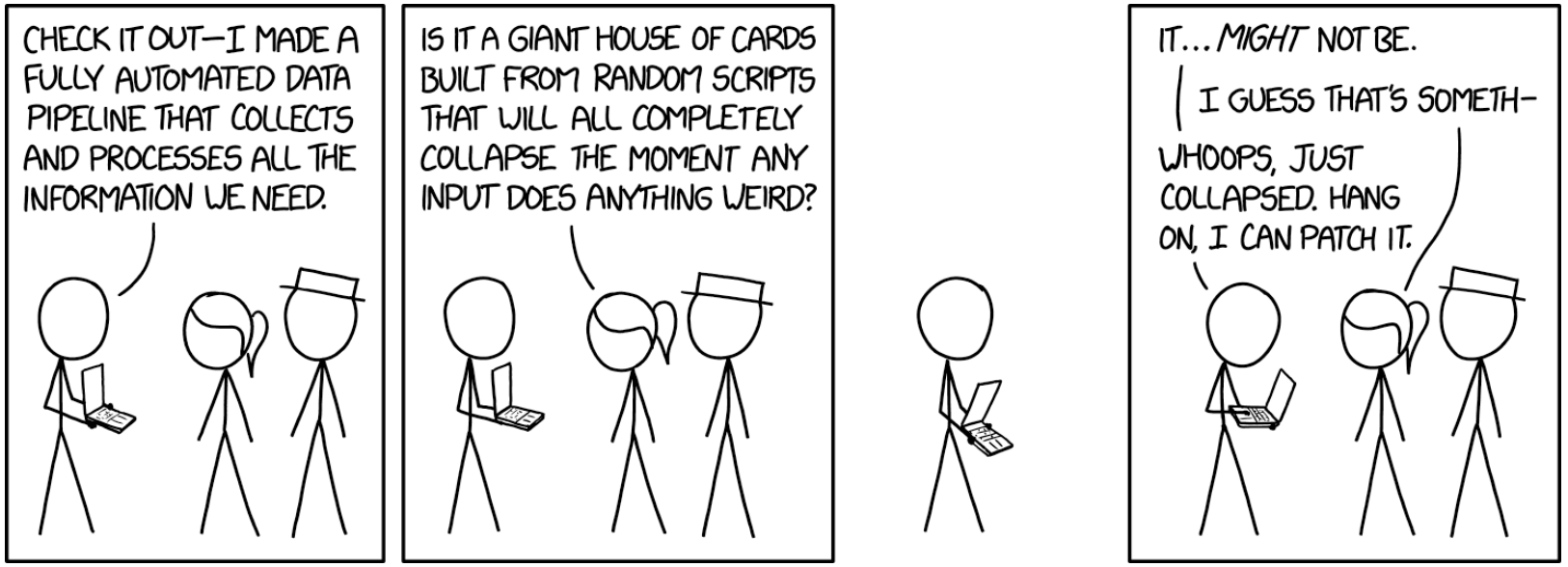
M1D6: Image and analyze high-throughput genome damage assay

1. Prelab
2. Use python to examine your CometChip data
3. Analyze CometChip data set to examine DNA damage repair



Homework

Prepare for Implications and Future Works

Answer questions to prepare for Implications and Future Works section

- ☐ What is the main conclusion for your γ-H2AX results?
  ☐ What is the main conclusion CometChip results?
  ☐ How do the results answer your research question?
  ☐ Did you observe any unexpected results or contradictory results?
  ☐ What follow-up experiments (include at least two!) would be helpful in further addressing your research question?

  - Follow up experiments can clarify results
  - Follow up experiments can build on results

Labwork

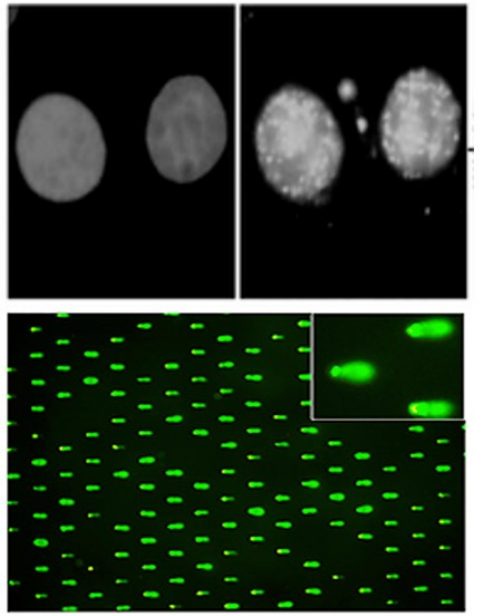
Analyze CometChip data

Mod1 Overview

Last lab:

This lab:

Next lab:



1. Use repair foci experiment to measure DNA breaks

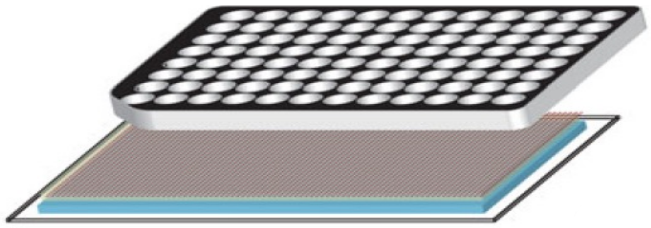
- Examine effect of H_2O_2 +/- As on double strand DNA breaks by measuring γH2AX foci formation

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

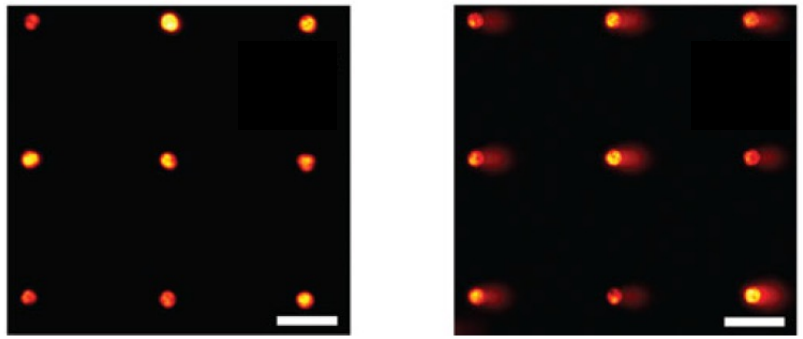
Overview of CometChip Assay: chemically treating cells and visualization

Treat captured cells in comet chip with H_2O_2 and As

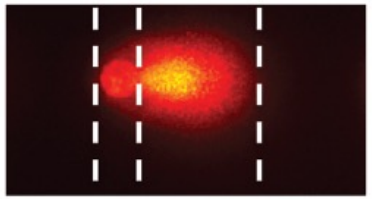


Lyse cells & unwind DNA
(DNA still captured agarose in overlay)

Agarose Electrophoresis

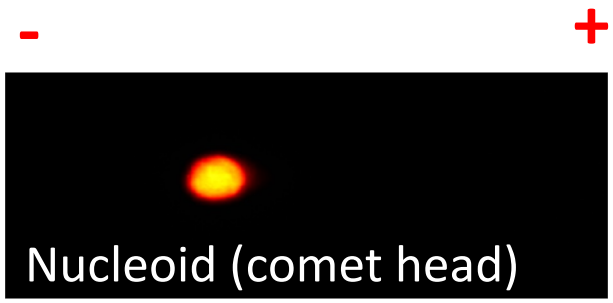


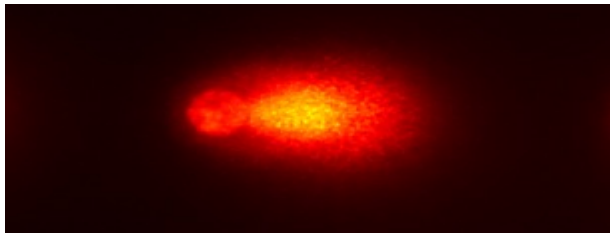
Stain DNA and image via fluorescence microscopy



Analysis via python

Output of the alkaline CometChip assay





No Damage

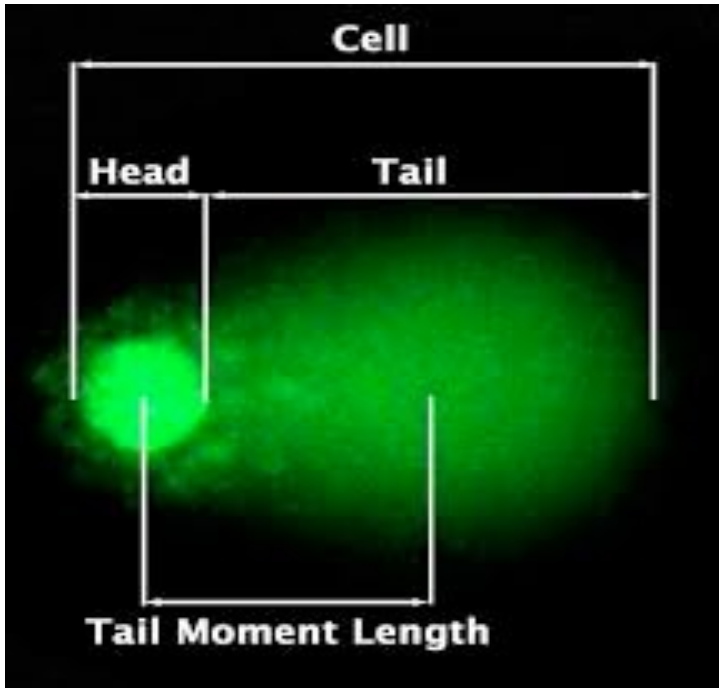
- Supercoiled nucleoid
- Little or no migration

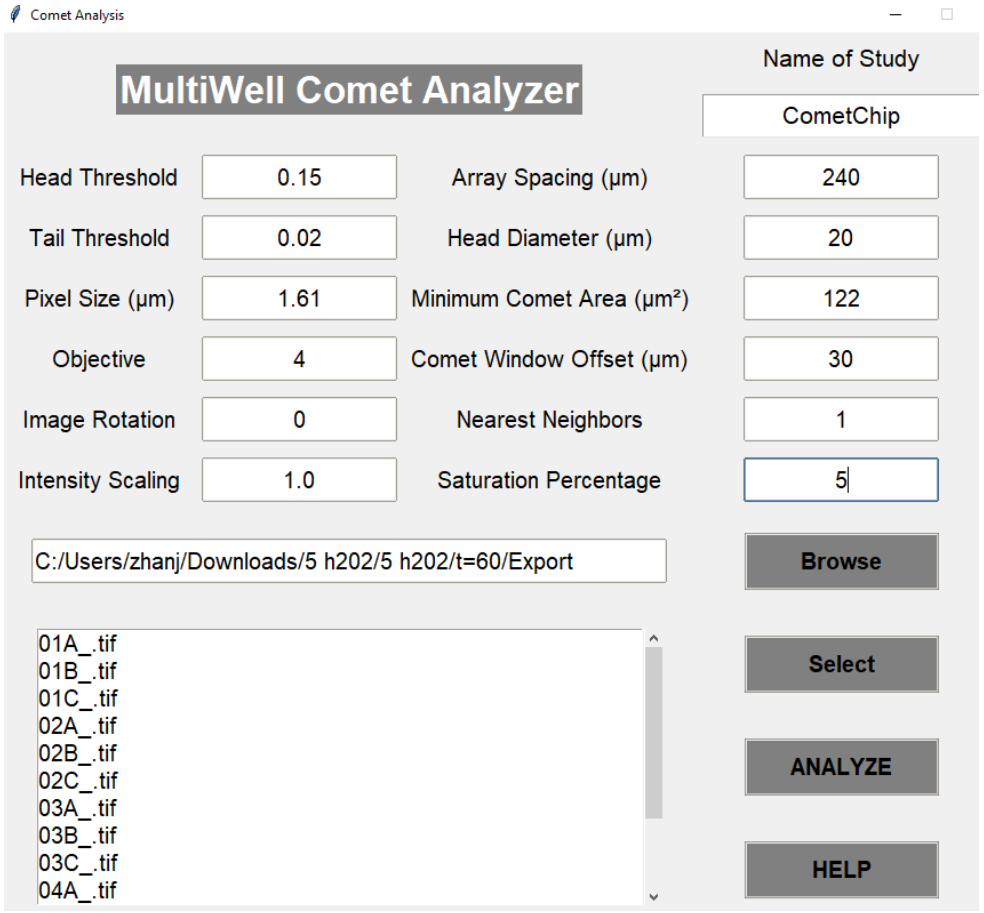
High Damage

- SSBs, DSBs, abasic sites, alkali labile sites, sites of incomplete excision repair
- Forms a "comet tail"

* Nuclear DNA normally supercoiled
    * DNA breaks and fragmentation releases tension
        * Unwound DNA will migrate in response to electrical current to create comet

How will you assess and analyze CometChip data?

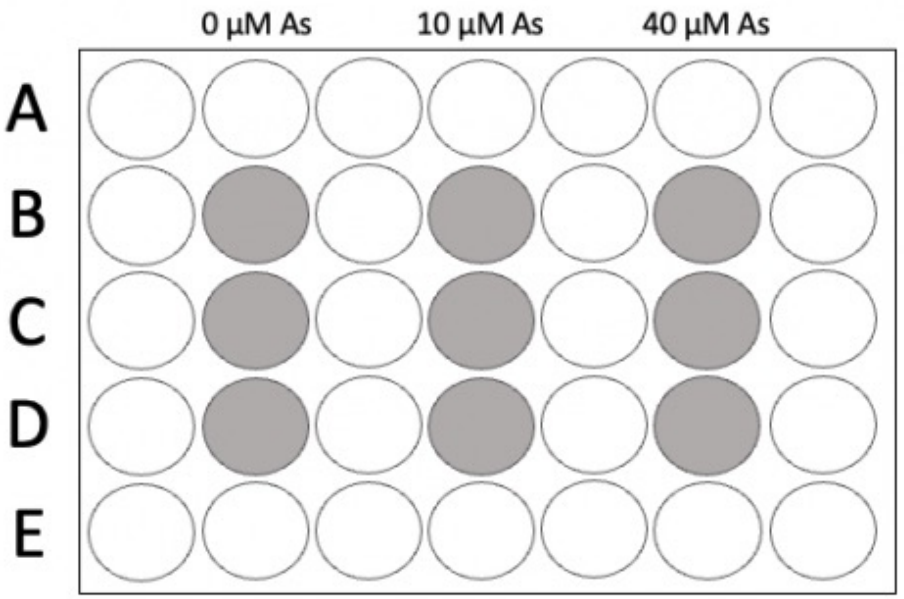




- Assess comet images with python code
  - Recommended parameters capture tail features

Identifying and processing CometChip images

- The naming scheme is similar to H2AX data image names
  - Each macrowell is an individual image
  - 0H40A_001



- Use Excel to analyze compiled CometChip data
  - Percent Head DNA
  - Percent Tail DNA
  - OTM
- First sheet will have summary of each image
  - Subsequent sheets will show numbers for each comet measured
- Graph % Tail DNA for Data Summary

How can CometChip be used in the lab beyond your experiment?

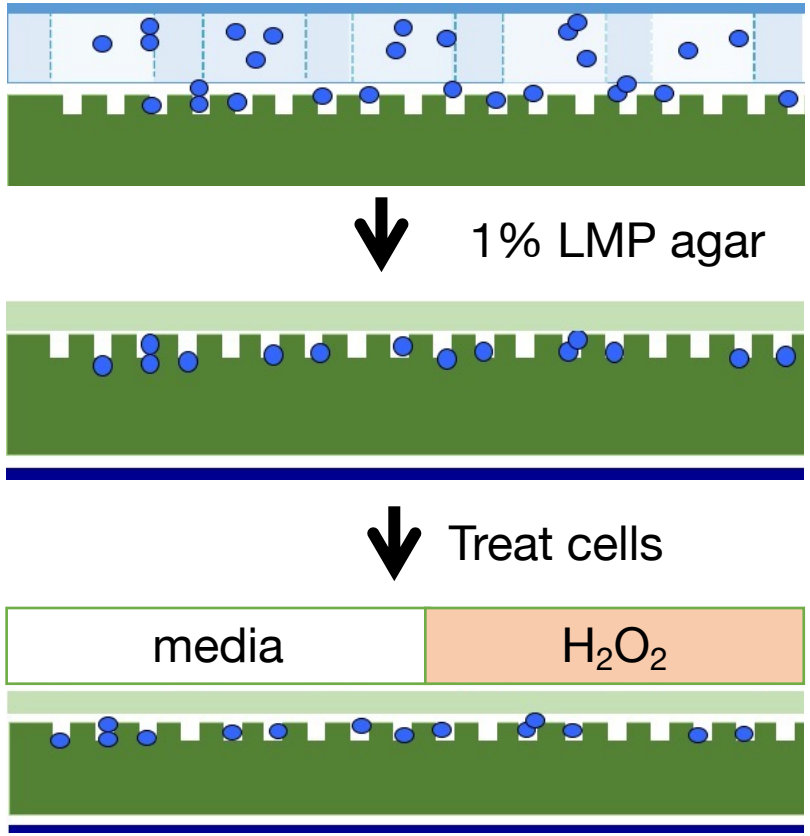
- High throughput compared to yH2AX assay
  - **H2AX**: image data coverslip by **coverslip**
  - **CometChip**: image well by well on a **large gel**

- Comet Chip can be loaded with the same cell population and then divided into different treatment conditions
  - Do a **dose-response curve** for different concentrations of arsenic
  - Do a **timecourse** to examine repair kinetics following DNA damage

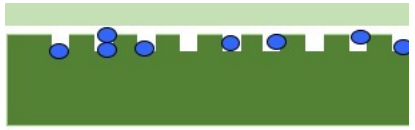
- You will analyze data from a timecourse with multiple [arsenic] for the **Data Summary**

Overview of the repair CometChip assay

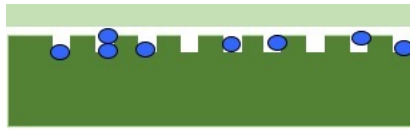
Treat with As for 4hrs



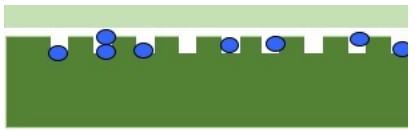
-15min recovery Baseline



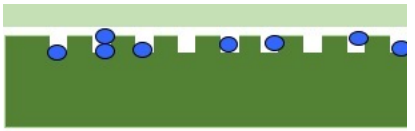
0min recovery
Place directly in lysis buffer



30min recovery



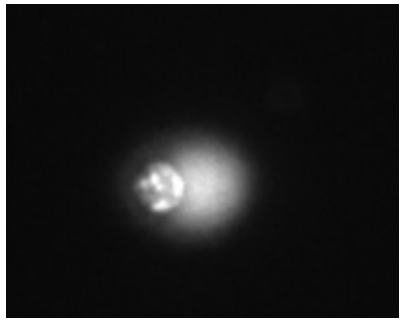
60 min recovery



Examine CometChip images for visual examples to include in Data Summary Figure

- Can use example individual comets for each condition
- Pull them out of ImageJ

No Treatment



40uM As + 10uM H_2O_2

For Today

1. Use python to analyze comets from CometChip experiments
2. Analyze repair CometChip data from linked Excel sheet
3. Begin work on Data Summary

For M1D7

- Answer the Homework questions to frame your Implications & Future Works section for the Data Summary

- With your lab partner, revise your methods draft and add methods for M1D3