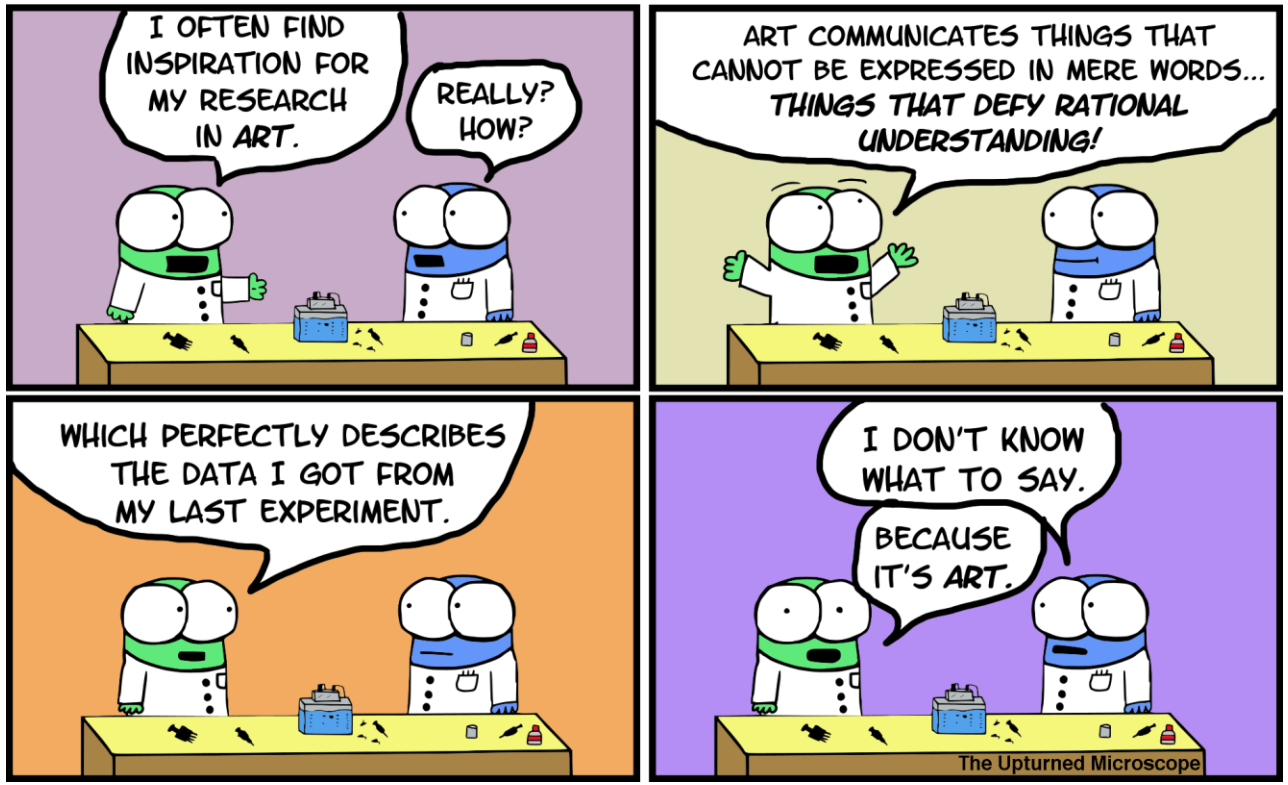
M1D4: Complete data analysis for γH2AX experiment

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



Mod1 Overview

Last lab:

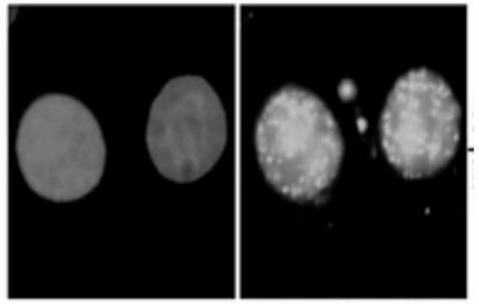
IF staining

This lab:

Analysis & Pouring CometChip

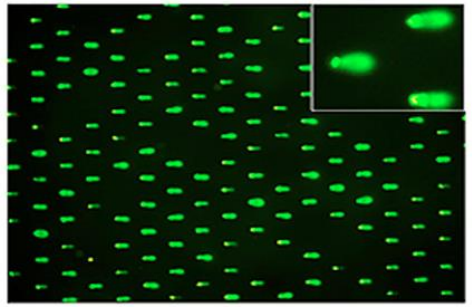
Next lab:

CometChip



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

Notes on bias in images

- 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)
  - ***<u>Do NOT blame bias for discrepancies in your data</u>***

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

Image naming format

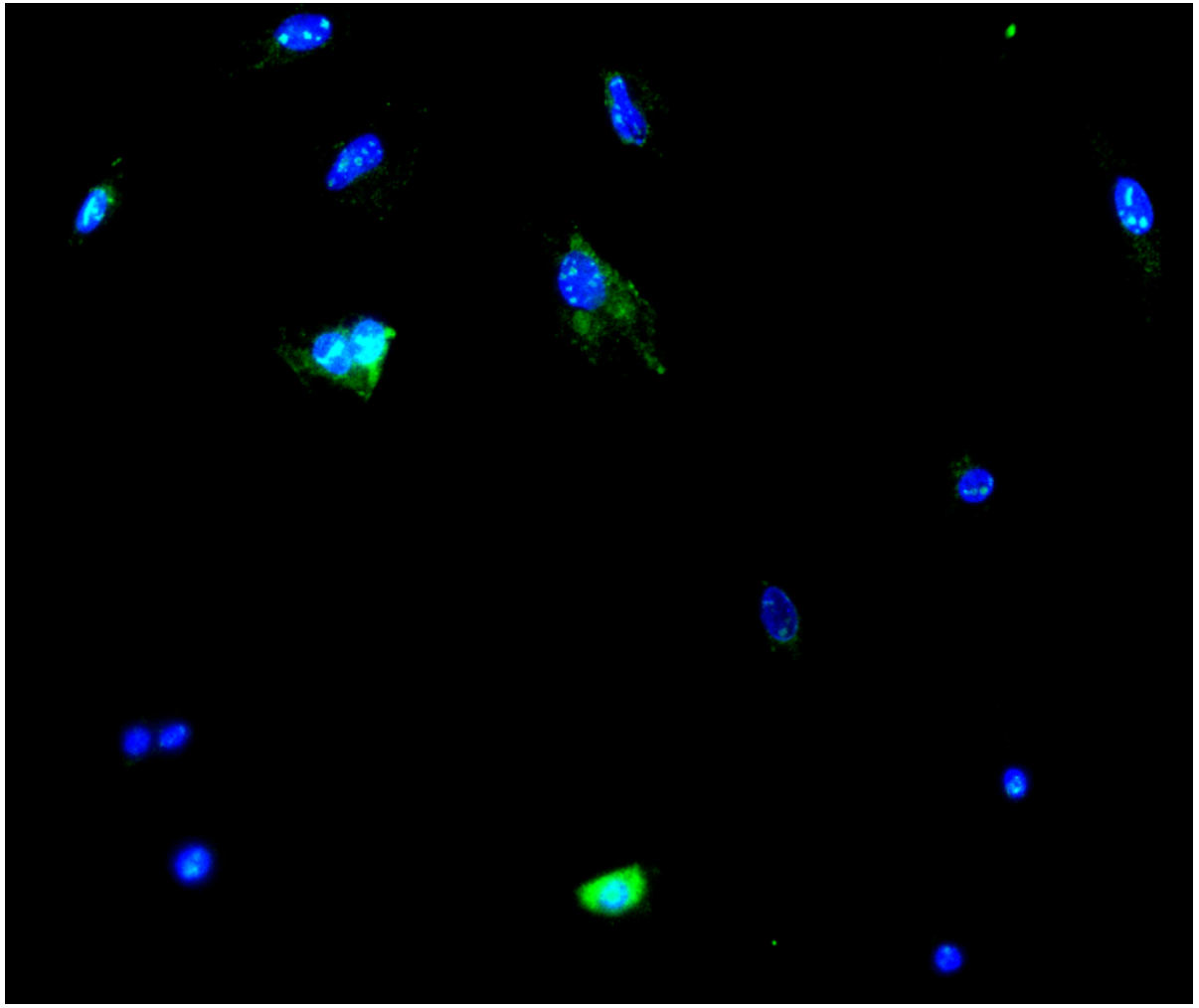
00As0H1001

uM Concentration of Arsenic

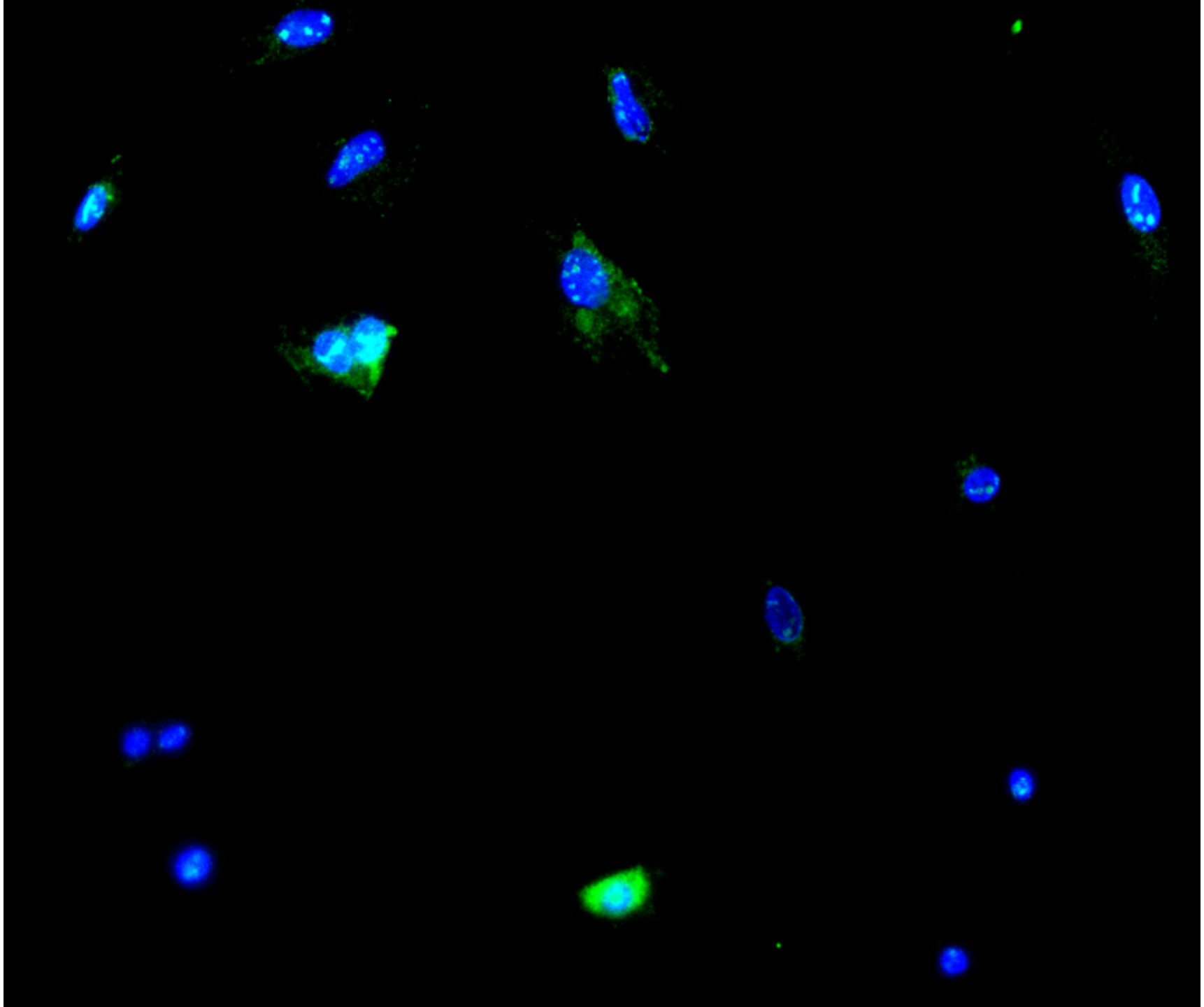
uM Concentration of H202

1 = Your mounted CS 2 = Instructor's mounted CS

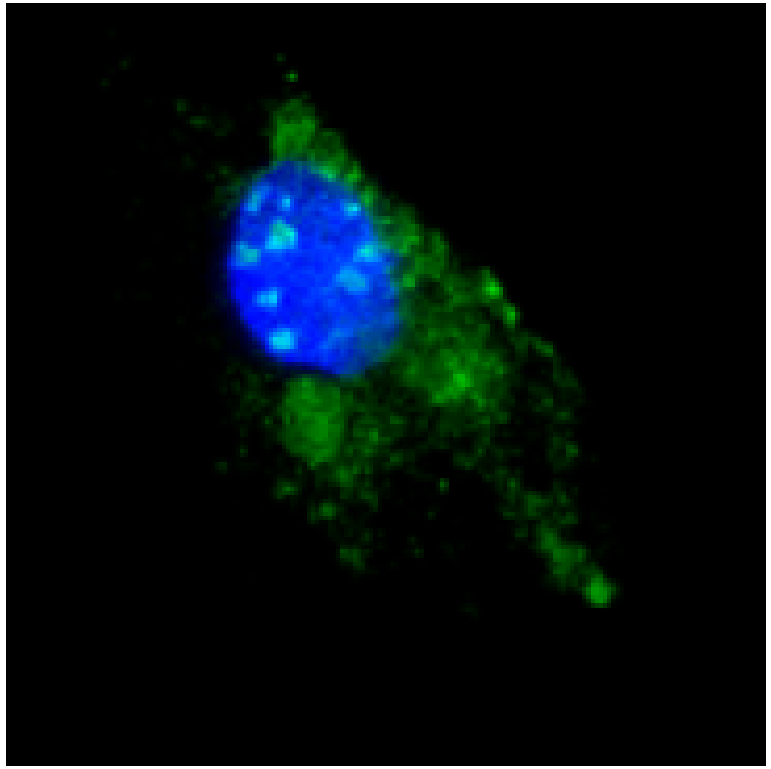
Problem: How do we count our nuclear yH2AX foci?

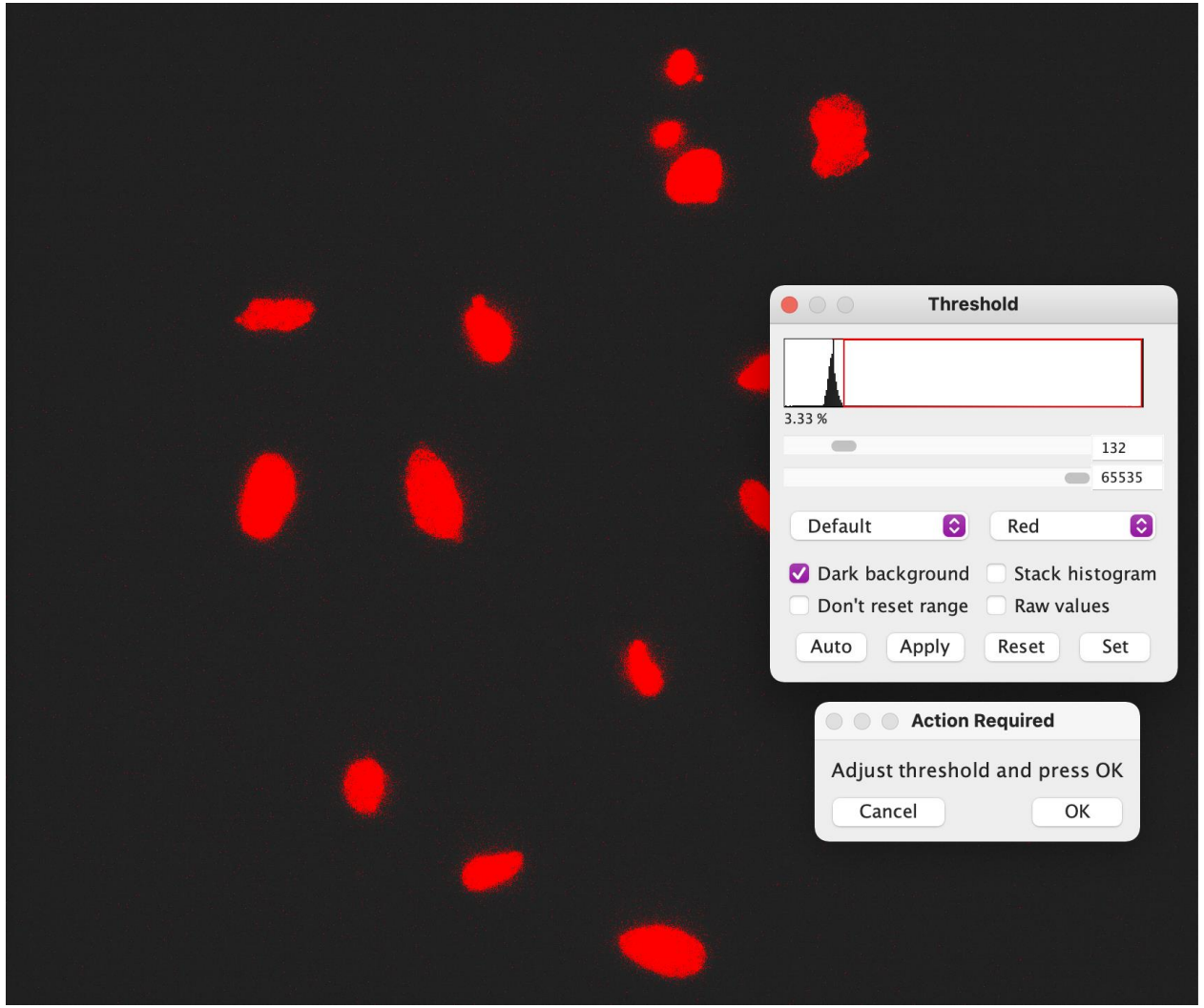


Come up with some plain language ways to solve this problem.

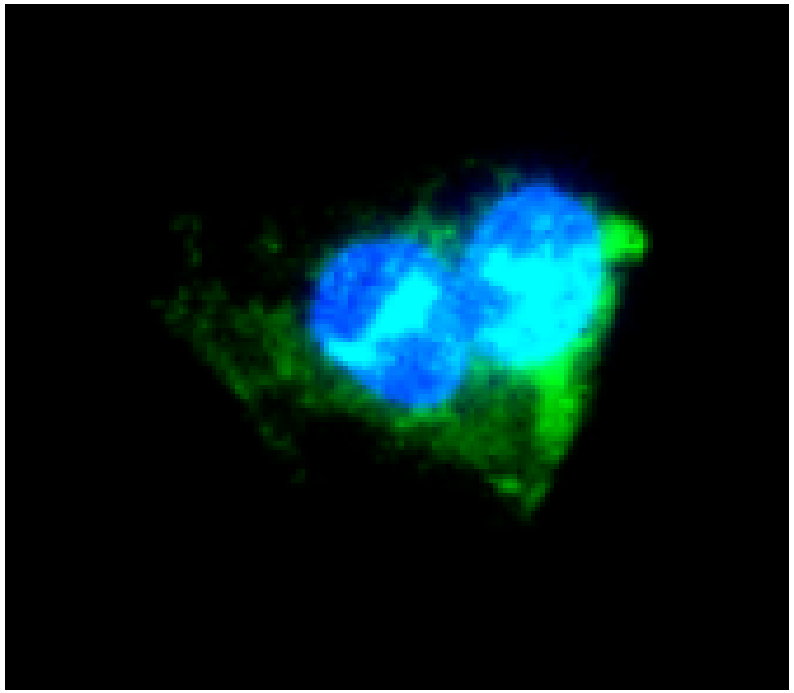


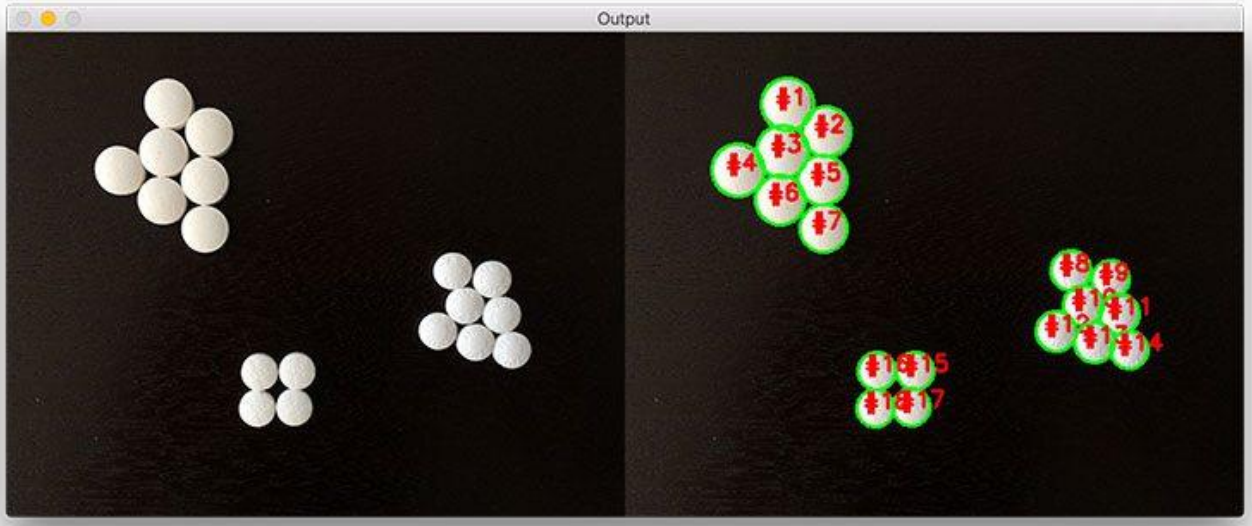
1) Threshold the nucleus - Masking

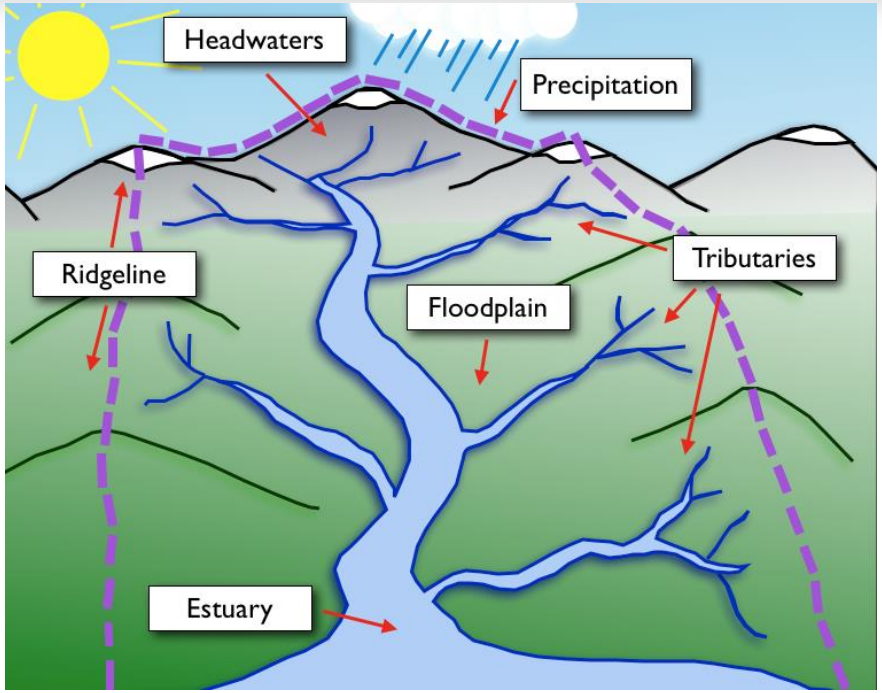




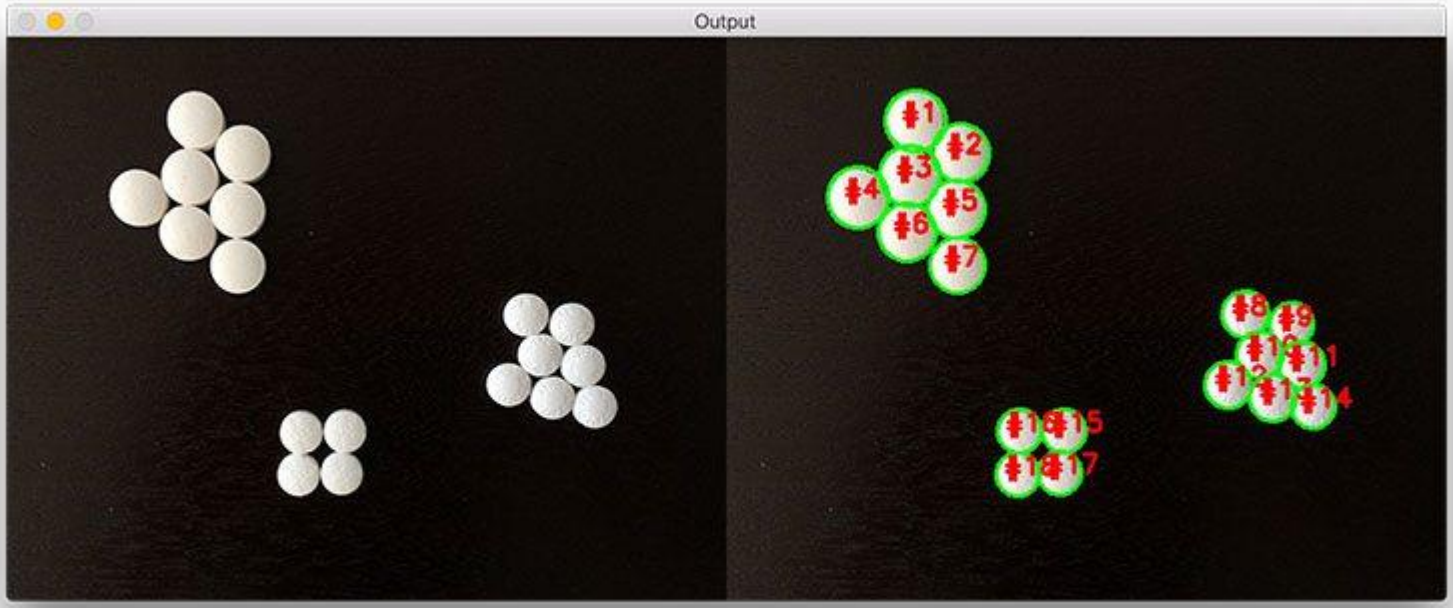
2) Watershed

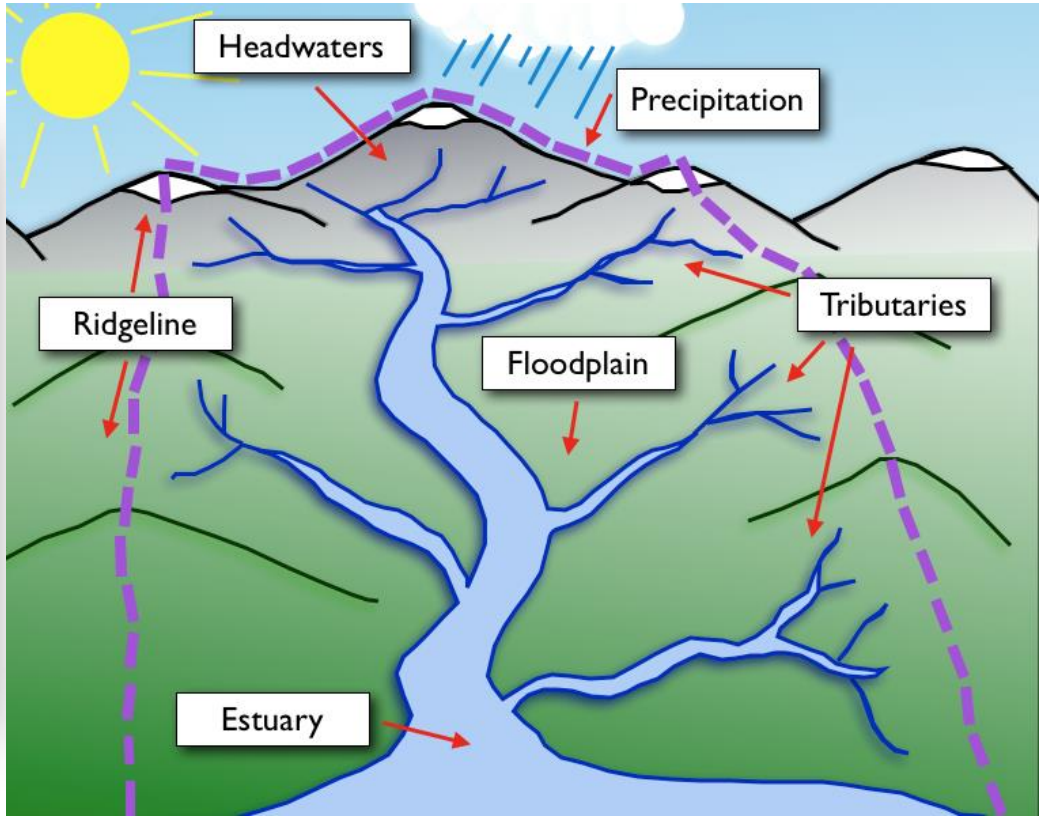



Output


Headwaters
Precipitation
Ridgeline
Tributaries
Floodplain
Estuary

Watershedding separates adjacent nuclei

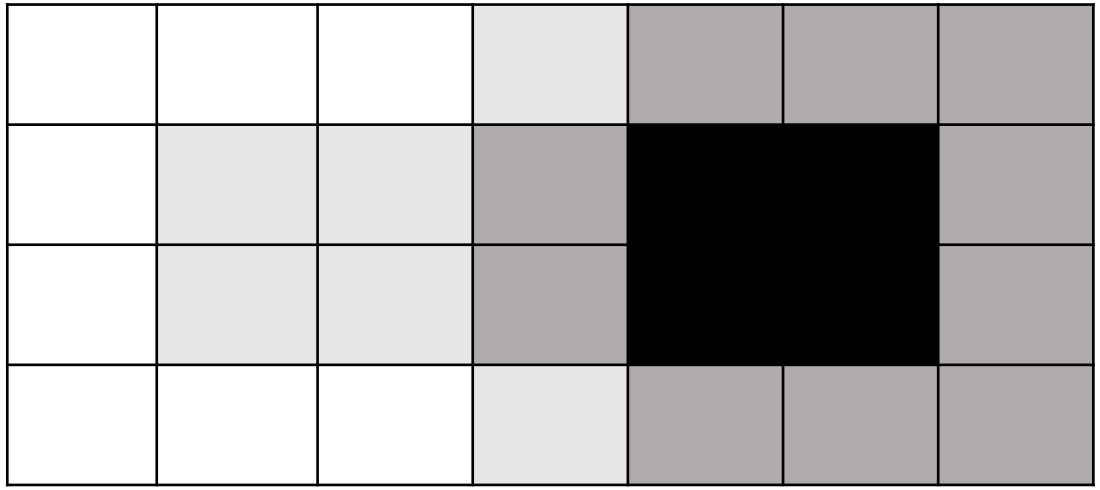




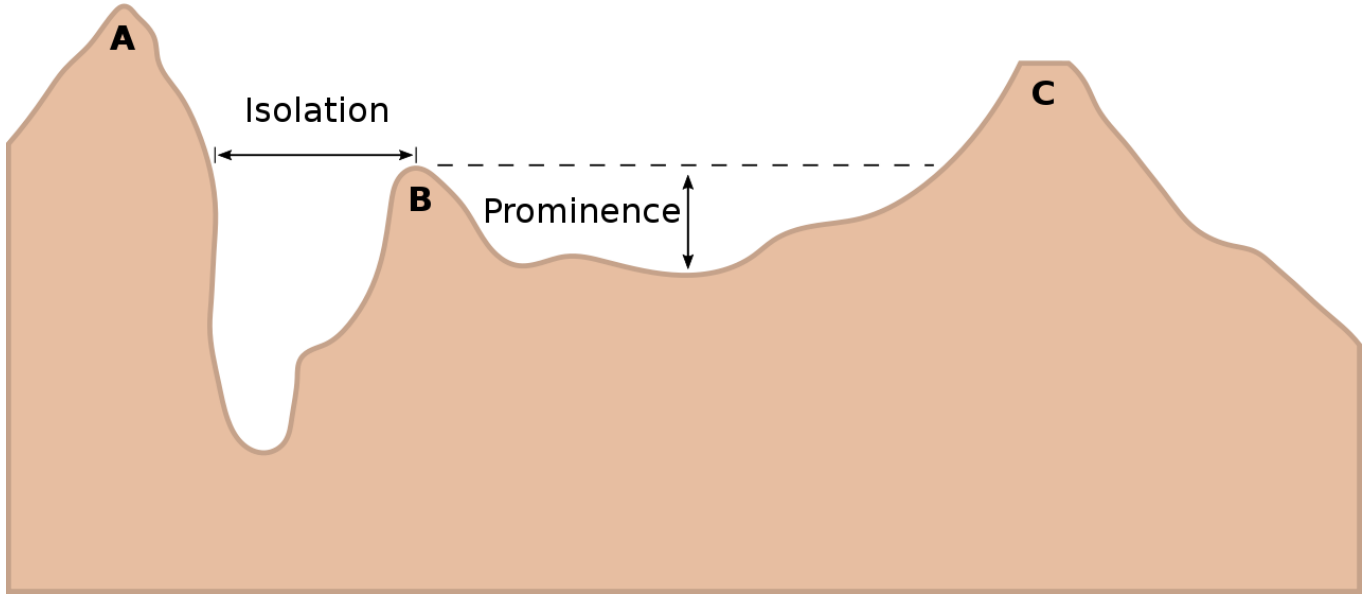
3) Find our peaks

0	0	0	0	0
0	5	5	5	0
0	5	10	5	0
0	5	5	5	0
0	0	0	0	0

3) Prominence finds the peaks

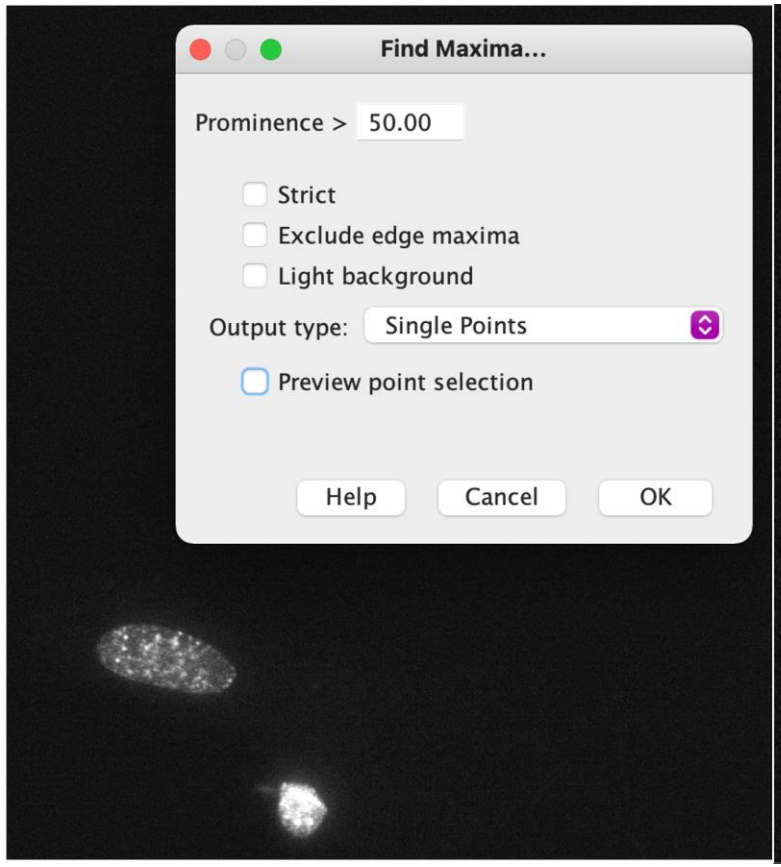


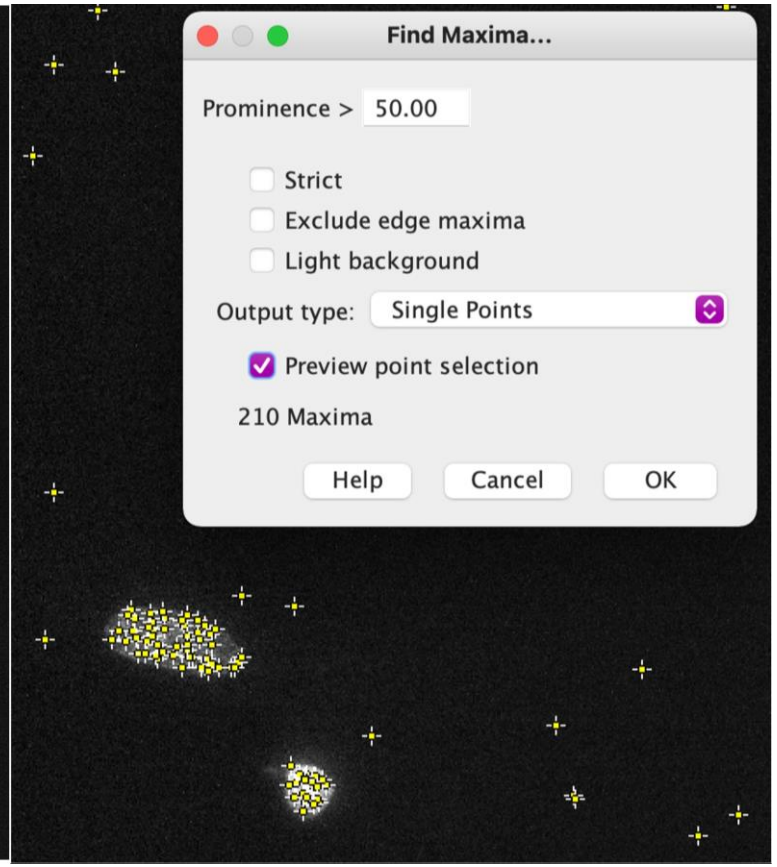
Topographic isolation and prominence of the summit "B"



How many peaks?

Set prominence for the FITC/488 channel image





- Find a prominence setting that allows most visible foci to be counted in a condition
  - Select output of Single Points
  - Check Preview point selection
- Set the prominence to 50 to start
- Once you decide on a prominence, stick with it
- Mess around with it, which image should you use to set your prominence?

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

Each line here is a different nucleus! Divide IntDen by 255 to get the foci count

Data analysis required for Data Summary

- Complete the analysis of images in all conditions (3 replicates from your team and 3 replicates from instructors)
  - Divide the work amongst your lab team!
  - **<u>Do your team's stuff first</u>**

- Once the numbers are recorded, take the average number of foci for <u>each</u> 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

After the analysis is done, feel free to explore the images/data!

- If you have time and interest, feel free to explore other aspects of the images (once you have established your baseline data using the wiki parameters)

Look at any differences in DAPI staining
Is there any relationship between DAPI intensity and foci number?

Play around with threshold and prominence settings
What effect does changing analysis parameters have on the numbers generated?

- <u>Note:</u> Only the original analysis (average # of foci under a single prominence) should be used in your Data Summary

In lab today:

1. Work on image analysis until 3pm
2. Paper discussion from 3-3:45ish
3. Demo on creating the CometChip gel
4. Work in teams to pour CometChip gels
5. Career Fare?

HW due M1D5

1. Create a data figure of H2AX results with title and caption
2. Visit Comm Lab before M1D5.

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)
  - This allows the reader to prime their brain for the new info and allows them to decide whether to believe you or not
- 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

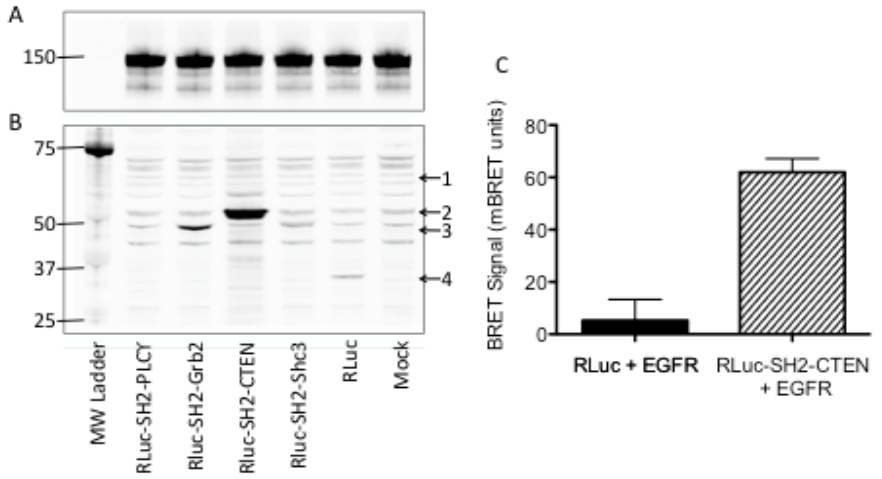


Figure 1: Development of BRET assay to monitor EGFR and SH2 domain interactions. CHO-K1 cells were transfected with Citrine-EGFR (A) and renilla luciferase (RLuc)-tagged SH2 domains from PLCg, Grb2, CTEN, and Shc3 (B). Western blots of CHO-K1 lysates were probed with anti-EGFR (A) or anti-RLuc (B) antibodies. 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