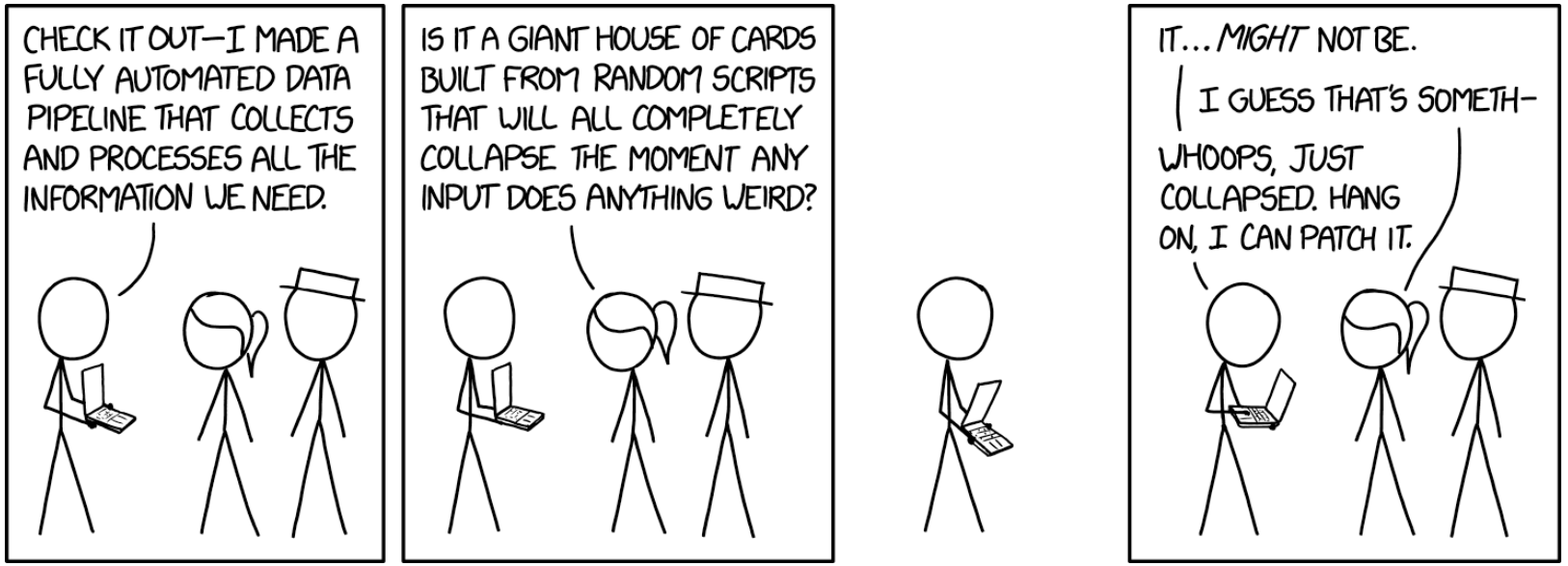
M1D6: Image and analyze high-throughput genome damage assay

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



Mod1 Overview

Last lab:

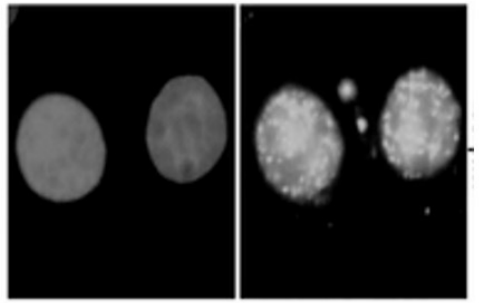
Comet Chip EXP

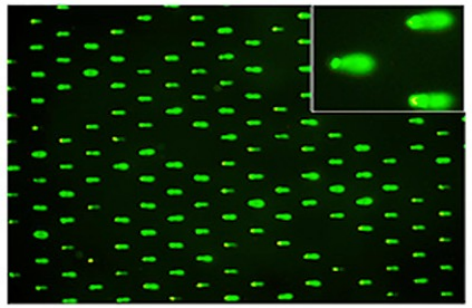
This lab:

MATLAB analysis

Next lab:

Stats / Wrap up module





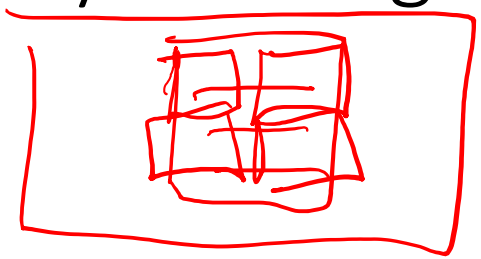
1. Use repair foci experiment to measure DNA breaks

- 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

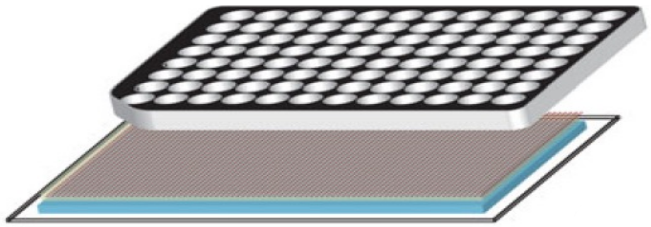
- Measure effects of H_2O_2 +/- As on DNA damage by measuring DNA migration in agarose matrix

Overview of CometChip Assay: chemically treating cells and visualization



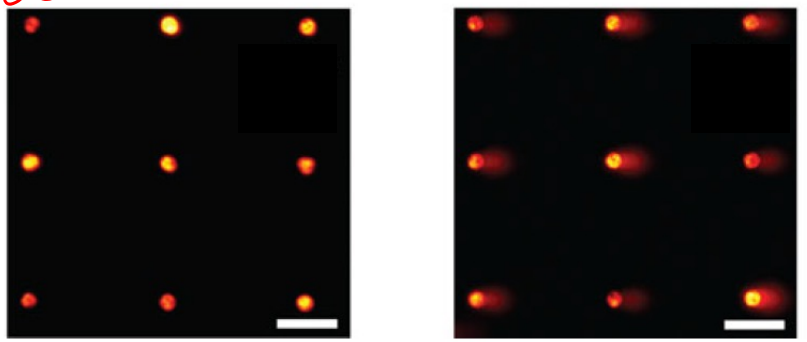
16V
300mA

Treat captured cells in comet chip with H_2O_2 and As



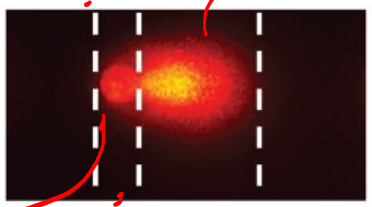
Lyse cells & unwind DNA
(DNA still captured agarose in overlay)

Agarose Electrophoresis



SYBR Gold

Stain DNA and image via fluorescence microscopy



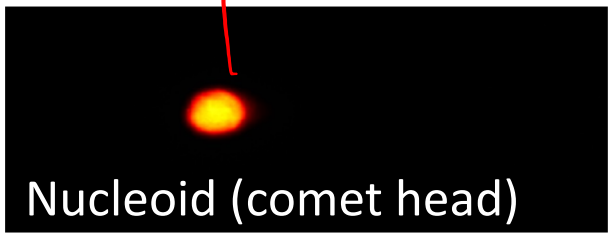
escaped DNA from microwell

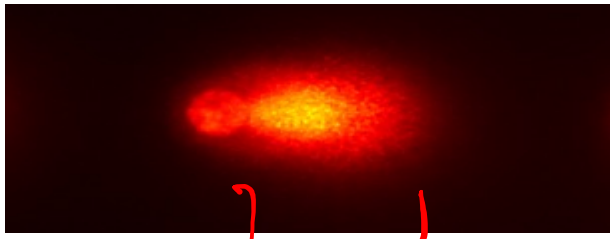
segment DNA

microwell

Analysis via Matlab

Output of the alkaline CometChip assay





No Damage

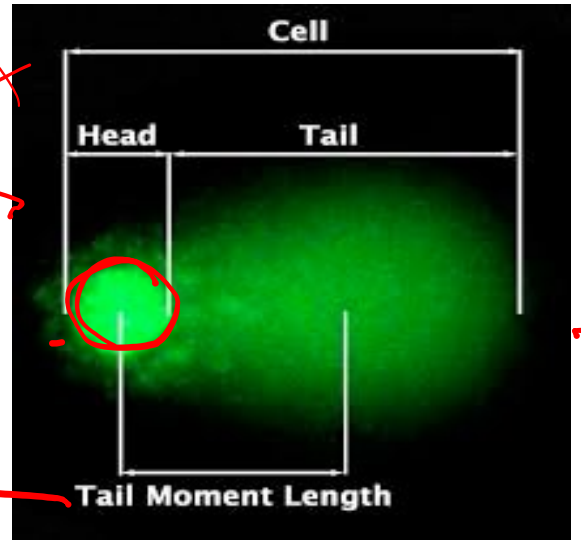
- Supercoiled nucleoid
- Little or no migration

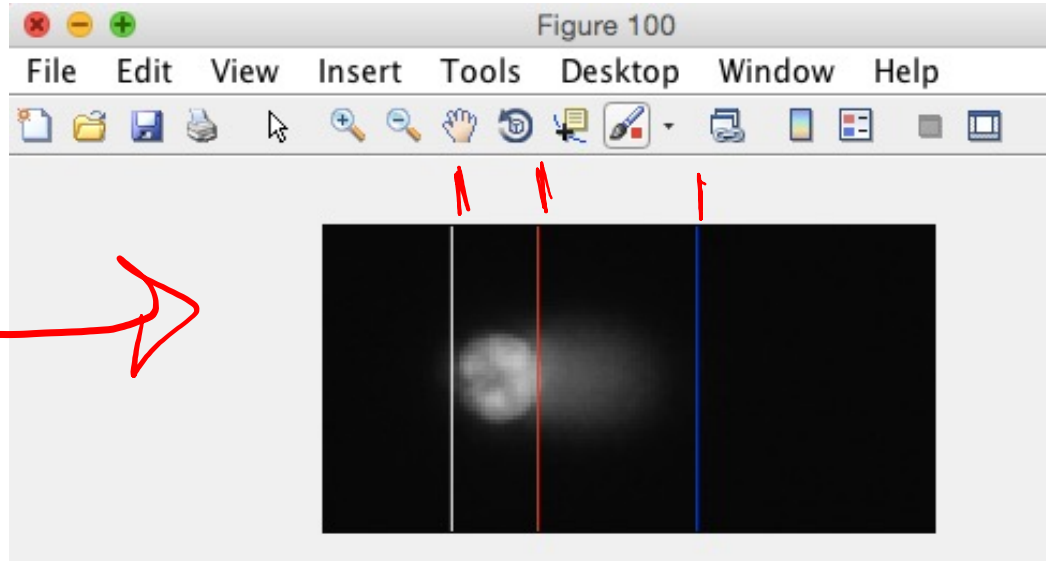
High Damage

- 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

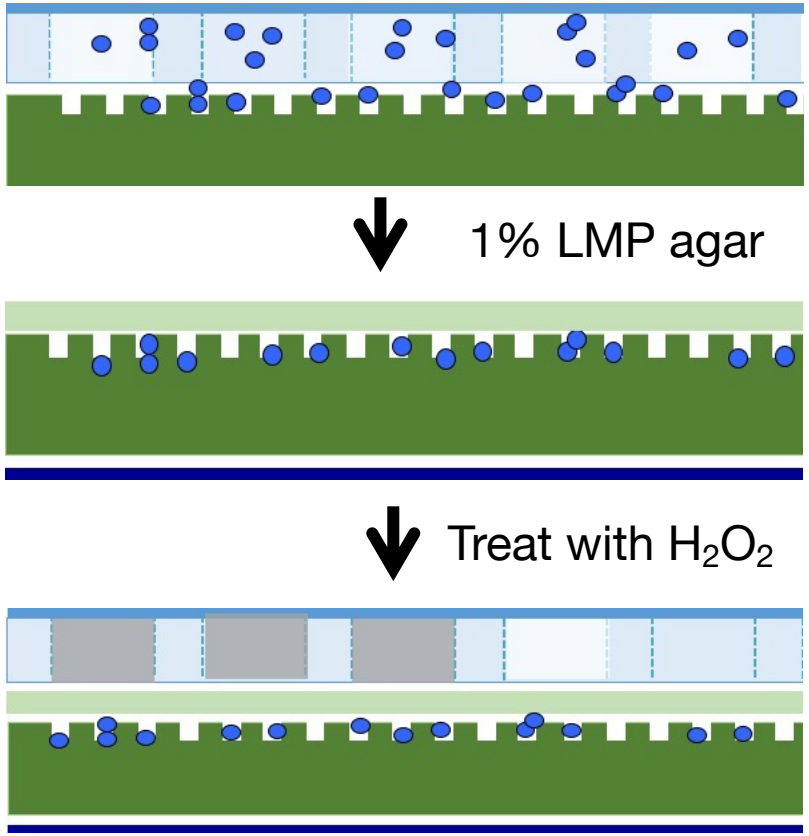
Data image labels

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

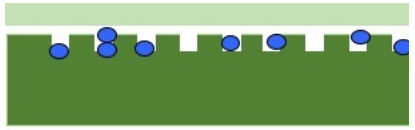
- _01A_ / _02A_ / _03A_ = column A = No H202 No As
- _01B_ / _02B_ / _03B_ = column B = No H202 10uM As
- _01C_ / _02C_ / _03C_ = column C = No H202 40uM As
- _01D_ / _02D_ / _03D_= column D = H202 No As
- _01E_ / _02E_ / _03E_ = column E = H202 10uM As
- _01F_ / _02F_ / _03F_ = column F = H202 40uM As

Overview of the repair CometChip assay

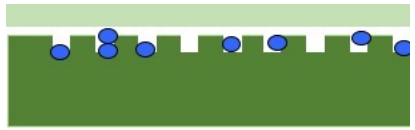
Treat with As for 24hrs



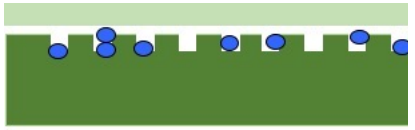
-15min recovery Baseline



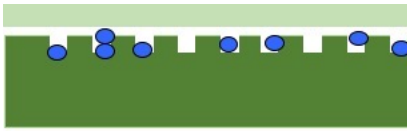
0min recovery
Place directly in lysis buffer



30min recovery



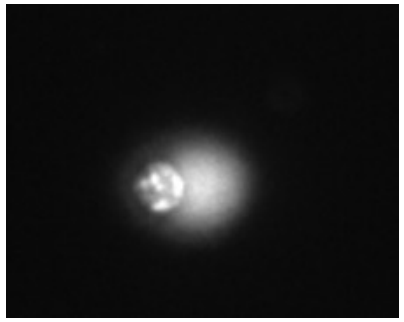
60 min recovery



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 H_2O_2

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!