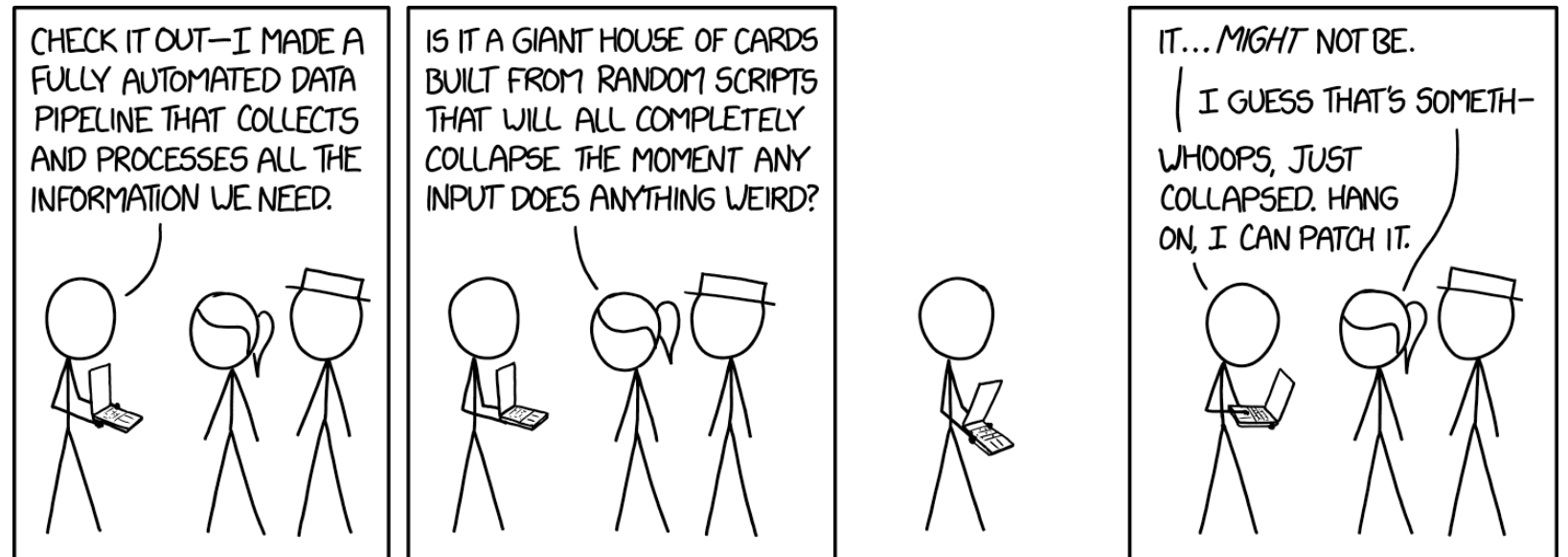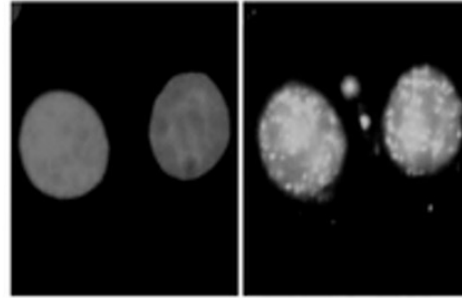# M1D6: Image and analyze high-throughput genome damage assay

1. Prelab
2. Use Matlab to examine your CometChip data
3. Analyze CometChip data set to examine DNA damage repair
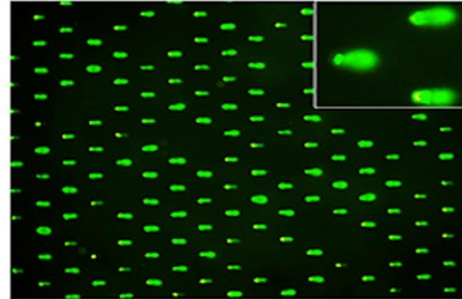
Mod1 Overview

Last lab:

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

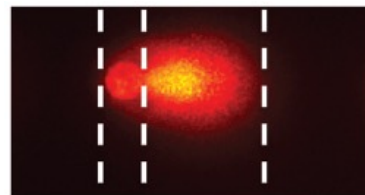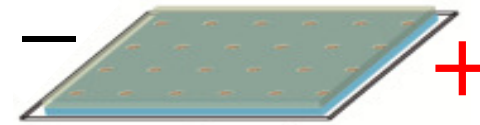# Overview of CometChip Assay: chemically treating cells and visualization

Treat captured cells in comet chip with H_2O_2 and As
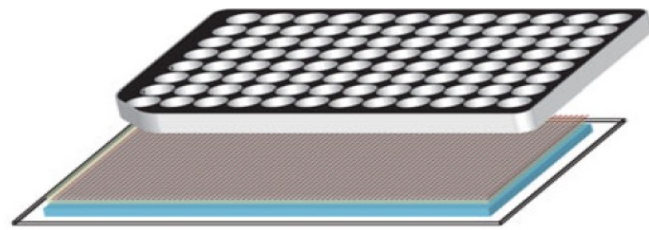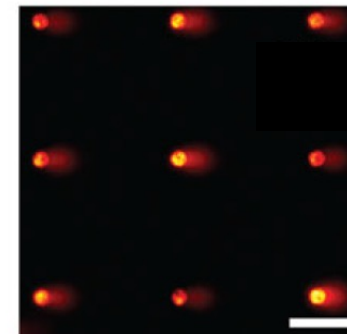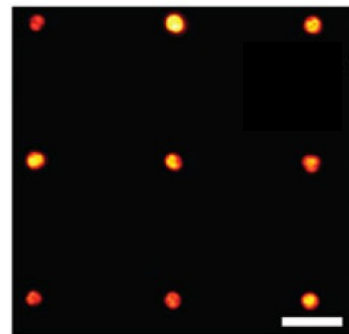

Lyse cells & unwind DNA
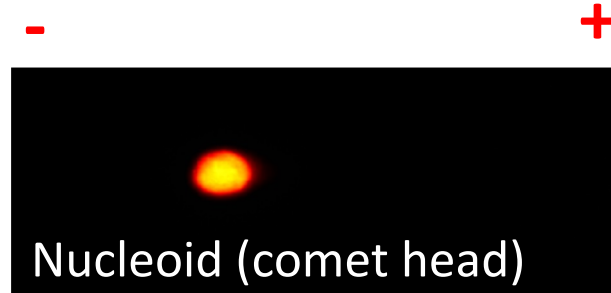(DNA still captured agarose in overlay)

Agarose Electrophoresis

Analysis via Matlab

Stain DNA and image via fluorescence microscopy

Output of the alkaline CometChip assay

- +

Nucleoid (comet head)

No Damage
- Supercoiled nucleoid
- Little or no migration
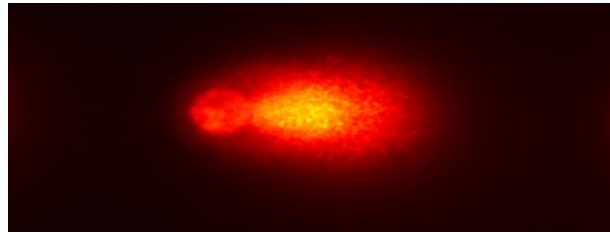
High Damage
- SSBs, DSBs, abasic sites, alkali labile sites, sites of incomplete excision repair
- forms a "comet tail"

* 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?

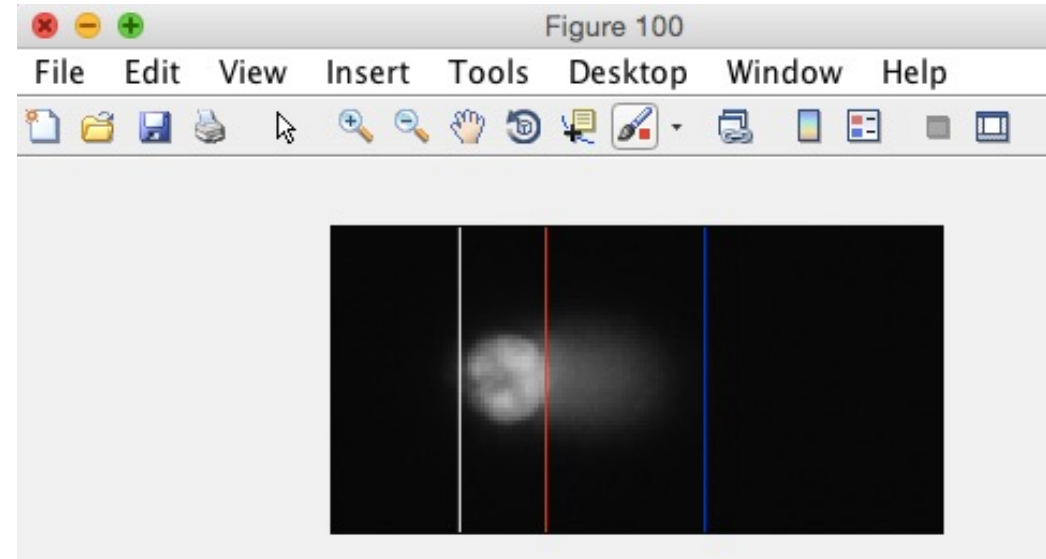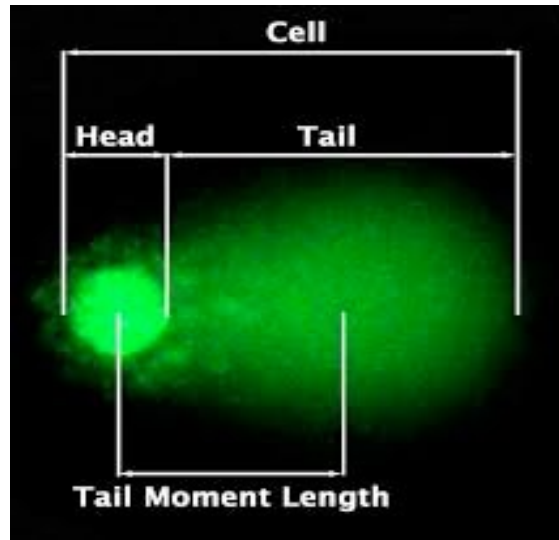- Assess comet images in MATLAB
  - Do recommended parameters (on wiki) accurately measure most comets in your sample?

- 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

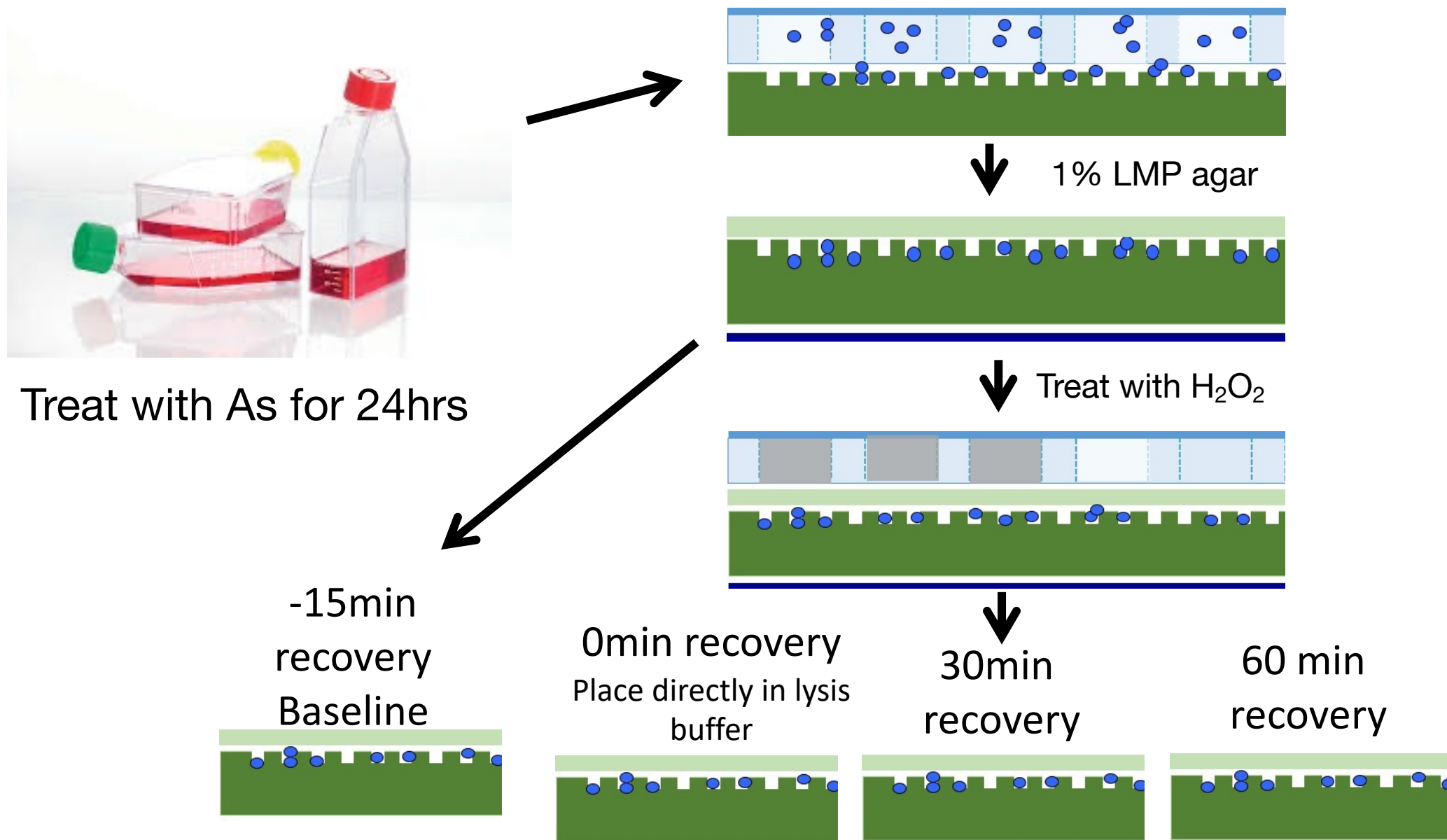# Overview of the repair CometChip assay

Treat with As for 24hrs

1% LMP agar

Treat with H_2O_2

-15min recovery
Baseline

0min recovery
Place directly in lysis buffer
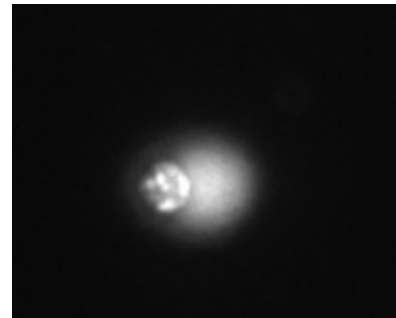
30min recovery

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)

- <u>Anytime</u> you want to talk about how you are doing in the class- just ask!