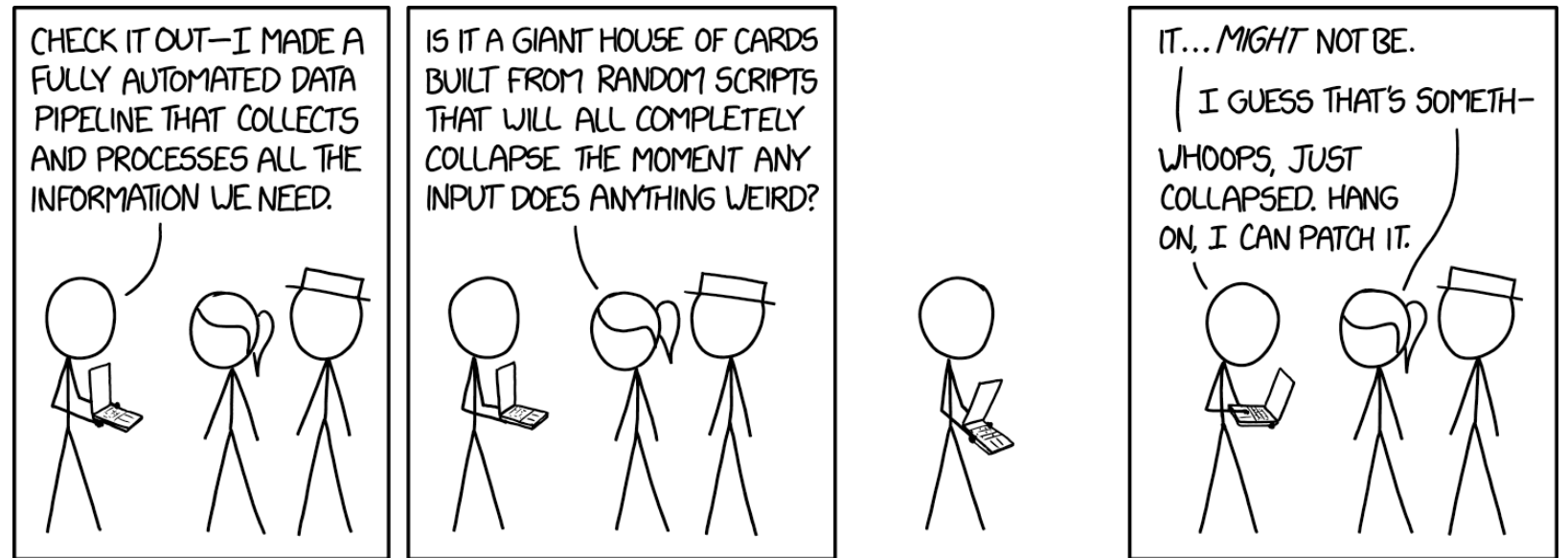


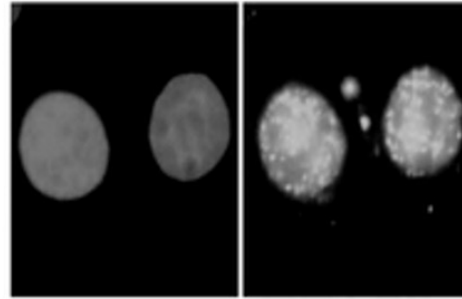
M1D6: Image and analyze high-throughput genome damage assay

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



Mod1 Overview

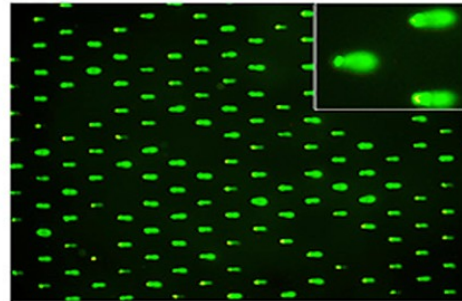
Last lab:



1. Use repair foci experiment to measure DNA breaks

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

This lab:



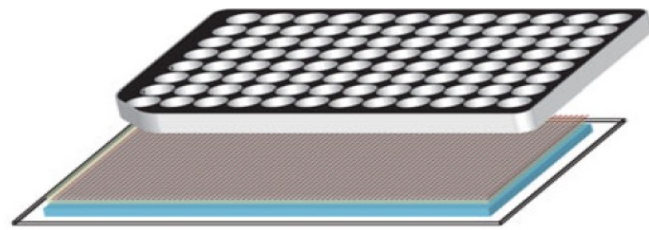
2. Use high-throughput genome damage assay to measure DNA damage

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

Next lab:

Overview of CometChip Assay: chemically treating cells and visualization

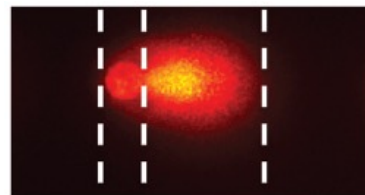
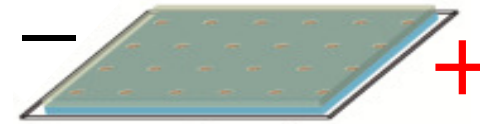
Treat captured cells in comet chip with H_2O_2 and As



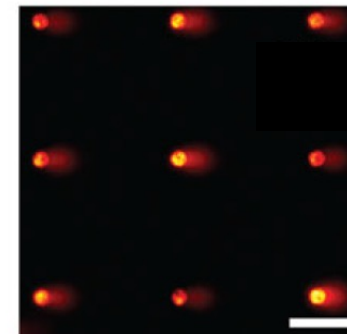
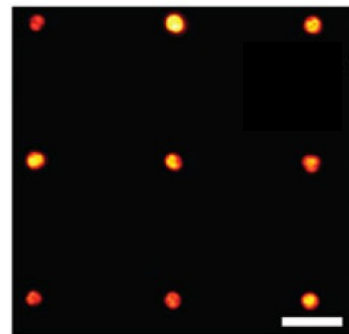
Lyse cells & unwind DNA
(DNA still captured
agarose in overlay)



Agarose Electrophoresis

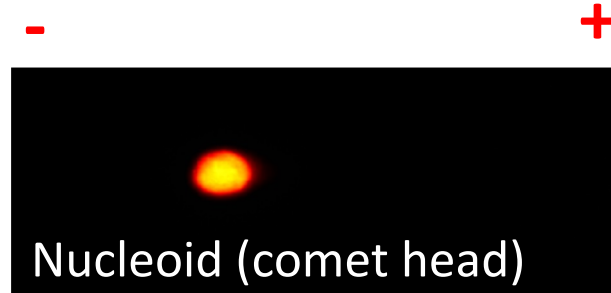


Analysis
via
Matlab



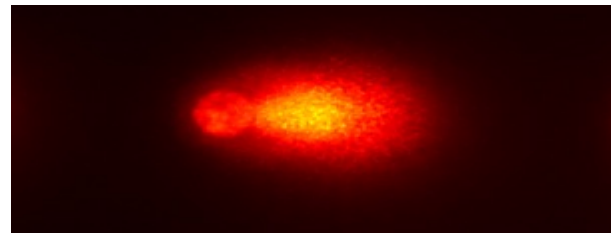
Stain DNA and image via
fluorescence microscopy

Output of the alkaline CometChip assay



No Damage

- Supercoiled nucleoid
- Little or no migration



High Damage

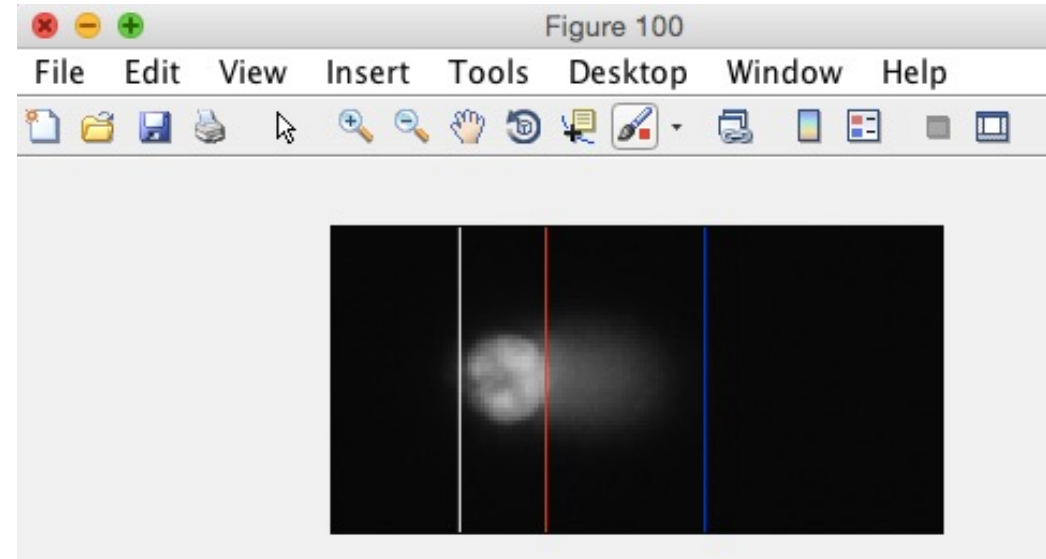
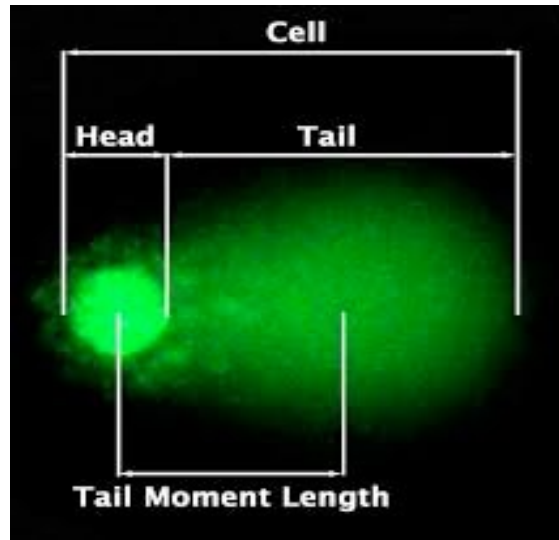
- SSBs, DSBs, abasic sites, alkali labile sites, sites of incomplete excision repair
- forms a "comet tail"

* 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 in MATLAB
 - Do recommended parameters (on wiki) accurately measure most comets in your sample?
- Have a “class data example” folder in Dropbox for analysis if your data is confusing
- Use Excel to analyze compiled CometChip data
 - Graph % Tail DNA for Data Summary

Data image labels

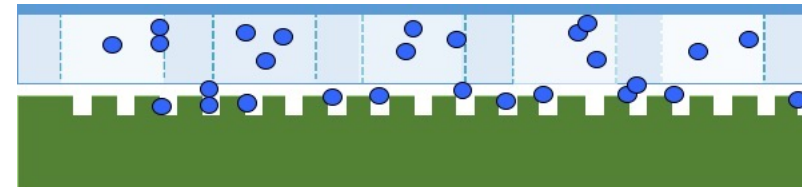
- The MATLAB script requires a specific naming scheme
- Use these image names to decode your data

- `_01A_ / _02A_ / _03A_` = column A = No H2O2 No As
- `_01B_ / _02B_ / _03B_` = column B = No H2O2 10uM As
- `_01C_ / _02C_ / _03C_` = column C = No H2O2 40uM As
- `_01D_ / _02D_ / _03D_` = column D = H2O2 No As
- `_01E_ / _02E_ / _03E_` = column E = H2O2 10uM As
- `_01F_ / _02F_ / _03F_` = column F = H2O2 40uM As

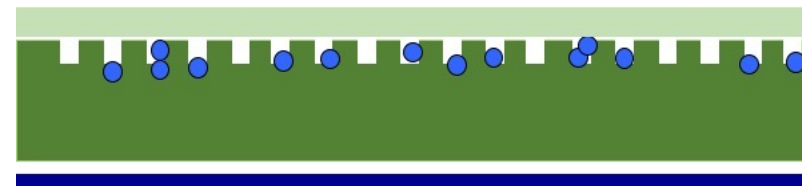
Overview of the repair CometChip assay



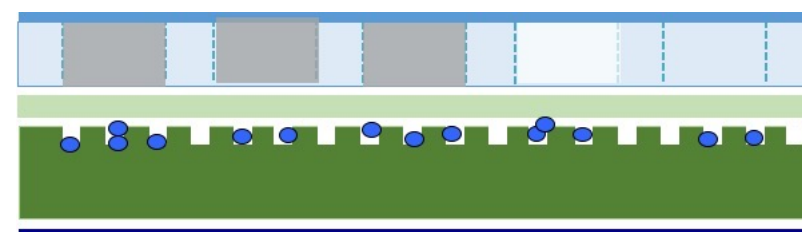
Treat with As for 24hrs



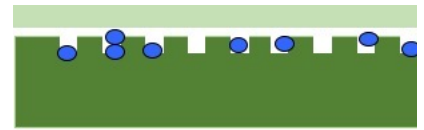
↓ 1% LMP agar



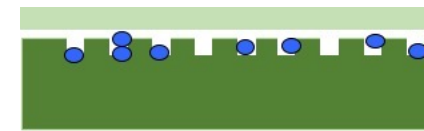
↓ Treat with H₂O₂



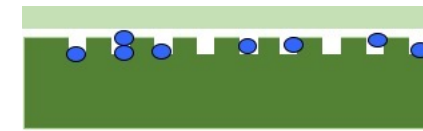
-15min
recovery
Baseline



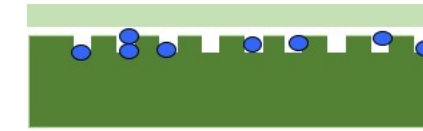
0min recovery
Place directly in lysis
buffer



30min
recovery



60 min
recovery

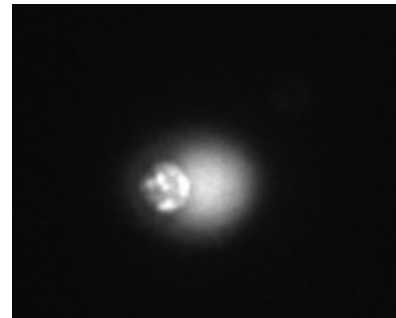


Examine CometChip images for visual examples to include in Data Summary Figure

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



No Treatment



40uM As + 5uM H₂O₂

For Today

1. Use Matlab 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

Notes on homework

- Homework in total = 10% of the final grade
- Goal:
 - tell you how to start
 - have you practice using wiki and prelab guidelines
 - grade as though it's a final assignment so you know where you need to get
- Homework grades are always low (past classes average ~ 80%)
 - Homework grades increase throughout the semester (repeat assignments)
- Anytime you want to talk about how you are doing in the class- just ask!