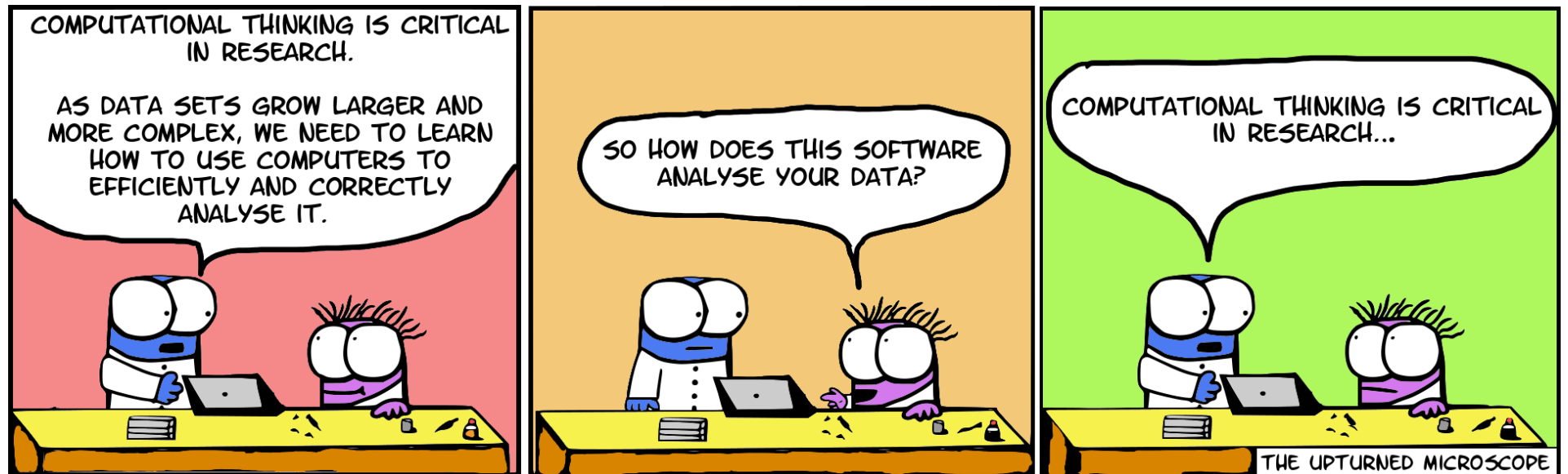


# M1D6: Image and analyze high-throughput genome damage assay

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

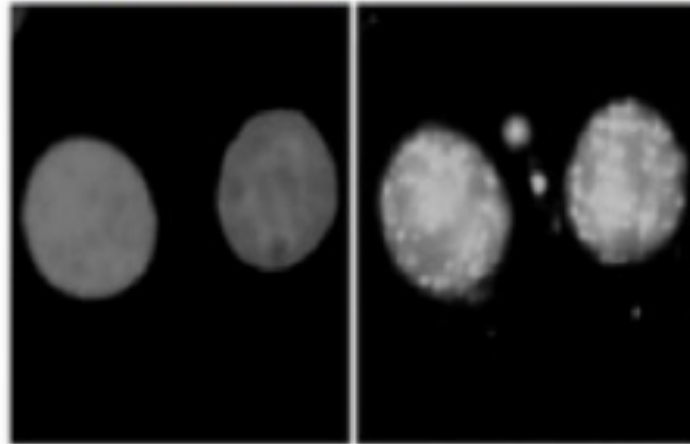
- Be sure your name is in the file name **and** in the document itself
- For group assignments, submit one copy of the homework using your team color for the file name

**BeckyM\_M1D7**

**TR\_Teal\_DataSummary**

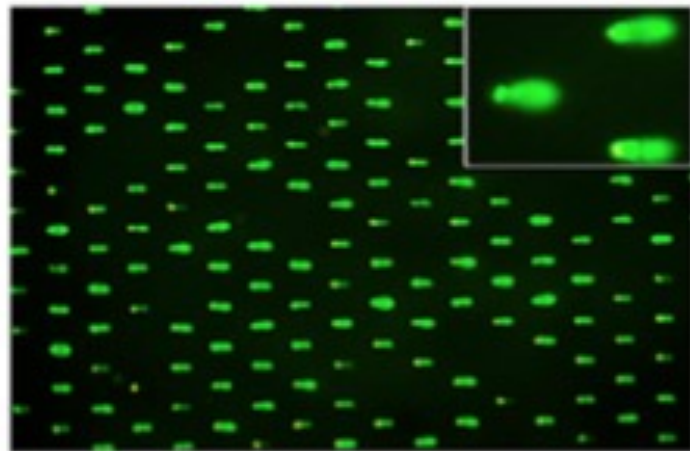
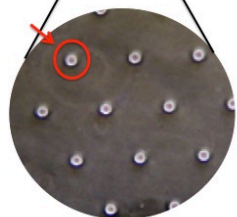
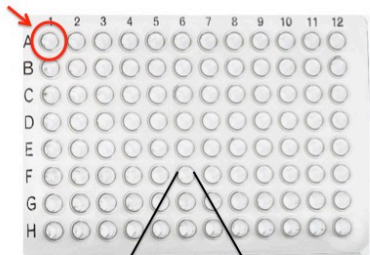
# Overview of Module 1: Measuring Genomic Instability

**Research question: Does exposure to As inhibit, or decrease, repair of H<sub>2</sub>O<sub>2</sub>-induced DNA damage, raising the possibility that combined exposure is an important risk to public health?**



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

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

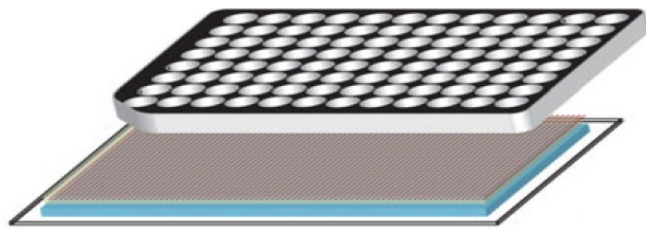


**Measure the effects of H<sub>2</sub>O<sub>2</sub> +/- As on DNA damage by measuring DNA migration in agarose matrix**

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

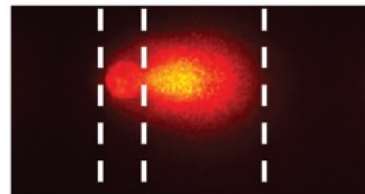
# Overview of CometChip Assay: chemically treating cells and visualization

Treat captured cells in comet chip with  
 $\text{H}_2\text{O}_2$  and As

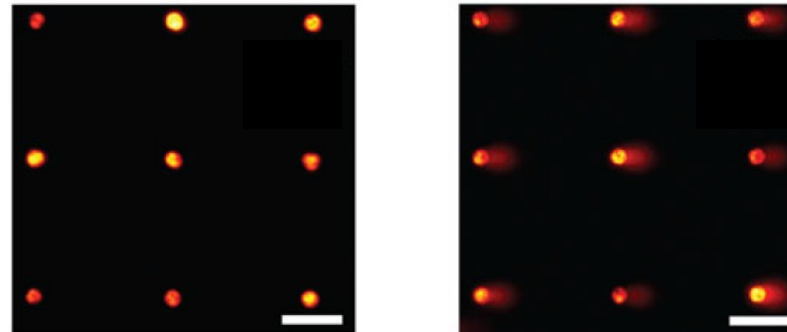


Lyse cells & unwind  
DNA  
(DNA still captured  
agarose in overlay)

Agarose Electrophoresis

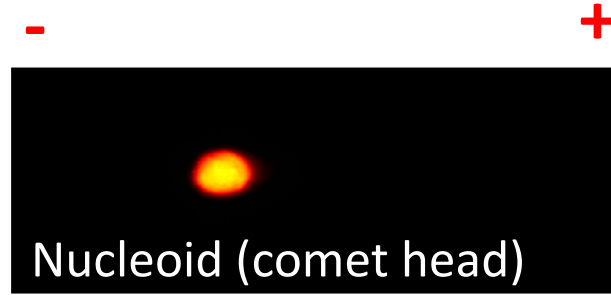


Analysis  
via  
Matlab



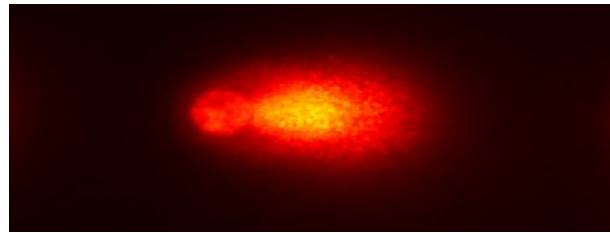
Stain DNA and image via  
fluorescence microscopy

# Output of the alkaline CometChip assay



## No Damage

- Supercoiled nucleoid
- Little or no migration



## High Damage

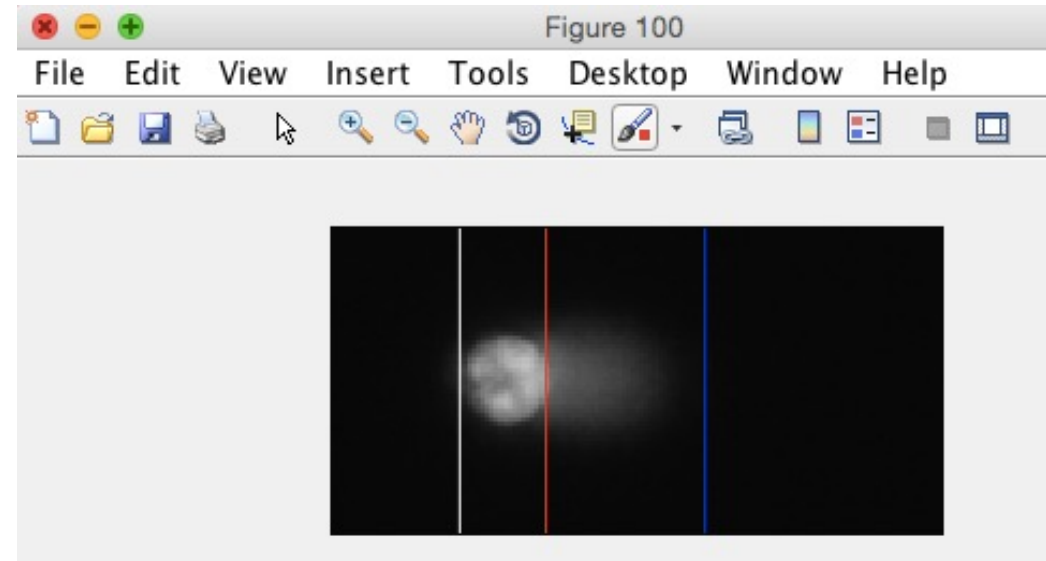
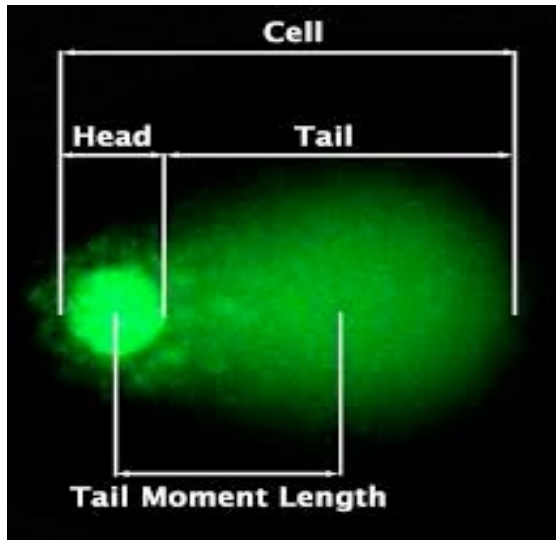
- SSBs, abasic sites, alkali labile sites, sites of incomplete excision repair
- forms a "comet tail"

\* 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?

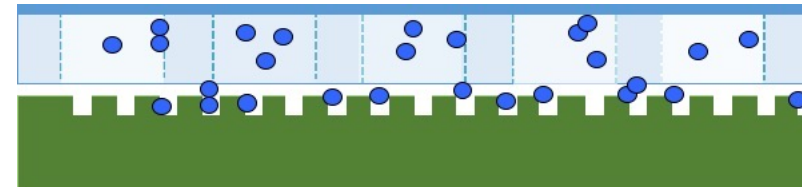


- Assess comet images in Matlab
  - Do recommended parameters (on wiki) accurately measure most comets in your sample?
- Compare % Tail DNA between comets from Matlab analysis
  - Does the data appear consistent and reliable?
- Use Excel to analyze compiled CometChip data

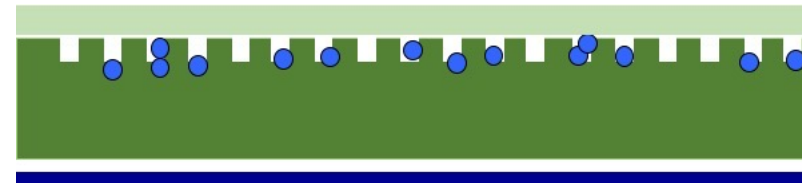
# Overview of the CometChip assay: treating cells



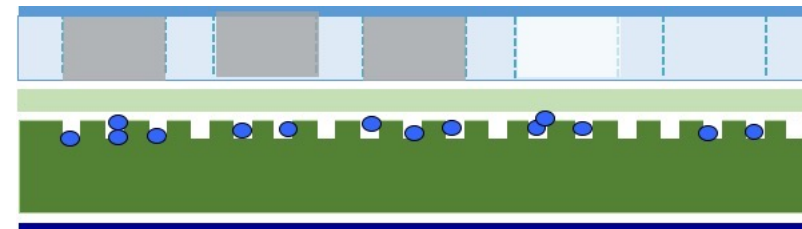
Treat with As for 24hrs



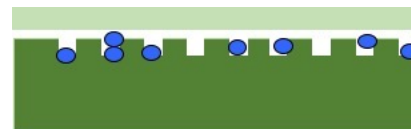
1% LMP agar



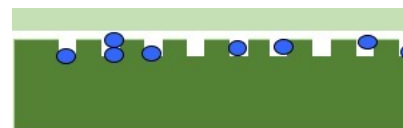
Treat with  $H_2O_2$



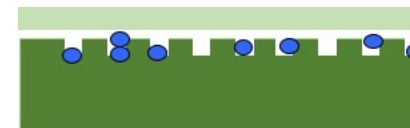
0min recovery  
Place directly in lysis  
buffer



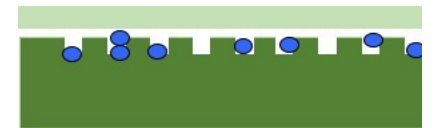
15min  
recovery



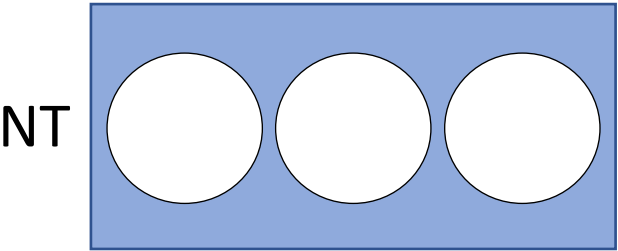
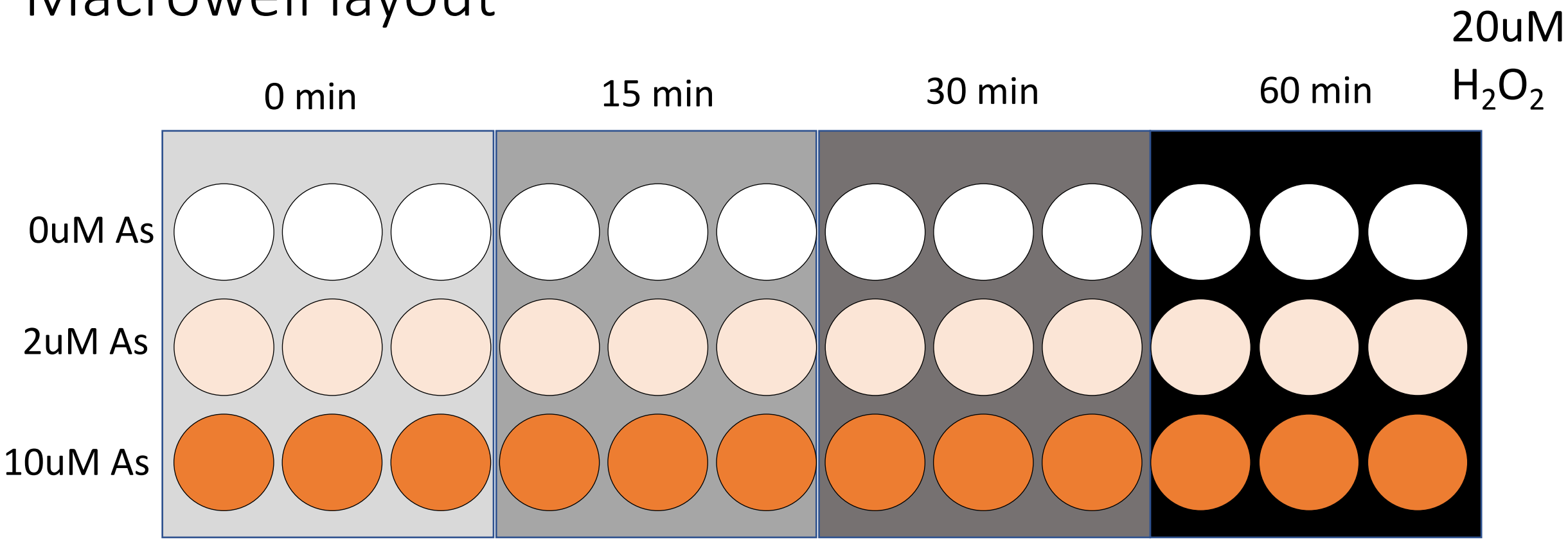
30min  
recovery



60 min  
recovery



# Macrowell layout

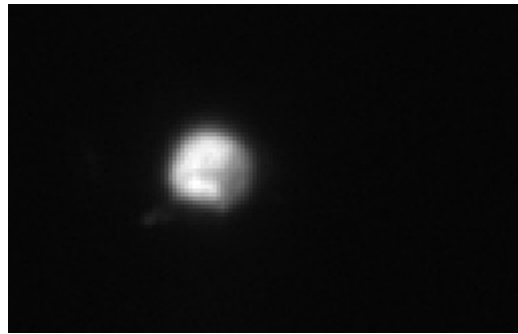


% Tail DNA in replicates for each condition at each timepoint  
Analyze data in Excel

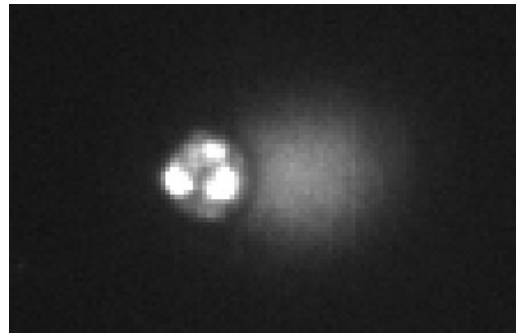


# Examine CometChip images for visual examples to include in Data Summary Figure

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

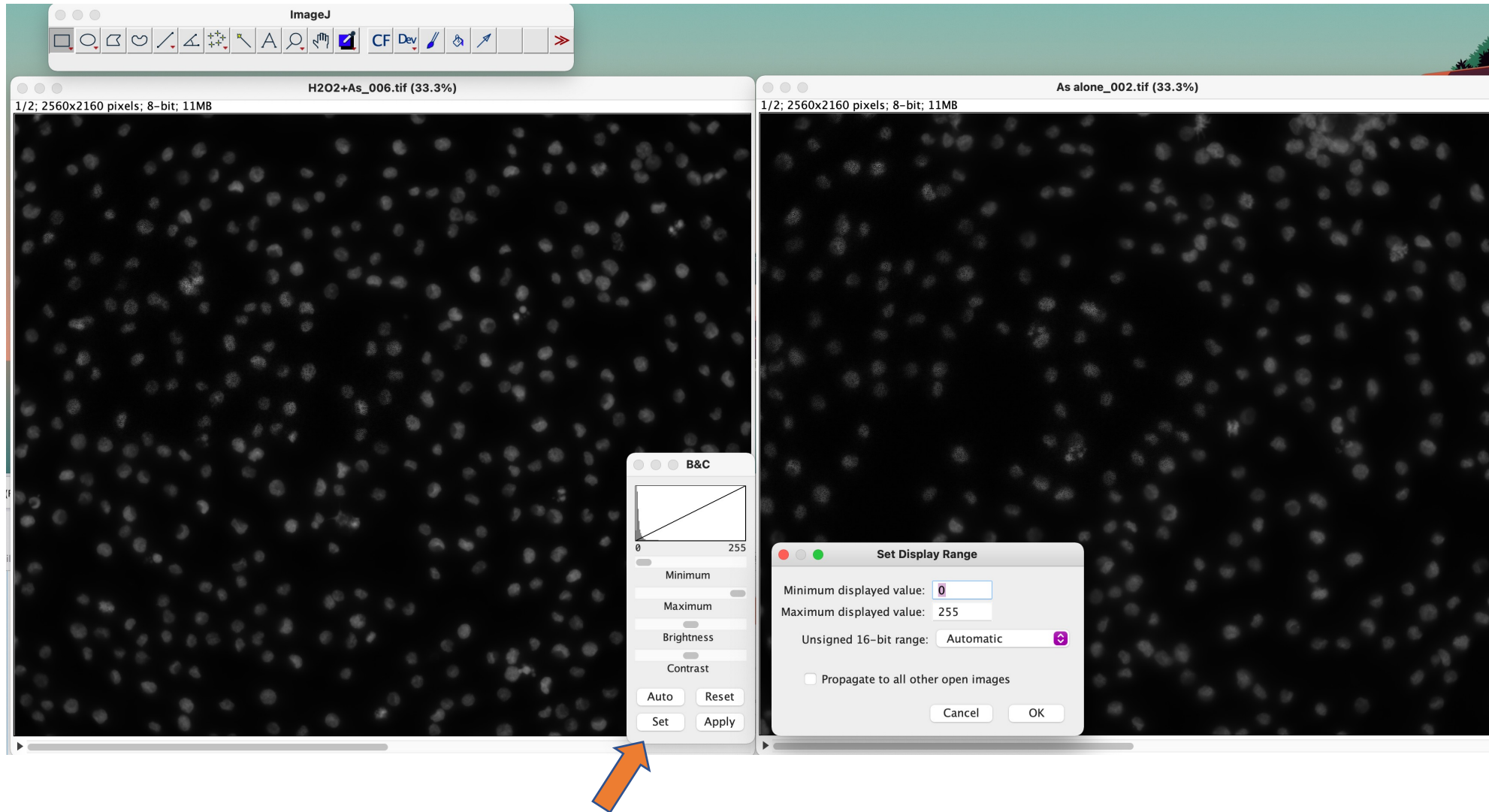


No Treatment



2uM As + 20uM H<sub>2</sub>O<sub>2</sub>

# Notes on adjusting ImageJ images evenly



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](mailto:bioeng20.109@gmail.com), with a specific filename  
**Name\_LabSection\_RT.extension** (for example, ImaStudent\_TR\_RT.mov)