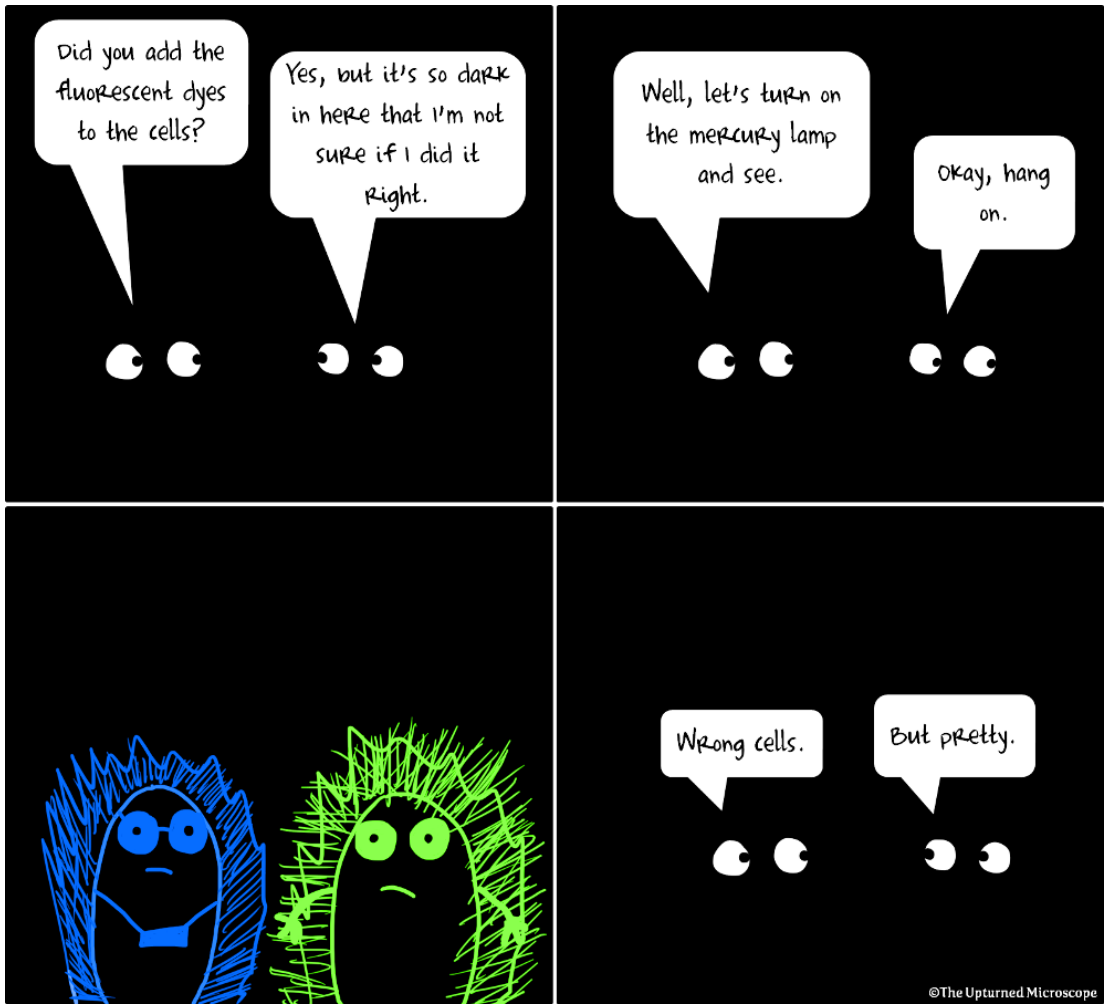
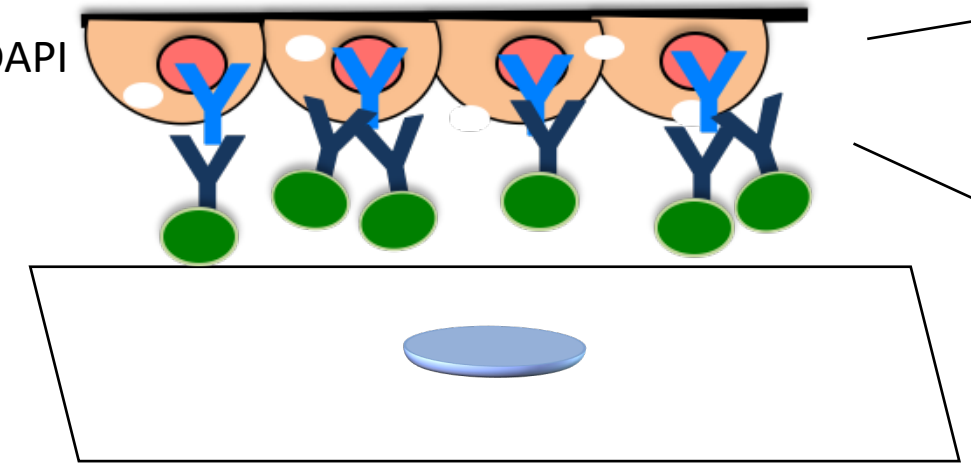
M1D4: Image foci experiment and quantify results

1. Prelab
2. Work on image analysis
3. Quiz
4. Comm Lab 4pm

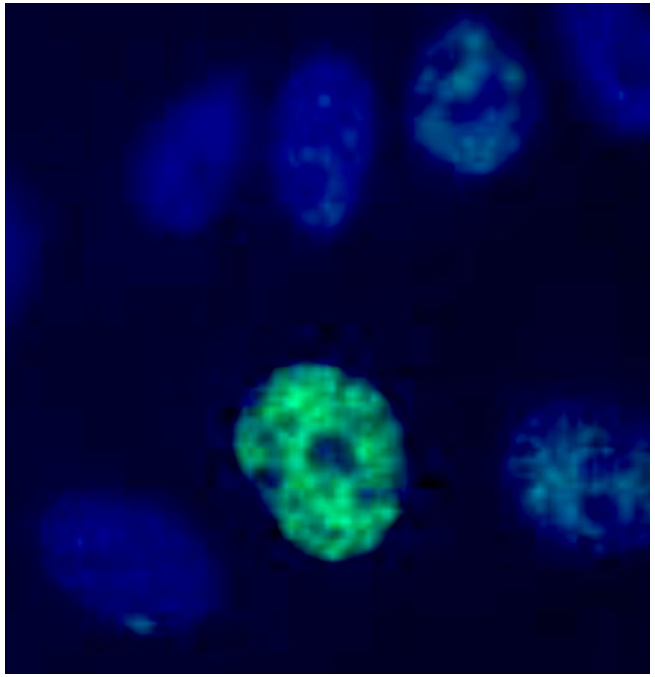


Finish IF by adding DAPI, then mount slides for imaging

Add DAPI



Mount coverslip on glass slide with mounting media



Blue= DAPI
Green= γH2AX

What are our controls for antibody staining?

DEMO TIME!

Advantages and Limitations of IF

Pros:

- Multiple colors
- Low background & good contrast
- Molecular and spatial specificity
- Specialized techniques for 3D and high-resolution imaging

Cons:

- Expensive equipment: laser, filters, sensitive cameras, …
- Toxicity to cells
- Need for fixing or gene manipulation

What type of images do we have?

Experimental Conditions

Fluorescent images

Two channels:

- DAPI
- FITC

Four conditions:

- NT
- 100uM H_2O_2
- 2uM As
- 2uM As + 100uM H_2O_2

Experimental Replication

Technical replicates:

- 6 images per condition

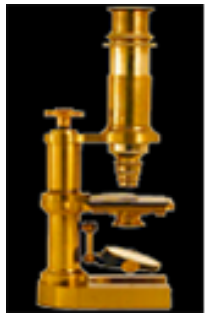
Biological replicates:

- 2 experiments performed on different days

How will we analyze our images?

ImageJ

- Open source software developed at the NIH and used around the world



ImageJ

Image Processing & Analysis in Java

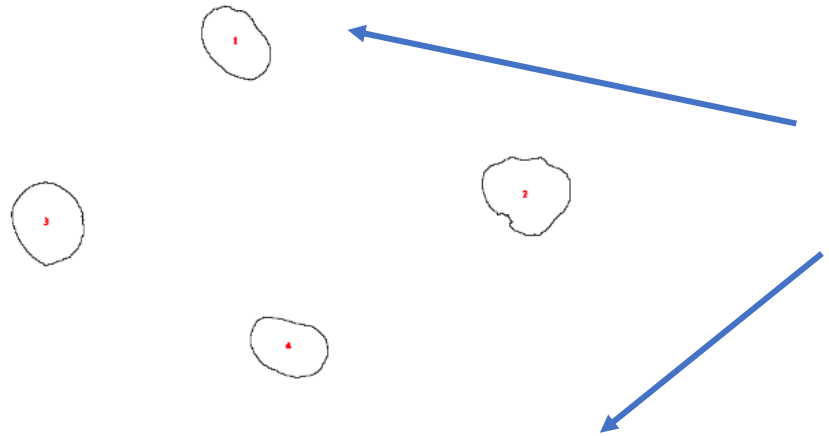
<u>Two types of ImageJ analysis</u>

1. Intensity
   - Measure average FITC signal intensity per nuclei

2. Foci counting
   - Measure average FITC punctae per nuclei

Perform ImageJ analysis for fluorescent intensity

- Set threshold and mask to only examine FITC signal within DAPI-stained nuclei
- Results should be copied into Excel sheet for analysis



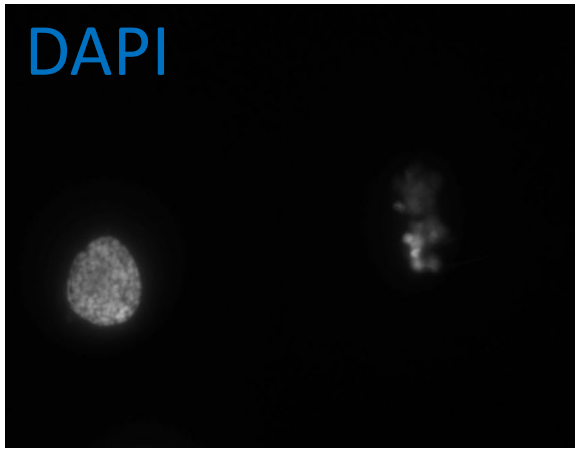
Outlines show nuclei corresponding to data

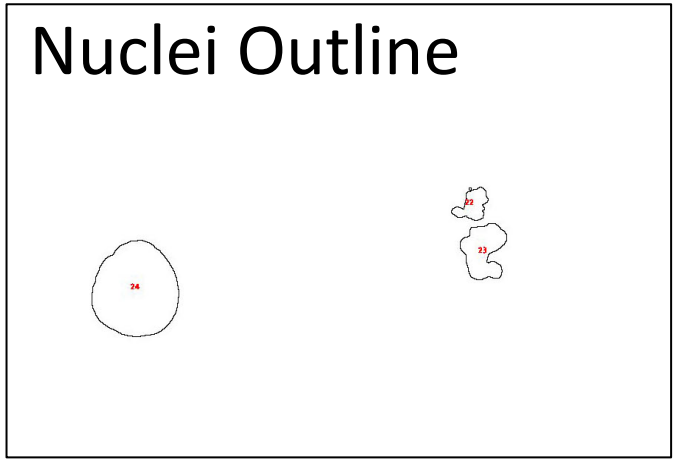
Results

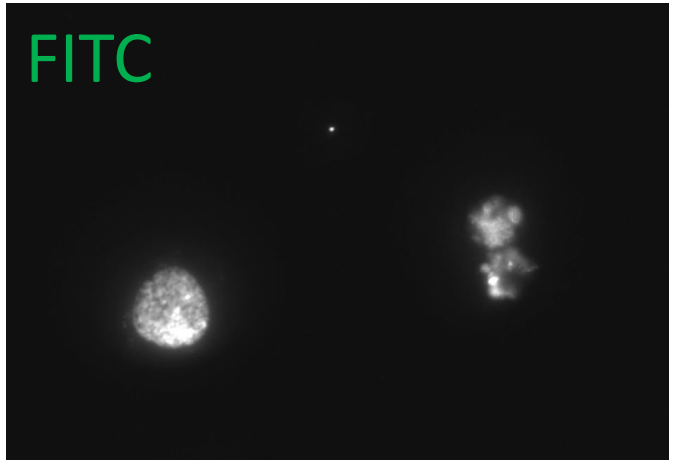
	Label	Area	Mean	Min	Max	Circ.	IntDen
1	082620_MCL5_notx_01007-0002	4291	11.407	10	14	0.829	48949
2	082620_MCL5_notx_01007-0002	5995	15.549	10	42	0.787	93215
3	082620_MCL5_notx_01007-0002	5316	20.894	12	53	0.868	111072
4	082620_MCL5_notx_01007-0002	4213	10.755	9	17	0.831	45309

Evaluate your data during image analysis

- Do the FITC measurement numbers make sense based on what you see?
- How much variability occurs between the nuclei?
- What are potential issues with the analysis you've used?







Notes on data figures in the Data Summary

Data Summary to be completed using **PowerPoint**

Each figure should relay one message

- Subpanels should be related to single conclusion
- Consider representative microscopy images and / or quantified representation of the data rather than a panel of multiple image examples
- Remember the title and caption

Text should be related to results in the figure

- See guidelines in homework description
- **Write in bullets!!**

Results slide example

- Image should not be the entire page
  - Only needs to be large enough to be clear / visible
- 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
- Bullet points should present and interpret the data

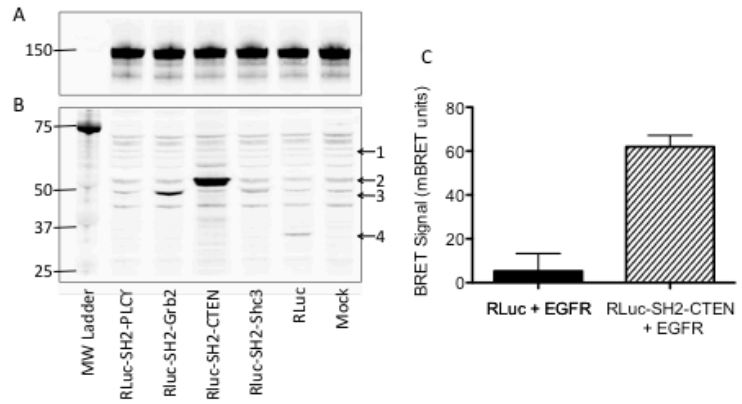


Figure 1: Development of BRET assay to monitor EGFR and SH2 domain interactions. CHO-K1 cells were transfected with Citrine-EGFR (A) and renilla luciferase (RLuc)-tagged SH2 domains from PLCg, Grb2, CTEN, and Shc3 (B). Western blots of CHO-K1 lysates were probed with anti-EGFR (A) or anti-RLuc (B) antibodies.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

BRET system effectively measures EGFR activation:

- To determine if the BRET system could be used to monitor EGFR activation, CHO-K1 cells were transfected with fluorescent EGFR and luciferase-tagged SH2 domains and a BRET assay was performed after growth factor stimulation.
- CHO-K1 were transfected with Citrine-EGFR in all conditions as indicated by correct molecular weight band at 150 kDa (Figure 1A).
- Several protein bands are present in Mock transfection lane suggesting off-target binding of the RLuc antibody (Figure 1B).
- RLuc alone, RLuc-SH2-Grb2, and RLuc-SH2-CTEN were successfully transfected as indicated by correct molecular weight bands (Figure 1B).
- RLuc-SH2-PLCg and RLuc-SH2-Shc3 did not appear by Western blot analysis -- bands different from those in the Mock lane are not identifiable. This outcome could be due to protein expression levels below the detection limit by Western blot or to unsuccessful transfection of cDNA.
- BRET signal increased in cells transfected with Citrine-EGFR and RLuc-SH2-CTEN versus Citrine-EGFR and RLuc alone after EGF stimulation. This difference suggests that the BRET signal is specific for an SH2-EGFR interaction versus randomly localized RLuc.
- In sum, these data suggest that the RLuc-SH2 constructs can be utilized to monitor EGFR phosphorylation, as SH2 domain-EGFR association occurs only at sites of EGFR tyrosine phosphorylation. Next, we determined the dynamic range of the BRET assay.

For Today

1. Work on image analysis from wiki
    1. Begin with FITC intensity protocol– use that for creating homework figure
2. Individually complete emailed quiz
3. Return to main room by 4pm for Comm Lab!

For M1D5

1. Write summary of BE Comm Lab meeting
2. Create figure of H2AX intensity analysis and write associated bullet points for Data Summary