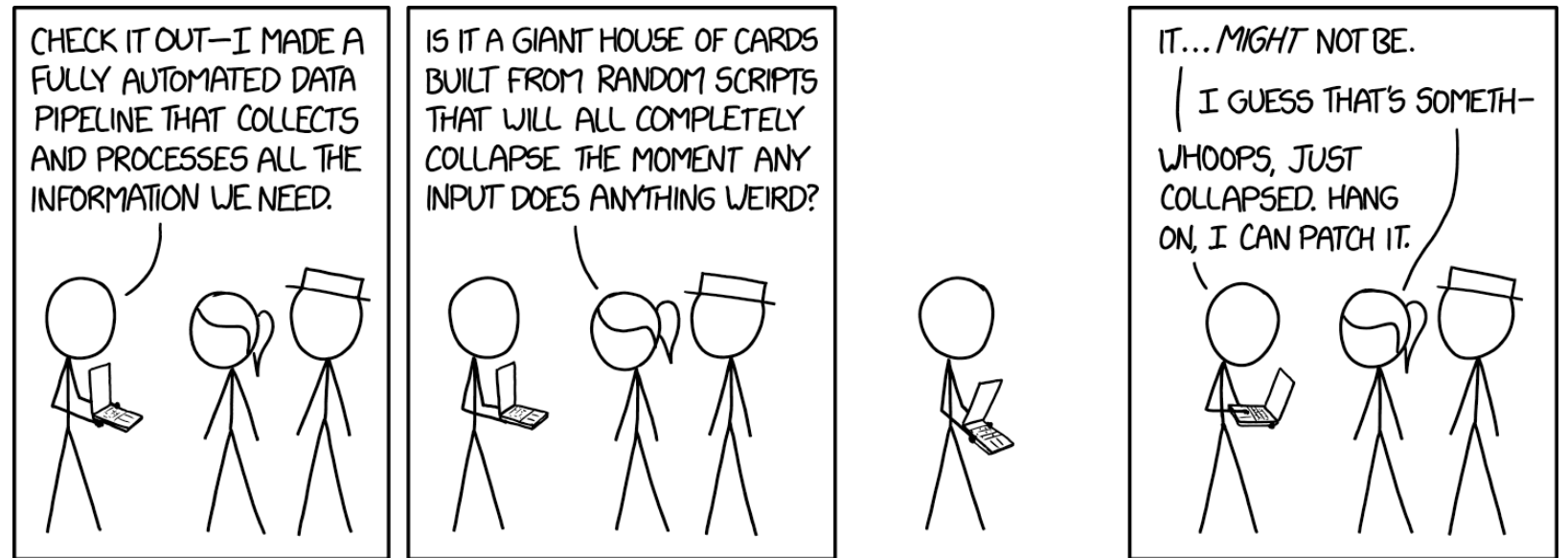


# M1D6: Image and analyze high-throughput genome damage assay

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



# Mod1 Overview

## Last lab:

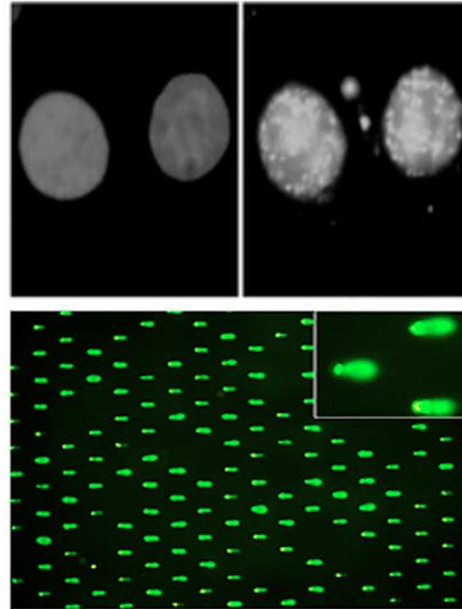
H<sub>2</sub>O<sub>2</sub> Treatment of As  
Pretreated Cells, Loaded into  
CometChip

## This lab:

MATLAB analysis

## Next lab:

Stats & Wrap up Module



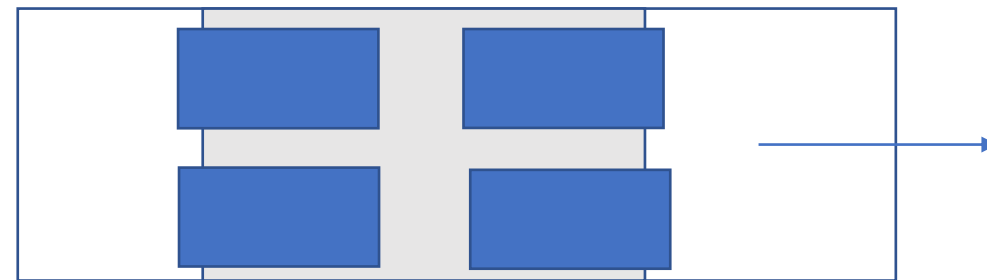
### **1. Use repair foci experiment to measure DNA breaks**

- Examine effect of H<sub>2</sub>O<sub>2</sub> +/- As on double strand DNA breaks by measuring  $\gamma$ H2AX foci formation

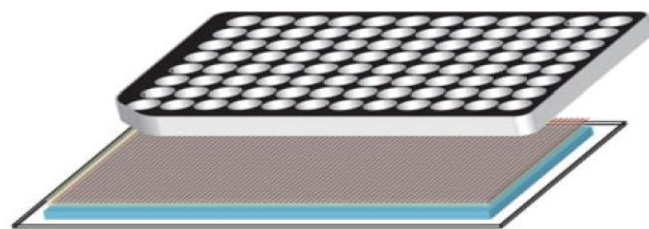
### **2. Use high-throughput genome damage assay to measure DNA damage**

- Measure effects of H<sub>2</sub>O<sub>2</sub> +/- 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

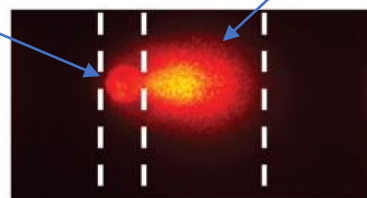


Lyse cells & unwind DNA  
(DNA still captured  
agarose in overlay)

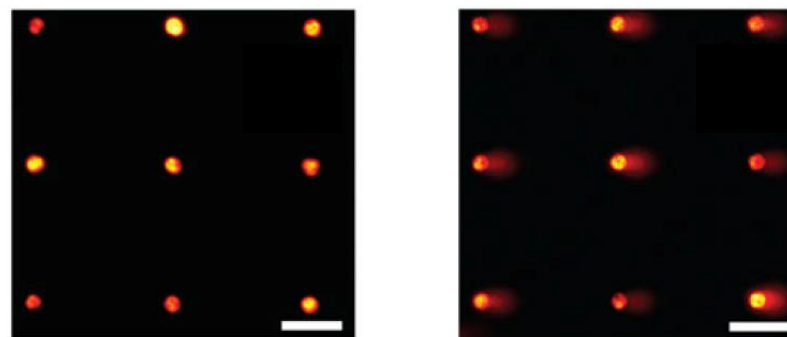
Agarose Electrophoresis



Microwell



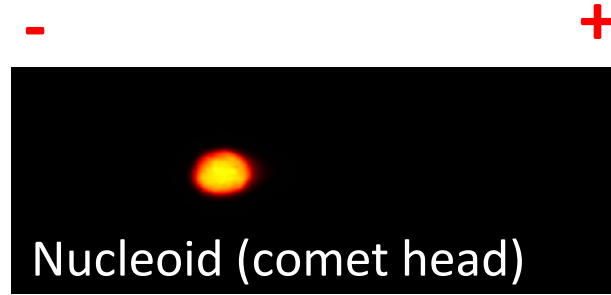
Analysis  
via  
Matlab



Stain DNA and image via  
fluorescence microscopy

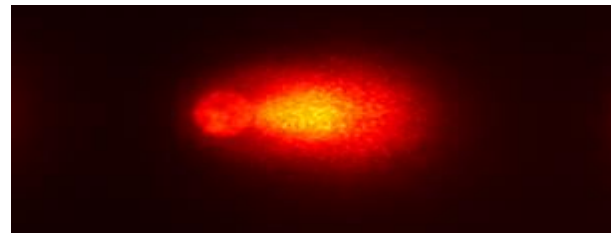
SYBR Gold  
staining

# Output of the alkaline CometChip assay



No Damage

- Supercoiled nucleoid
- Little or no migration



High Damage

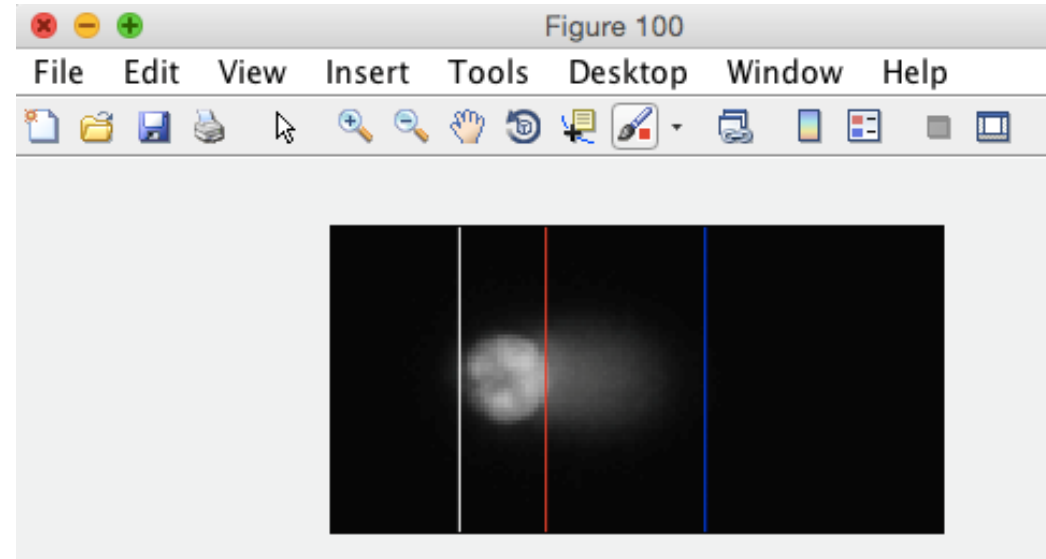
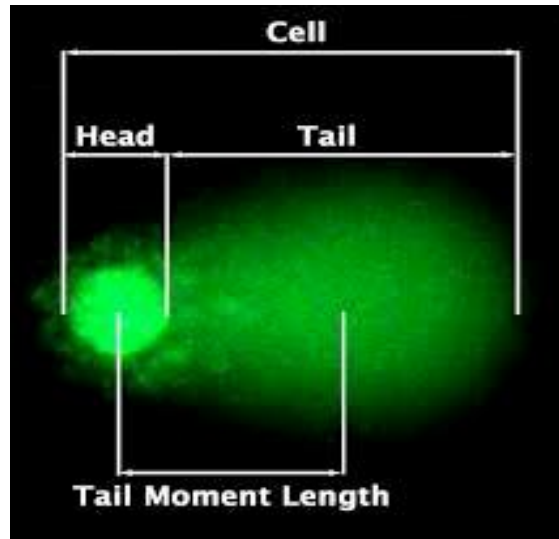
- SSBs, DSBs, abasic sites, alkali labile sites, sites of incomplete excision repair
- forms a “comet tail”

\* 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?



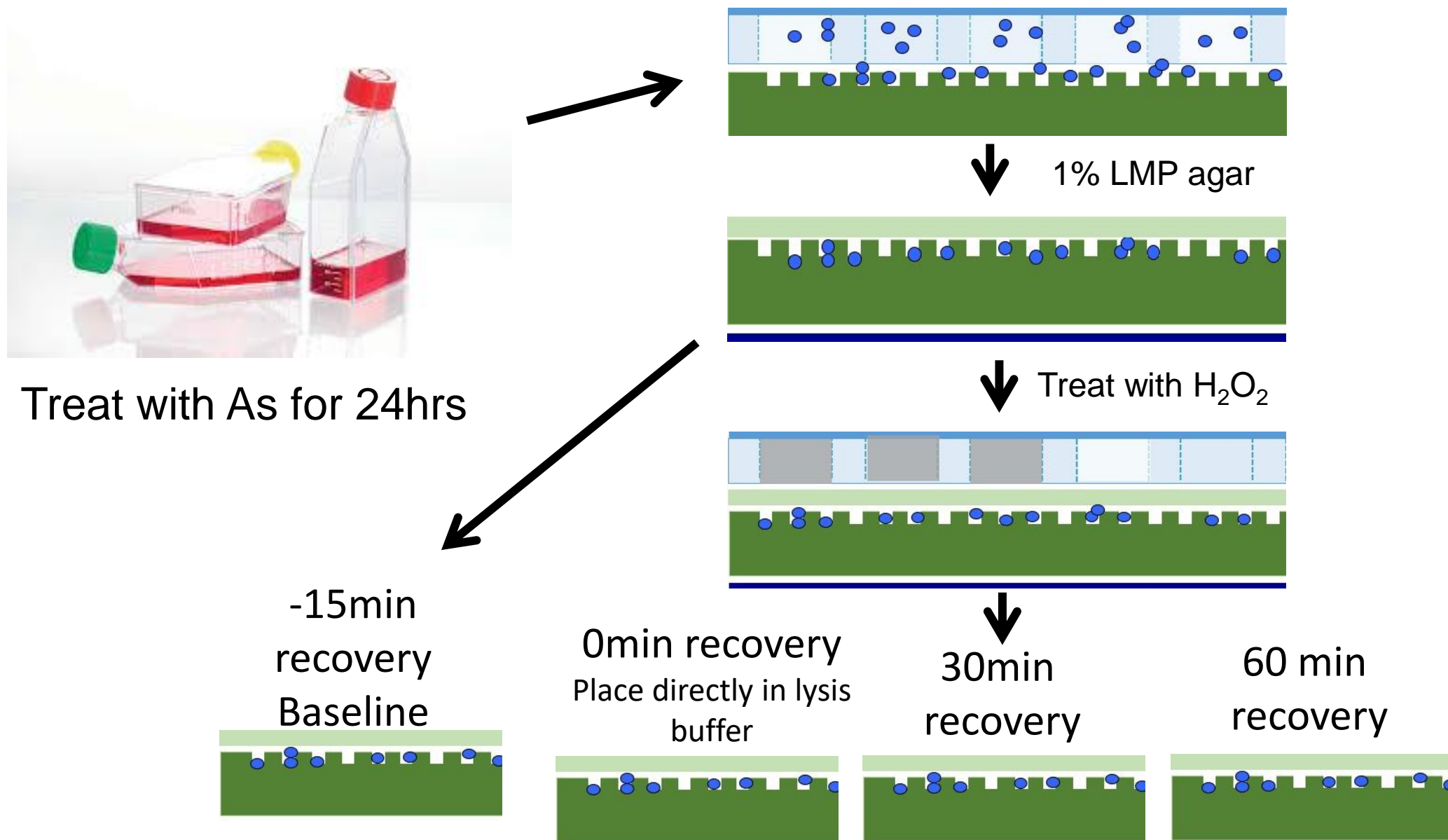
- Assess comet images in MATLAB
  - Do recommended parameters (on wiki) accurately measure most comets in your sample?
- Have a “class data example” folder in Dropbox for analysis if your data is confusing
- Use Excel to analyze compiled CometChip data
  - Graph % Tail DNA for Data Summary

Median?

# 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

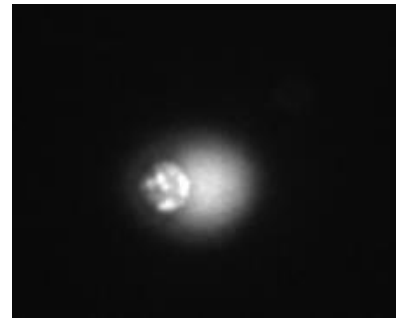


# Examine CometChip images for visual examples to include in Data Summary Figure

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



No Treatment



40uM As + 5uM H<sub>2</sub>O<sub>2</sub>



# Prepping your data for MATLAB Online

To adjust the parameters MATLAB needs to accurately determine head vs tail, you must feed it ONE sample image containing comets

Unfortunately, MATLAB online will only allow a max of 20 comets to be analyzed

Workaround – Crop an image containing  $< 20$  comets in ImageJ and use that to set your comet parameters.

# 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