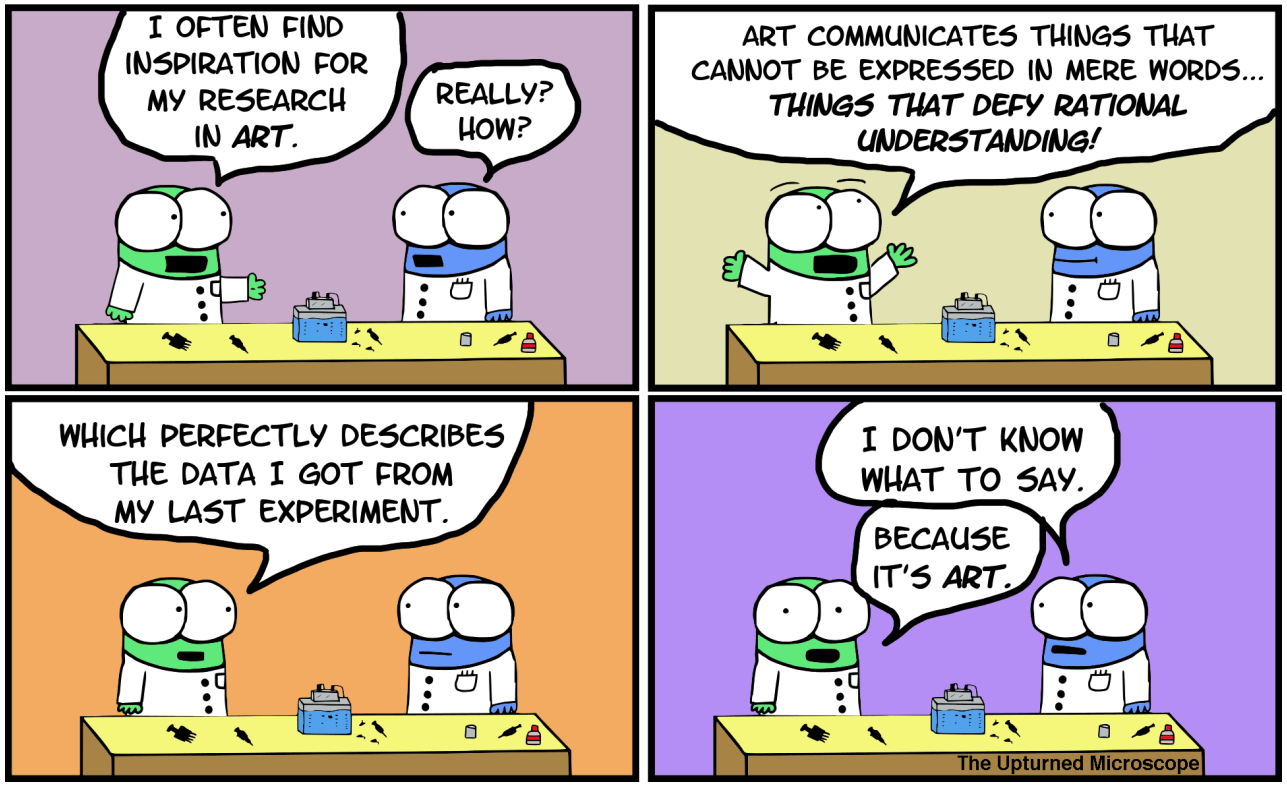
M1D4: Complete data analysis for γH2AX experiment

1. Quiz
2. Prelab, part 1
3. Image analysis for γH2AX assay
4. Paper discussion with Noreen
5. Prelab, part 2
6. Make a CometChip



Mod1 Overview

Last lab:

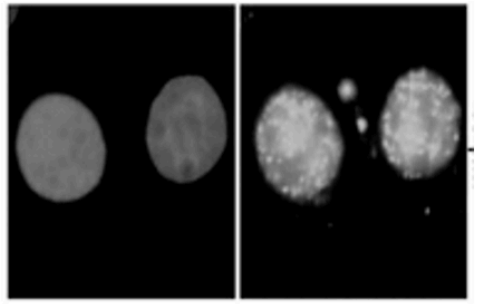
Completed yH2AX staining

This lab:

yH2AX data analysis
Paper Discussion
Pouring Comet Chip

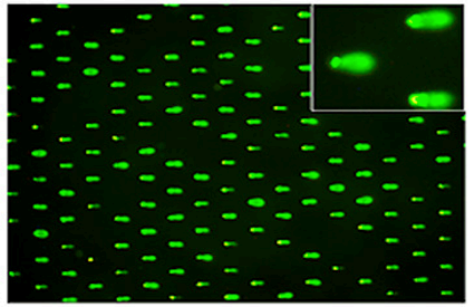
Next lab:

Completing Comet Chip



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

Image analysis has some potential pitfalls

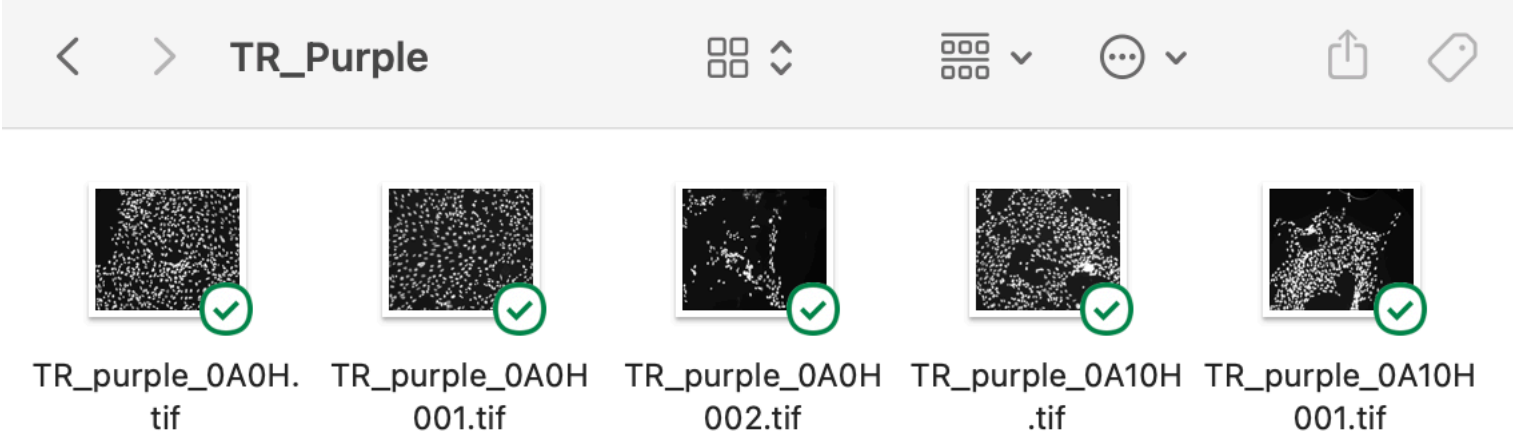
- Data can be skewed dramatically by **bias** (conscious or unconscious)
  - Microscopy images are vulnerable to this because they are often used as representative of a much larger population

- 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**

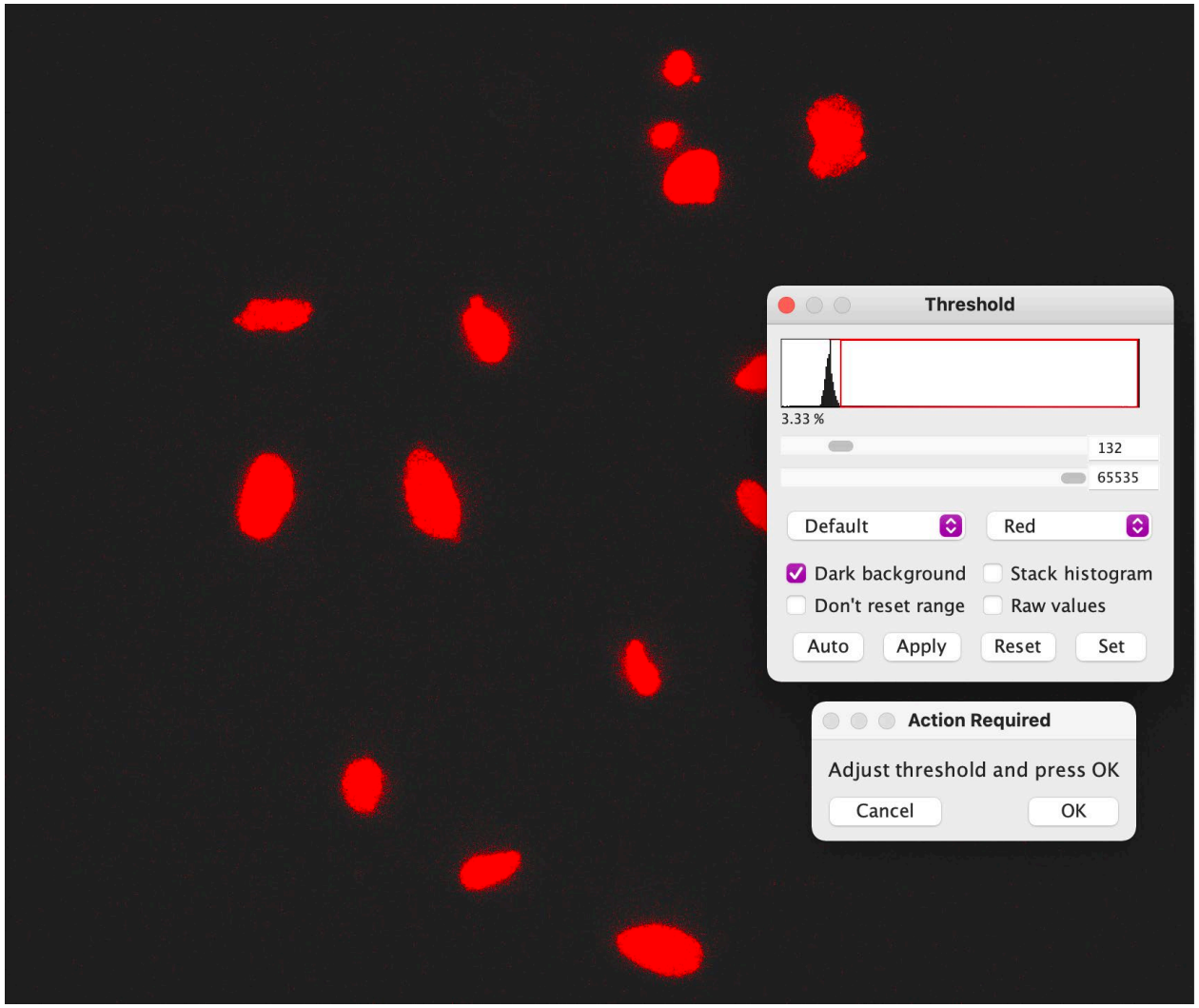
How will you analyze your images for the Data Summary?

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

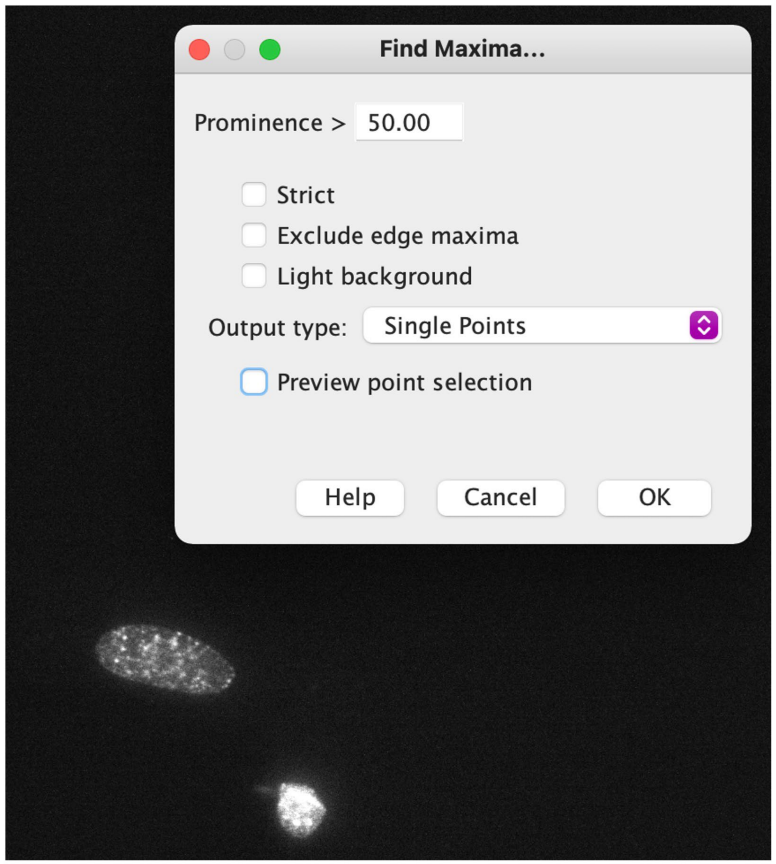


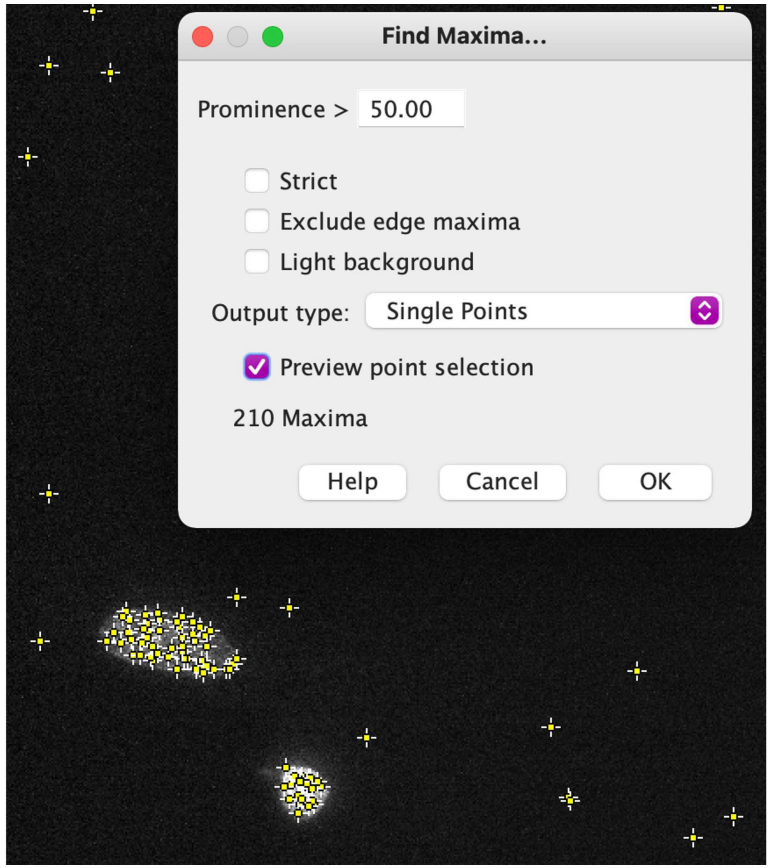
Set nuclei threshold to create region of interest to count foci



- 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

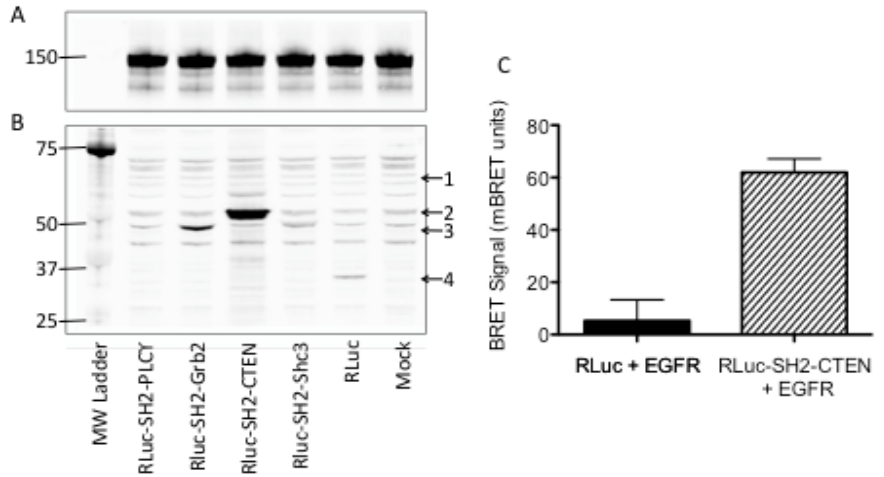


Figure 1: Development of BRET assay to monitor EGFR and SH2 domain interactions. CHO-K1 cells were transfected with Citrine-EGFR (A) and renilla luciferase (RLuc)-tagged SH2 domains from PLCg, Grb2, CTEN, and Shc3 (B). Western blots of CHO-K1 lysates were probed with anti-EGFR (A) or anti-RLuc (B) antibodies. Arrowheads indicate the expected molecular weight of the RLuc-tagged proteins; (1) RLuc-SH2-PLCg, (2) RLuc-SH2-CTEN, (3) RLuc-SH2-Grb2 and RLuc-SH2-Shc3, and (4) RLuc alone. Mock indicates no cDNA was utilized during transfection. (C) For CTEN only, BRET signal was quantified using a luminometer after stimulation of CHO-K1 with 100 ng/mL EGF for 15 min.

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.