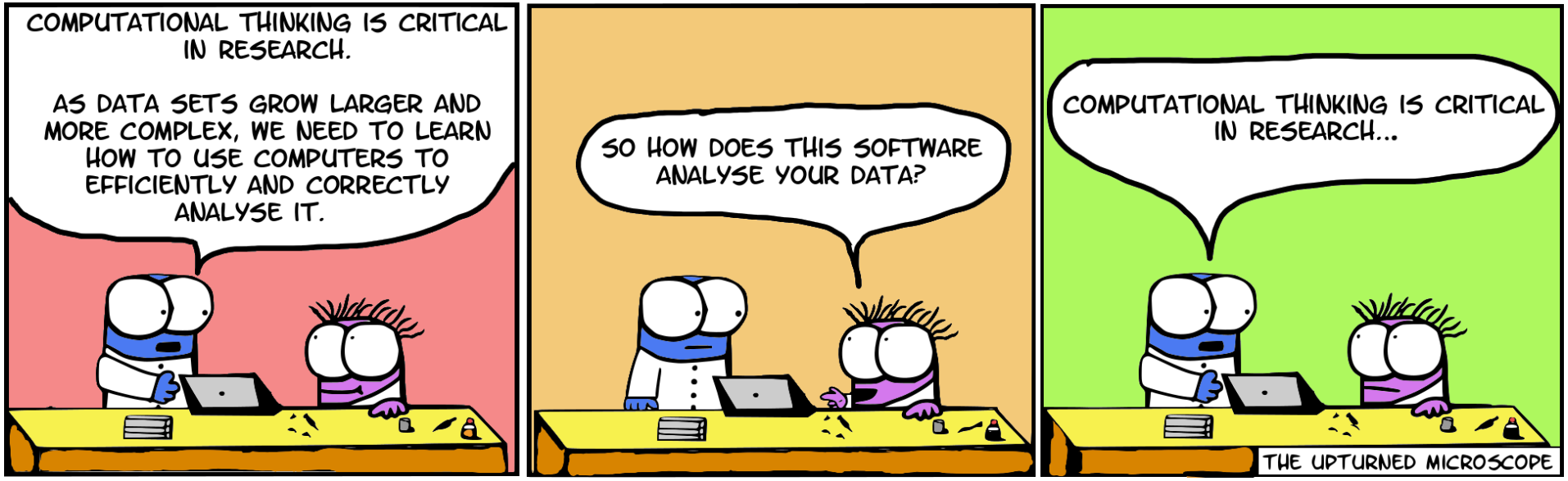
M1D6: Image and analyze high-throughput genome damage assay

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



Reiterating notes on Homework submission

- Be sure your name is in the file name **and** in the document itself

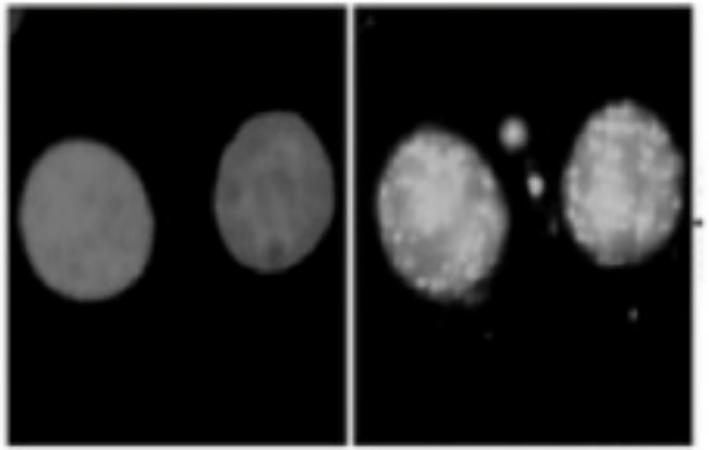
- For group assignments, submit one copy of the homework using your team color for the file name

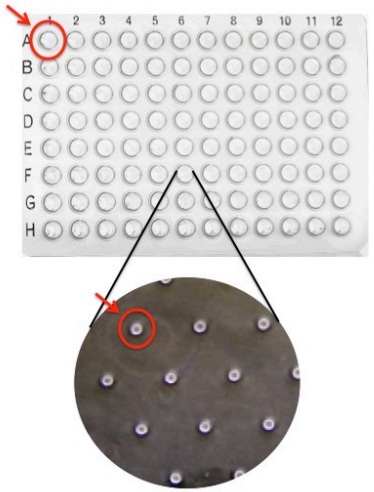
BeckyM_M1D7

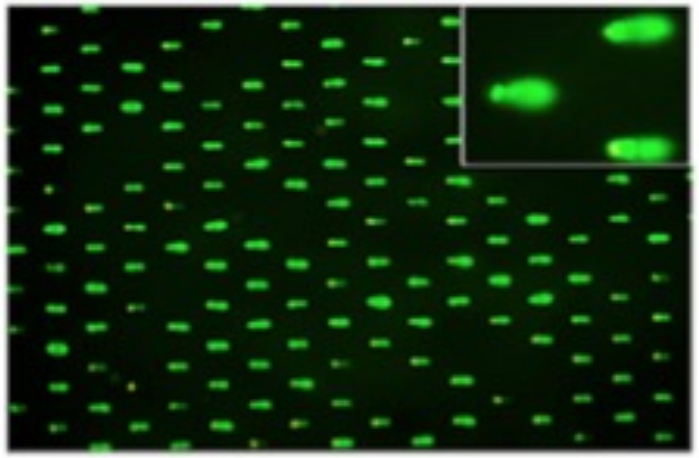
TR_Teal_DataSummary

Overview of Module 1: Measuring Genomic Instability

Research question: Does exposure to As inhibit, or decrease, repair of H_2O_2-induced DNA damage, raising the possibility that combined exposure is an important risk to public health?







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

- Immunofluorescence (IF)
  - Cells attached to glass coverslips
- Cellular response to DNA damage

Analysis: Intensity Foci

DSBs in context

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

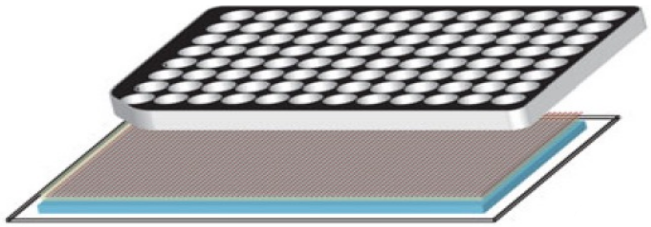
- CometChip assay
  - single cell gel electrophoresis in 96 well format
- Single strand DNA breaks

DSBs SSBs

Damage & Repair

Overview of CometChip Assay: chemically treating cells and visualization

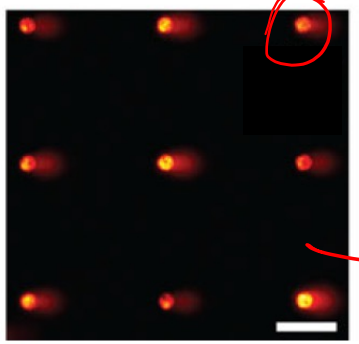
Treat captured cells in comet chip with H_2O_2 and As



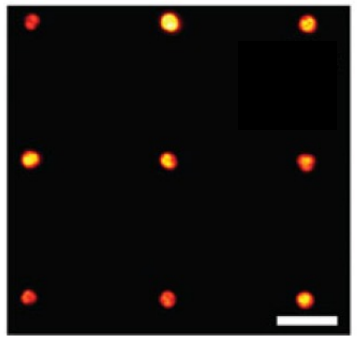
Lyse cells & unwind DNA

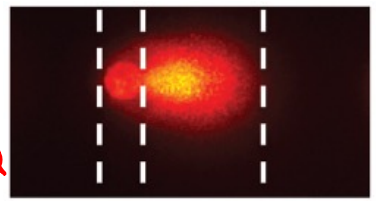
(DNA still captured agarose in overlay)

Agarose Electrophoresis



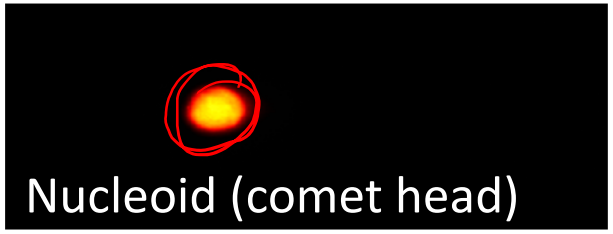
Stain DNA and image via fluorescence microscopy





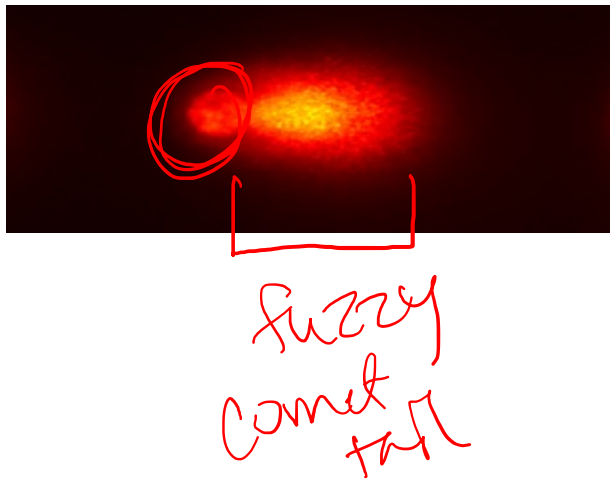
Analysis via Matlab

Output of the alkaline CometChip assay



No Damage

- Supercoiled nucleoid
- Little or no migration



High Damage

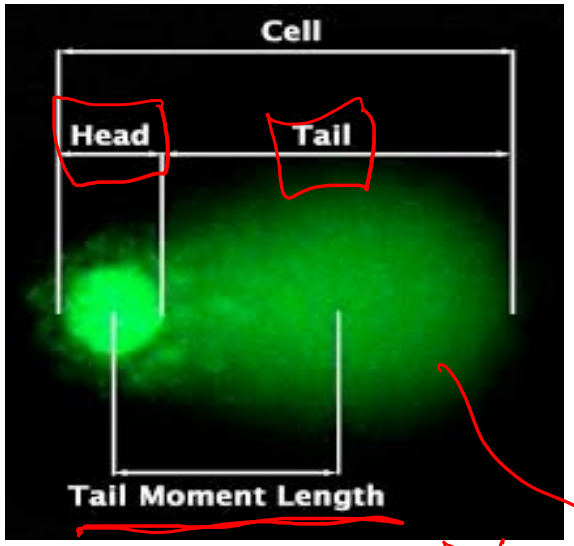
- SSBs, abasic sites, alkali labile sites, sites of incomplete excision repair
- forms a "comet tail"

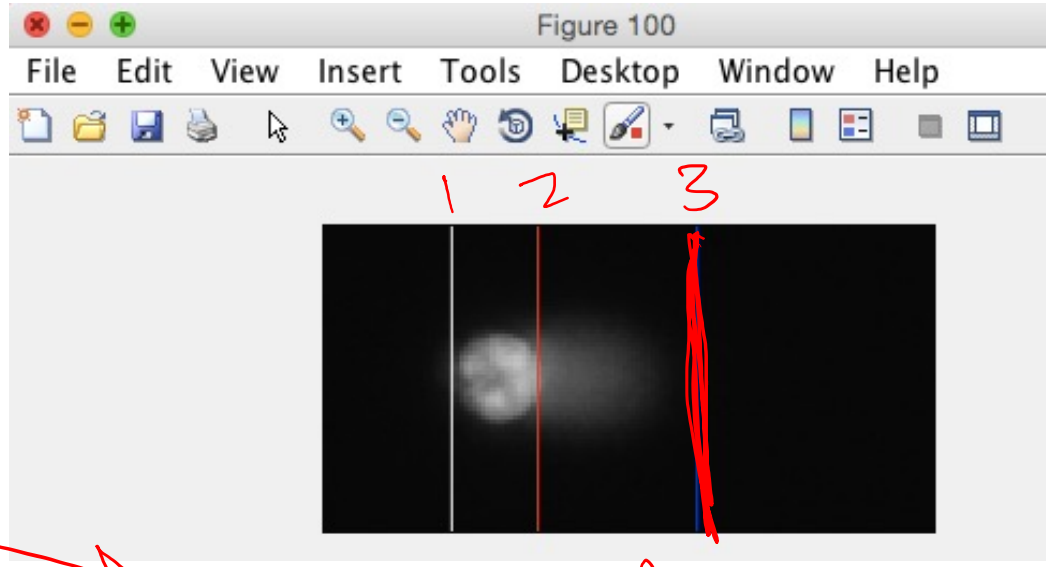
* Nuclear DNA normally supercoiled

* 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?





Matlab

no worries

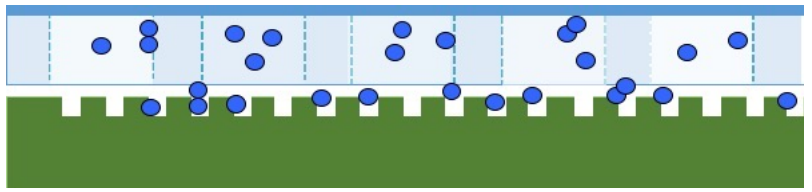
% Tail DNA

- Assess comet images in Matlab
  - Do recommended parameters (on wiki) accurately measure most comets in your sample?
- Compare % Tail DNA between comets from Matlab analysis → QC
  - Does the data appear consistent and reliable?
- Use Excel to analyze compiled CometChip data → Analysis

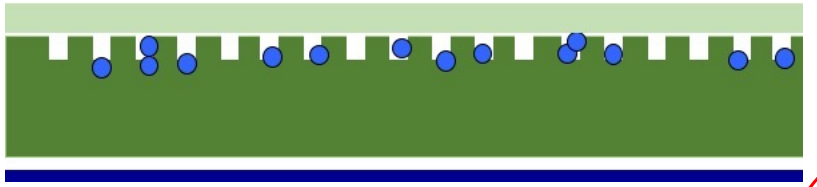
Overview of the CometChip assay: treating cells

Treat with As for 24hrs

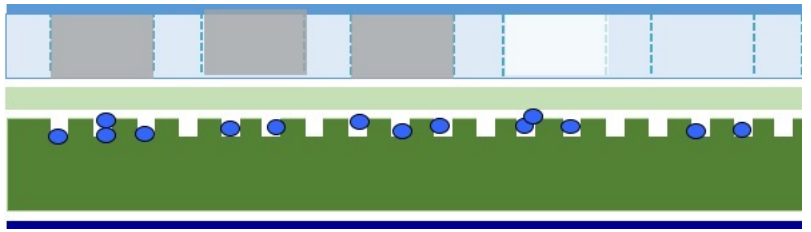
0
2 uM
10 uM



1% LMP agar

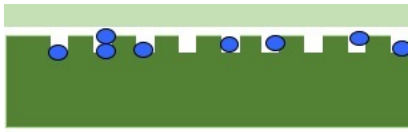


Treat with H_2O_2

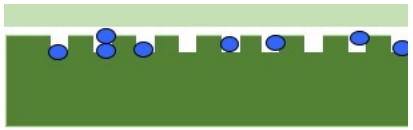


0min recovery

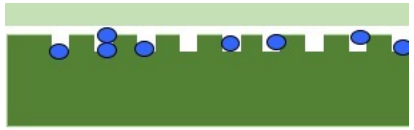
Place directly in lysis buffer



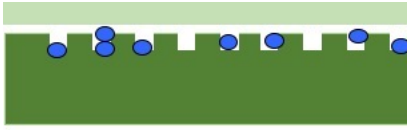
15min recovery



30min recovery



60 min recovery



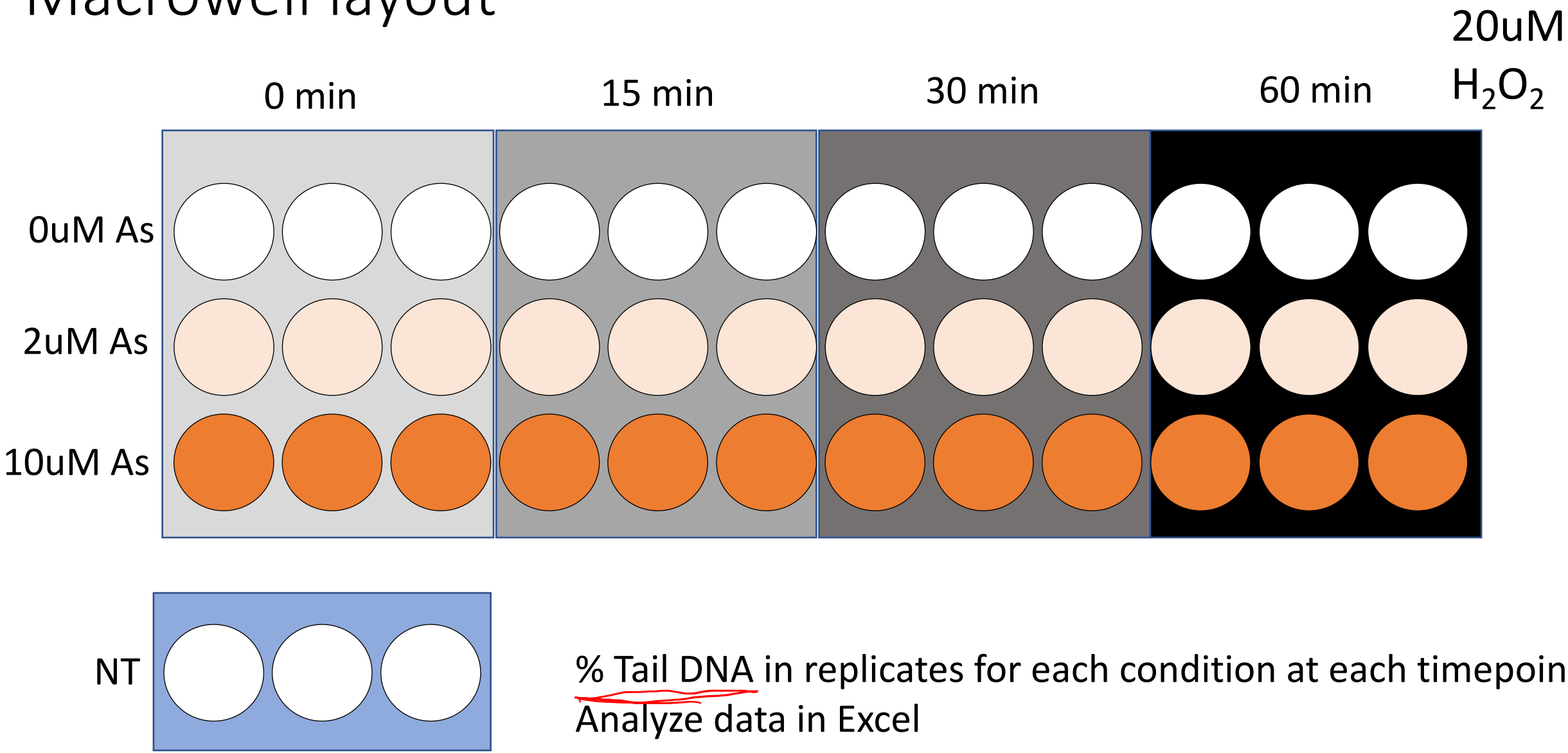
Cometchip

20 uM

similar to yours

Recovery/ Repair

Macrowell layout

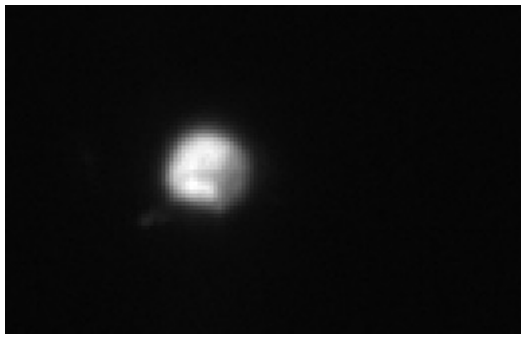


% Tail DNA in replicates for each condition at each timepoint

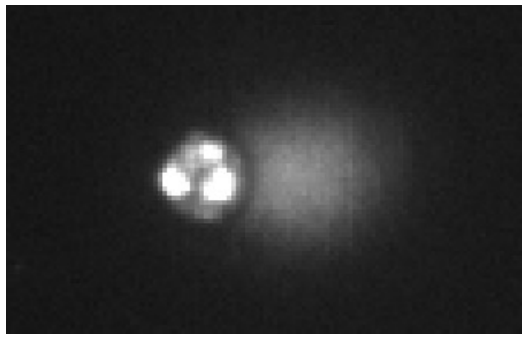
Analyze data in Excel

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

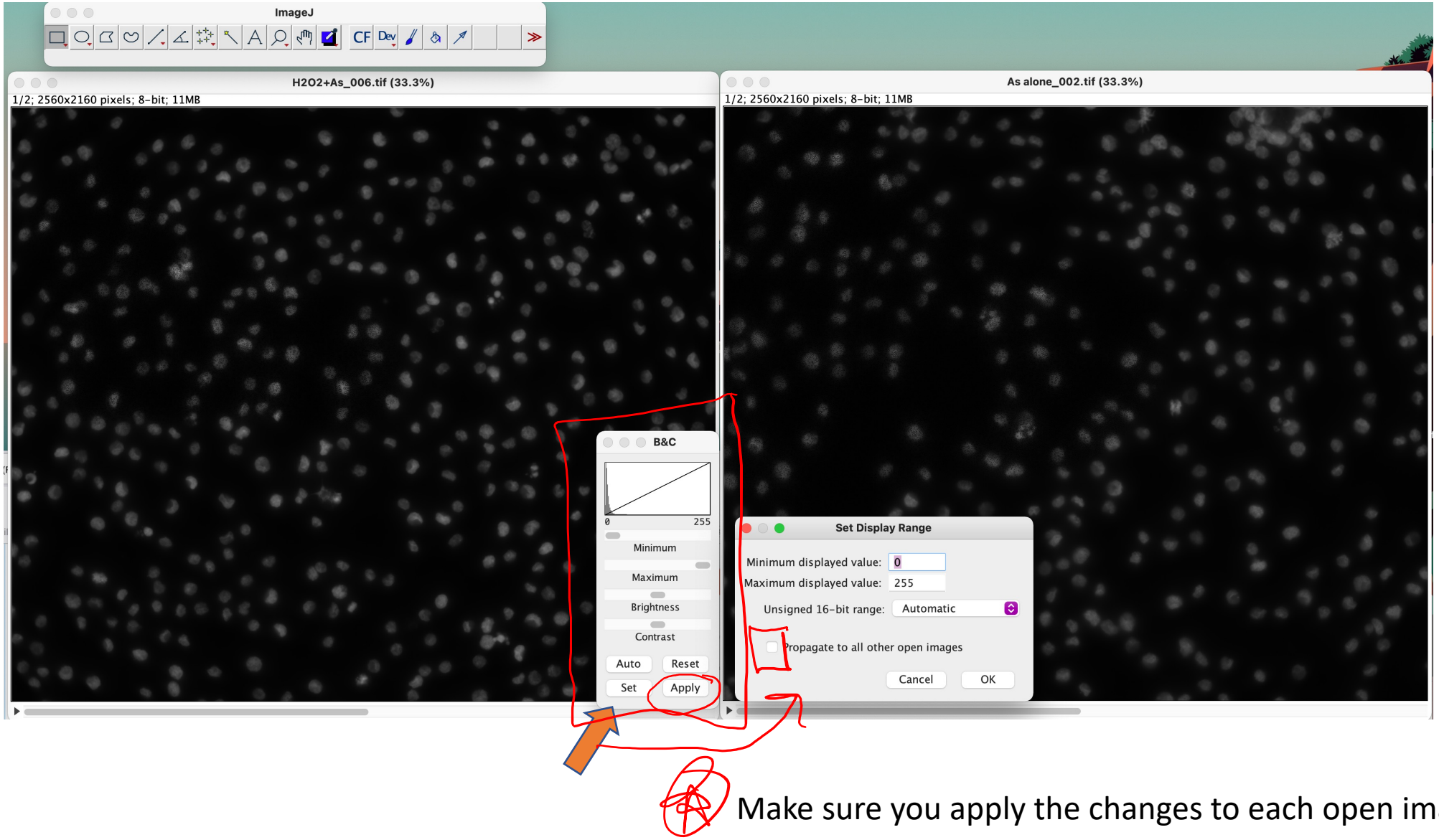


No Treatment



2uM As + 20uM H_2O_2

Notes on adjusting ImageJ images evenly



open multiple images
- Adjust
- Threshold
Brightness
Contrast

Make sure you apply the changes to each open image individually

For Today

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

For M1D7

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

- Outline your Research Talk (see Assignments page for details and Homework page for checklists)

Notes on the Research Talk

- Individual assignment
- Three (3) minute video of you talking directly into the camera
- No visual aids allowed
  - Introduce yourself and your project
  - Highlight key results with quantitative information
  - Place your work in the scope of the larger field
- No need to state you are doing a class project or anything about 20.109

- DO NOT submit to Stellar! Instead submit the video file to bioeng20.109@gmail.com, with a specific filename

Name_LabSection_RT.extension (for example, ImaStudent_TR_RT.mov)