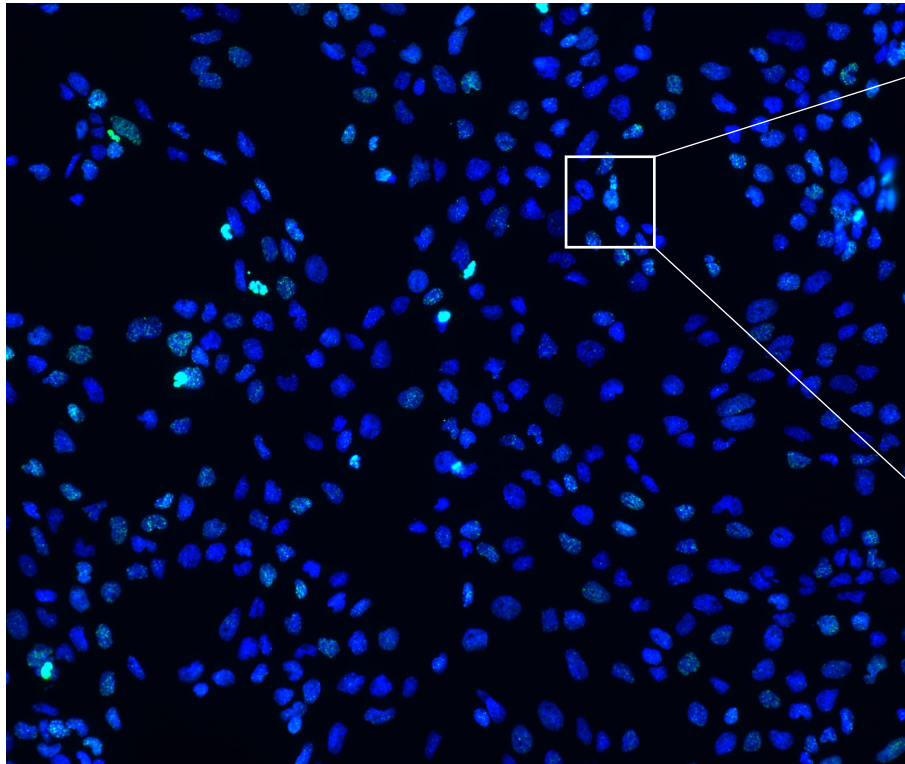
yH2AX



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

A **FIGURE:** Be sure the image is large enough to clearly read, but only large enough to see! If sub-panels are used, label them as A, B, etc., but do not include titles. Include labels on the image if needed, but be sure they are clear and do not obstruct the data.

B

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.

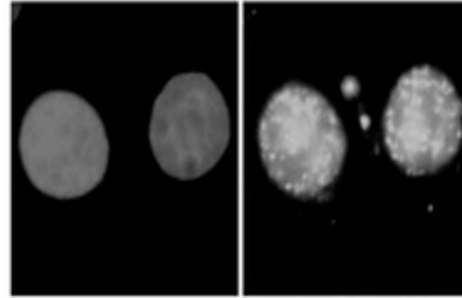
- What is the overall goal of your experiment?
- What was your expected result according to your hypothesis?
- What evidence do you have that your result is 'correct' or 'incorrect'?
 - What controls did you include and for what did these control?
 - Did the controls work as expected?
- What was the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

Lab work

The CometChip assay

Mod1 Overview

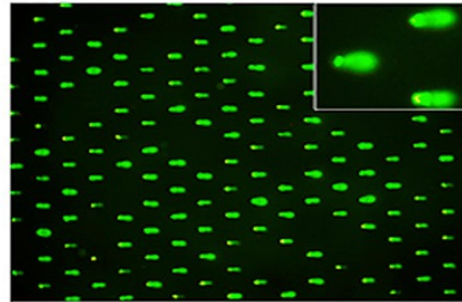
Last lab:



1. Use repair foci experiment to measure DNA breaks

- Examine effect of H_2O_2 +/- As on double strand DNA breaks by measuring γ H2AX foci formation

This lab:

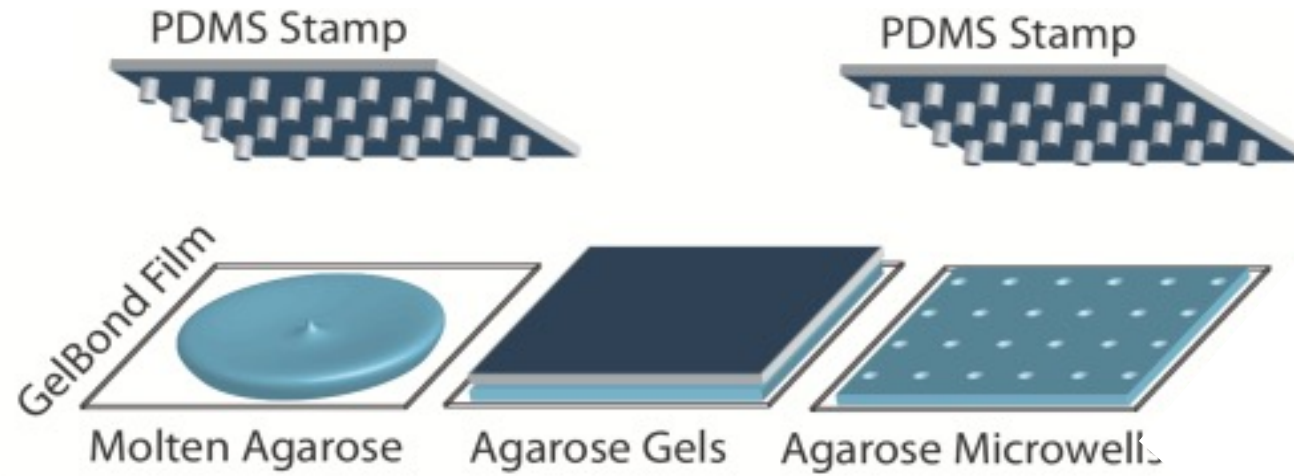


2. Use high-throughput genome damage assay to measure DNA damage

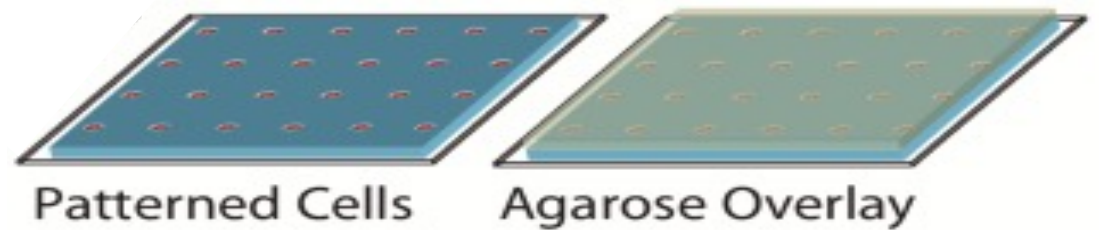
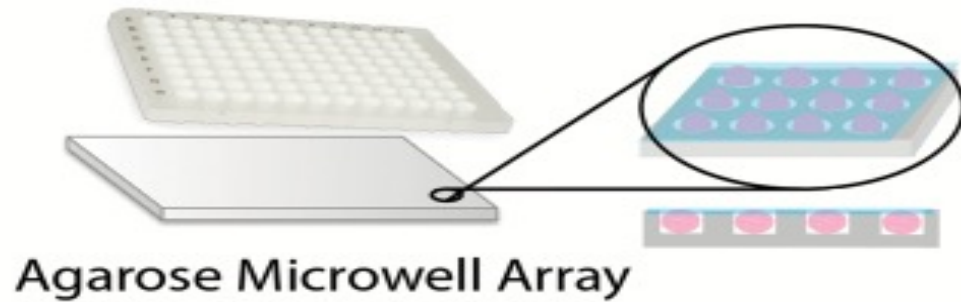
- Measure effects of H_2O_2 +/- As on DNA damage by measuring DNA migration in agarose matrix

Next lab:

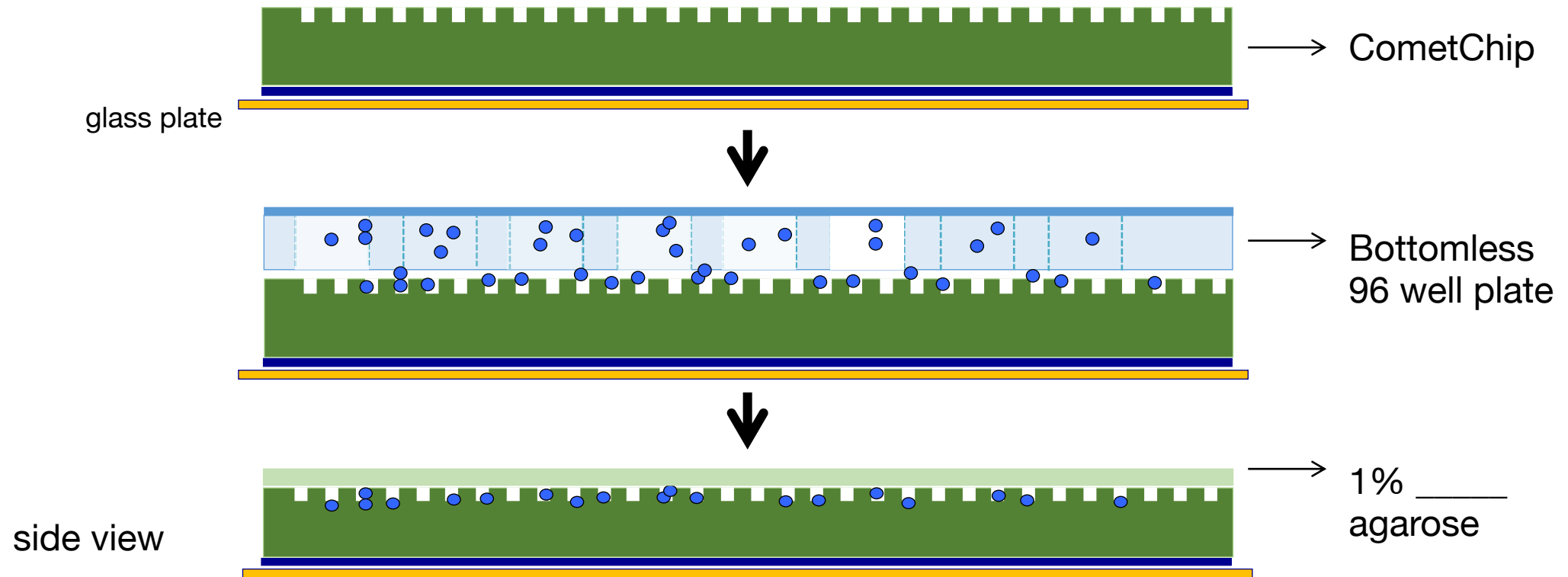
Review: CometChip creation and loading cells into wells



Macrowell Former



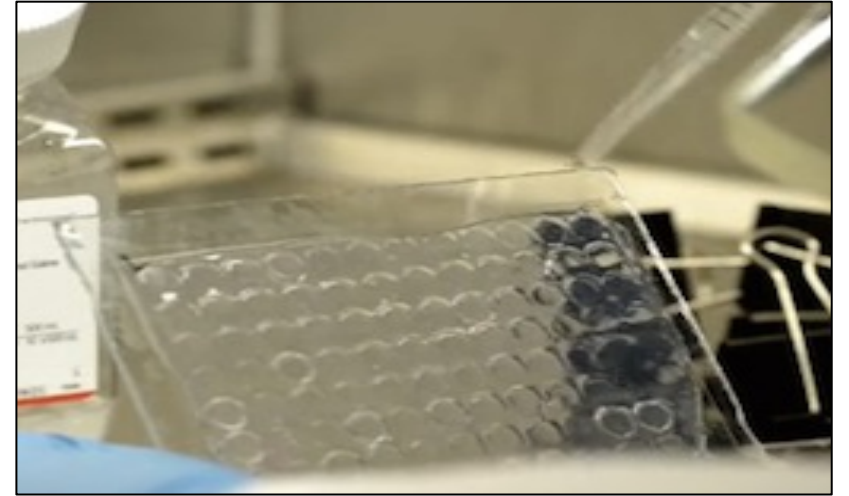
Load cells onto the CometChip



Major critical steps

- Cell loading

- Line up macrowells carefully so they are straight across gel
- Load cells and place in 37deg incubator
- Leave for 15 minutes (*why?*)

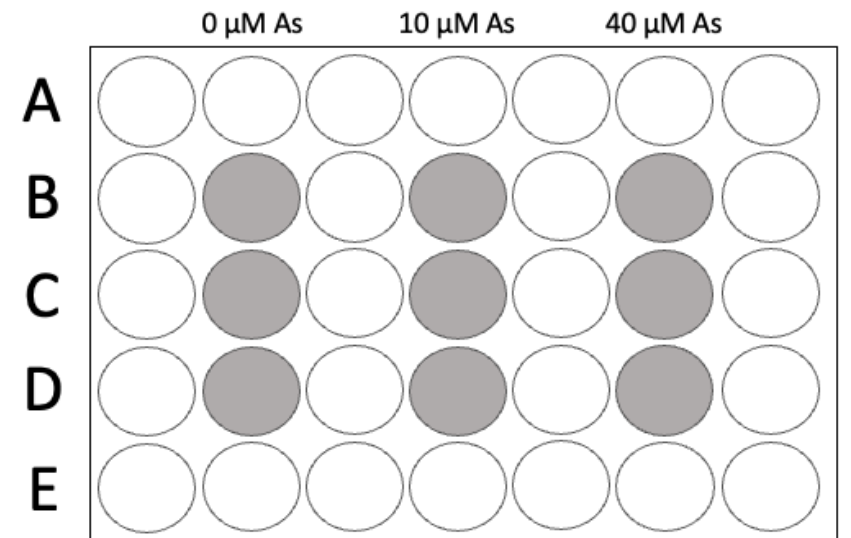


- Washing

- Not too much!
- Across the top of the glass plate
- Wash from _____ to _____ concentration

- Cover the cells

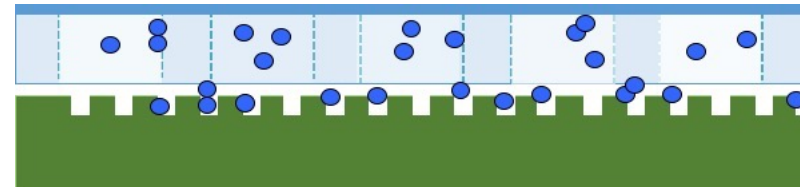
- Dispense 1ml of 1% LMP agarose over the gel with a P1000
- **1% LMP agarose gels quickly**
- Leave it undisturbed for 3 min then move to 4°C for 3 min



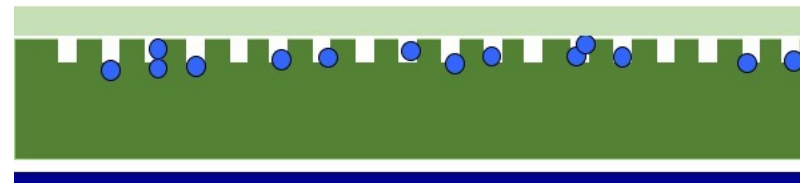
Overview of the CometChip assay: treating cells



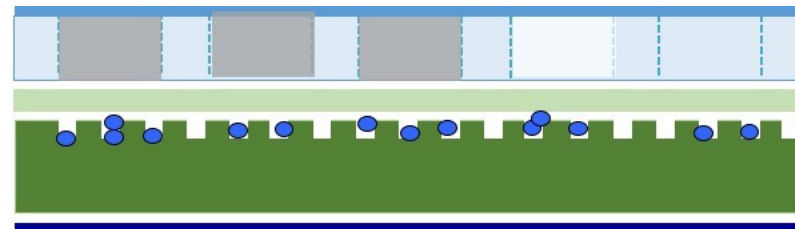
Treat with As for 4hrs



↓ 1% LMP agar



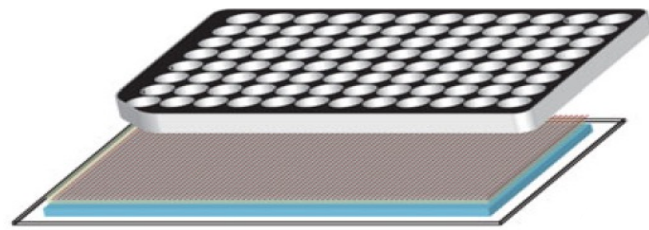
↓ Treat with H₂O₂



↓
Place in lysis buffer overnight

Overview of CometChip Assay: electrophoresis & visualization

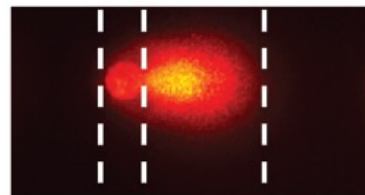
Treat captured cells in comet chip with H_2O_2 and As



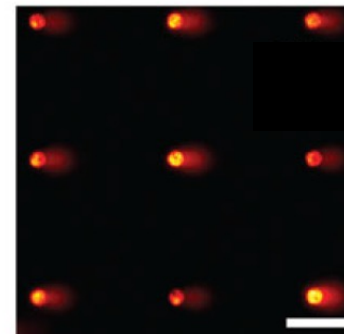
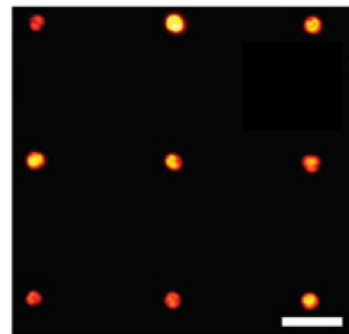
Lyse cells & unwind DNA
(DNA still captured
agarose in overlay)



Agarose Electrophoresis



Analysis
via
Python



Stain DNA and image via
fluorescence microscopy

For Today

- Seed cells for CometChip assay
- Treat cells with H₂O₂
- 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