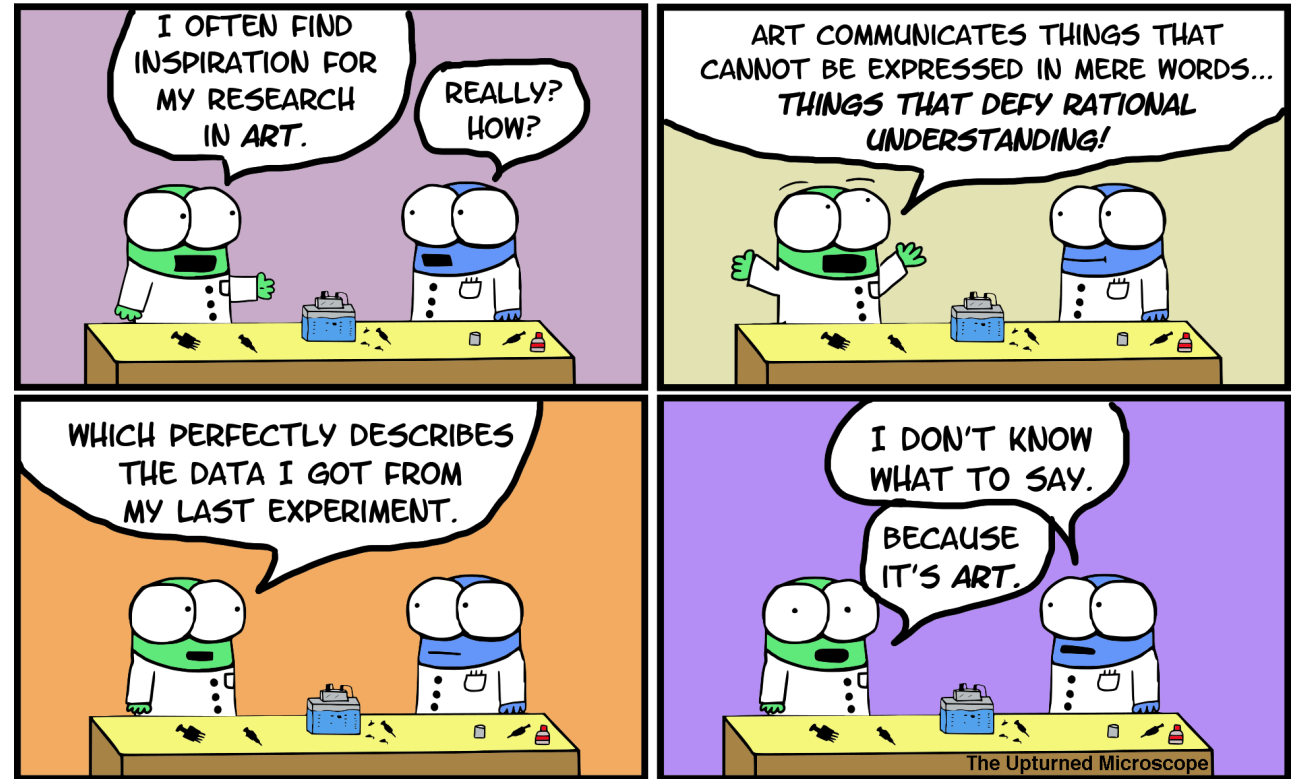


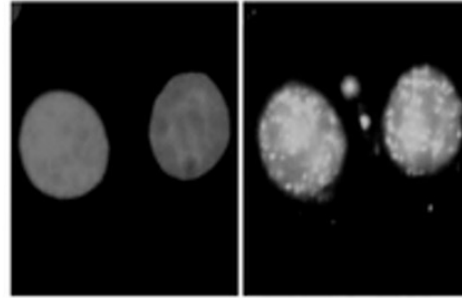
M1D4: Complete data analysis for γ H2AX experiment

1. Prelab, part 1
2. Image analysis for γ H2AX assay
3. Paper discussion with Noreen
4. Prelab, part 2
5. Make a CometChip



Mod1 Overview

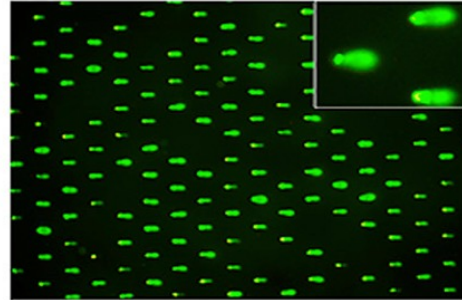
Last lab:



1. Use repair foci experiment to measure DNA breaks

- Examine effect of H_2O_2 +/- As on double strand DNA breaks by measuring γ H2AX foci formation

This lab:



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

Next lab:

Image analysis has some potential pitfalls

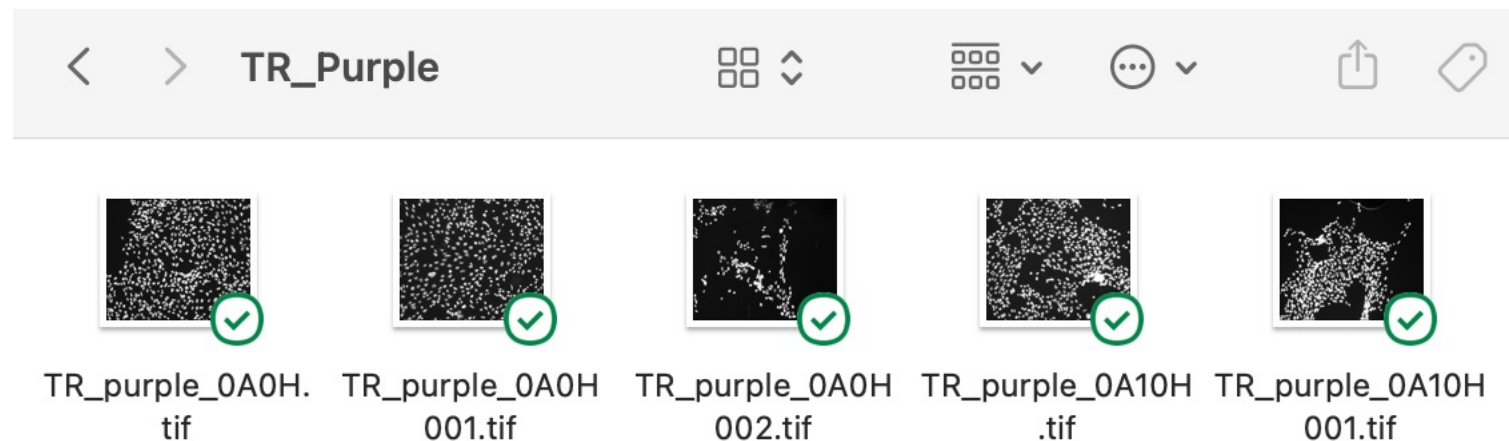
- Data can be skewed dramatically by **bias** (conscious or unconscious)
 - Microscopy images are vulnerable to this because they are often used as representative of a much larger population
- How do we mitigate bias when taking and analyzing images?
 - **Blind** imaging or analysis
 - **Set parameters** ahead of time (i.e. select images randomly in the DAPI channel without looking at H2AX staining)
 - Try to create a field of view that encompasses **multiple cells**

How will you analyze your images for the Data Summary?

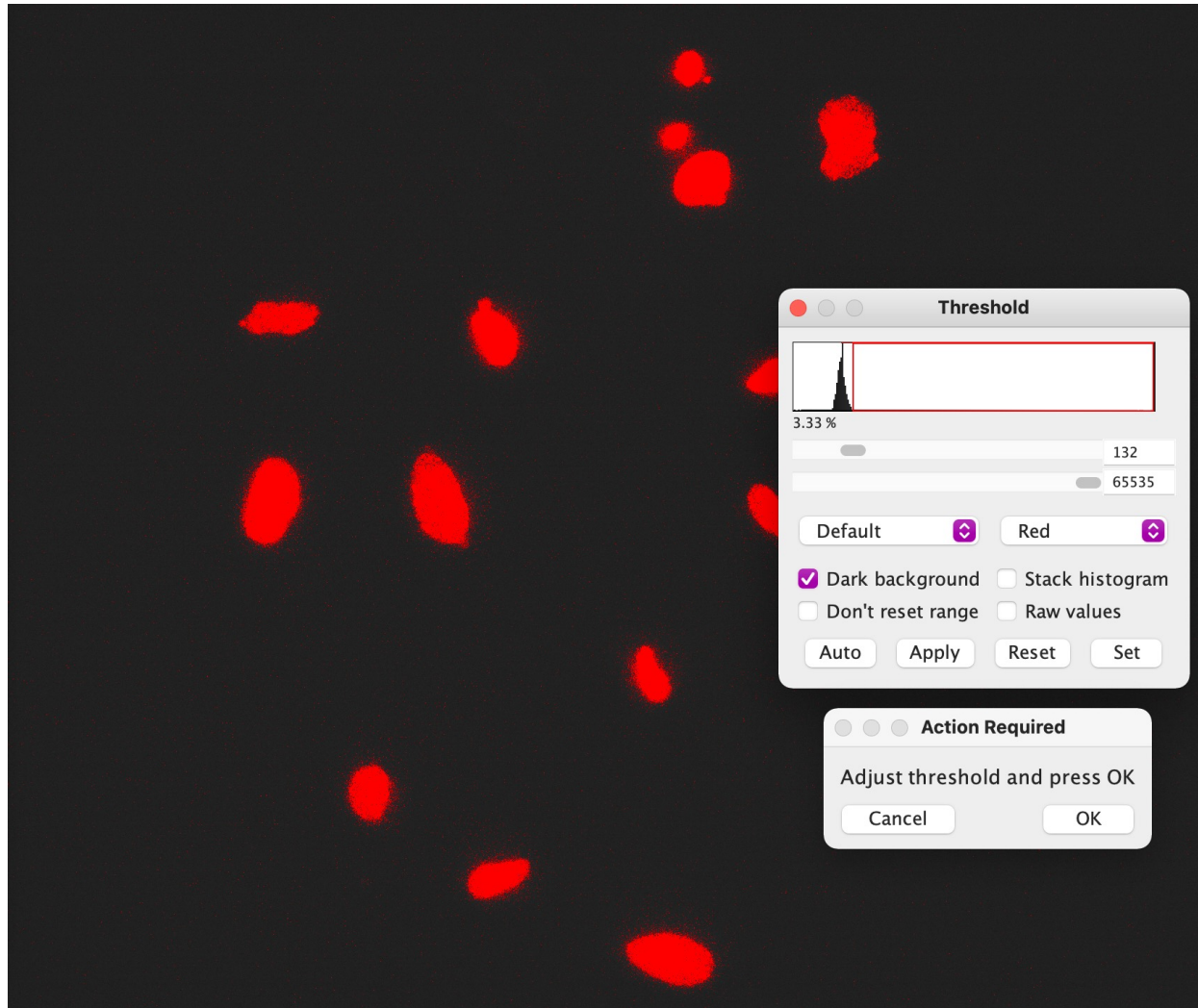


ImageJ
Image Processing & Analysis in Java

- Use macro developed by Joshua Corrigan in Engelward lab
 - The DAPI channel used to create a "mask" of the nuclei
 - Gamma-H2AX foci are identified by pixel maxima readings in the FITC channel
 - You will be able to compare you "by eye" assessment of punctae to the count identified by the program
 - Average the number of foci per nuclei per image to get data point

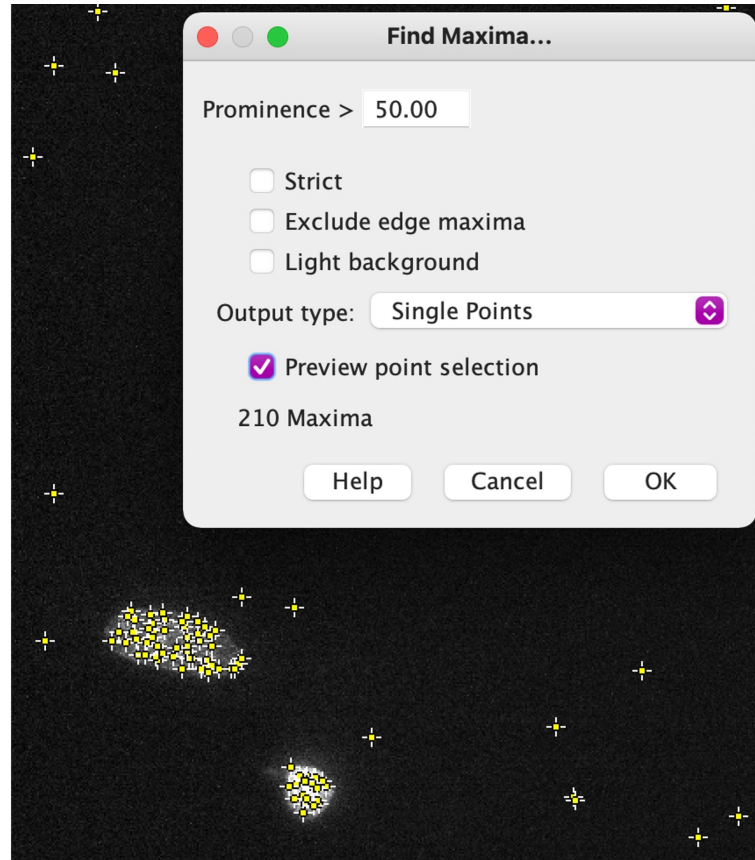
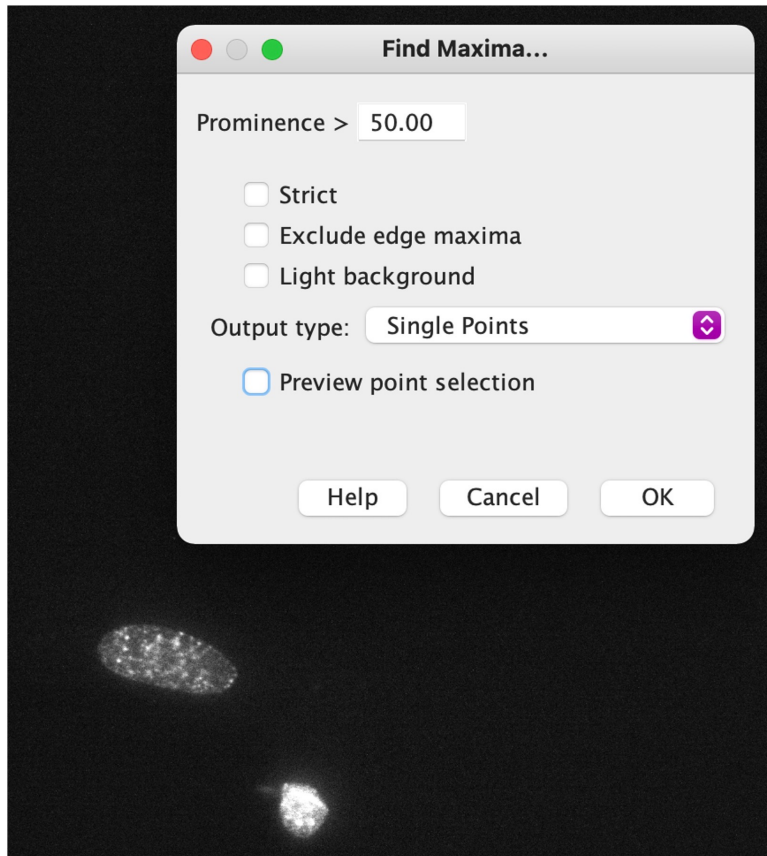


Set nuclei threshold to create region of interest to count foci



- Adjust threshold to capture discrete nuclei
 - May not be perfect!
- Program will also watershed the images to separate nuclei that are close together

Set prominence for the FITC/488 channel image



- Find a prominence setting that allows most visible foci to be counted in a condition while minimizing background counting
 - Select output of **Single Points**
 - Check **Preview point selection**

Compile results in Excel

Results							
	Label	Area	Mean	Min	Max	Circ.	IntDen
1	5H10As_40x__117-0002 Maxima:0004-0548	5972	0.000	0	0	0.267	0
2	5H10As_40x__117-0002 Maxima:0005-0630	8132	0.000	0	0	0.287	0
3	5H10As_40x__117-0002 Maxima:0007-0936	9354	0.000	0	0	0.359	0
4	5H10As_40x__117-0002 Maxima:0009-1017	8844	0.000	0	0	0.321	0
5	5H10As_40x__117-0002 Maxima:0013-1653	12860	0.000	0	0	0.412	0
6	5H10As_40x__117-0002 Maxima:0014-1681	9359	0.000	0	0	0.264	0
7	5H10As_40x__117-0002 Maxima:0017-2047	10956	0.000	0	0	0.423	0
8	5H10As_40x__117-0002 Maxima:0002-0252	8709	0.029	0	255	0.326	255
9	5H10As_40x__117-0002 Maxima:0008-1004	21650	0.012	0	255	0.371	255
10	5H10As_40x__117-0002 Maxima:0015-1952	8416	0.030	0	255	0.301	255
11	5H10As_40x__117-0002 Maxima:0001-0230	9846	0.052	0	255	0.495	510
12	5H10As_40x__117-0002 Maxima:0003-0307	10179	0.050	0	255	0.295	510
13	5H10As_40x__117-0002 Maxima:0006-0938	13402	0.038	0	255	0.233	510
14	5H10As_40x__117-0002 Maxima:0011-1481	13157	0.058	0	255	0.260	765
15	5H10As_40x__117-0002 Maxima:0010-1038	14512	0.176	0	255	0.229	2550
16	5H10As_40x__117-0002 Maxima:0016-1983	15859	0.338	0	255	0.325	5355
17	5H10As_40x__117-0002 Maxima:0012-1541	24834	0.226	0	255	0.354	5610

- Results should have a Max of 0 or 255
- Integrated Density should be in multiples of 255

Data analysis required for Data Summary

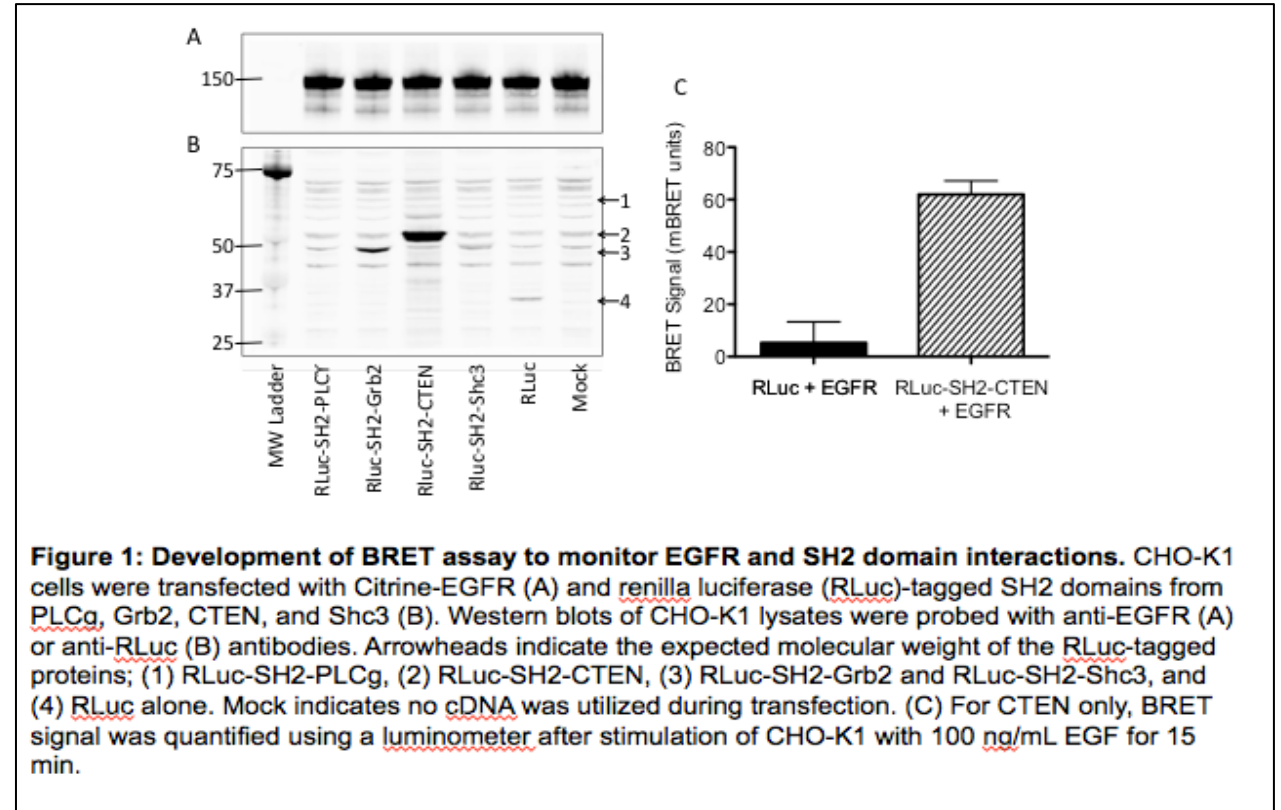
- Complete the analysis of images in all conditions for **your group** (3 replicates per condition)
- Then complete the image analysis for a biological replicate of **pilot data** from instructors
 - Divide the work amongst your lab team!
- Once the numbers are recorded for each experiment, take the average number of foci for each image (i.e. **treat each image as n=1**)
 - This is a special circumstance for this class!
 - Statistics are another lab session
- The average number of foci in each treatment condition will become a figure in the Data Summary

Homework

Data figure

Data figure example

- Image **should not** be the entire page
 - Only needs to be large enough to be clear / visible
 - 1/3 – 1/2 of a page in portrait orientation
- Title **should** be conclusive
 - Don't include what you did, rather state what you found (take home message)
- Caption **should not** detail the methods or interpret the data
 - Define abbreviations, symbols, etc.
 - Info needed to “read” figure
 - Figure captions with multiple panels need to start with a topic sentence



Data Summary =
pptx file with slides set at 8.5 x 11" portrait

In lab today:

1. Work on image analysis until 2:45pm
2. Paper discussion from 2:45-3:30ish
3. Prelab part 2: making a CometChip gel
4. Work in teams to pour CometChip gels

HW due M1D5

1. Create a data figure of H2AX results with title and caption
2. Write up a short summary of your Comm Lab visit.