M1D7: Visualize and analyze data for sub-nuclear foci assay

10/06/17

1. Quiz
2. Prelab Discussion
3. Mount coverslips and image on microscope
4. Analyze H2AX images
5. Draft data slide

Eric will grade M1D2 Notebook page
Last edits by 10pm tonight

Assignments for M1

- Data summary draft
  - due by 10pm on Wed., October 11
  - revision due by 10pm on Sun., October 22

Summary content

1. Title
2. Abstract
3. Background, Motivation
4. Figures, Results & Discussion, Interpretation
5. Implications, Future Work

- Mini presentation due by 10pm on Sat., October 14
- Blog post for M1 due by 10pm on Mon., October 23

Extra office hours

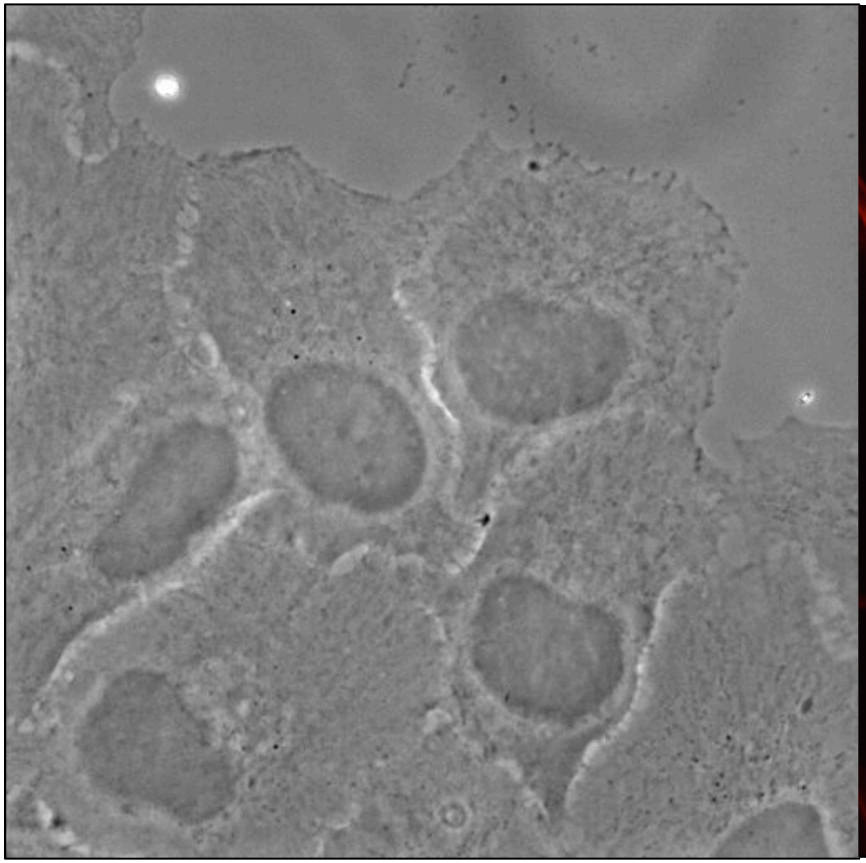
- Saturday 10/7, 10am-2pm **in 56-302**
- Monday 10/9 **(Prof. Engelward)** 1-3pm, 16-743c
- Wednesday, 10/11, 11am-4pm **in 56-302**

Regular office hours

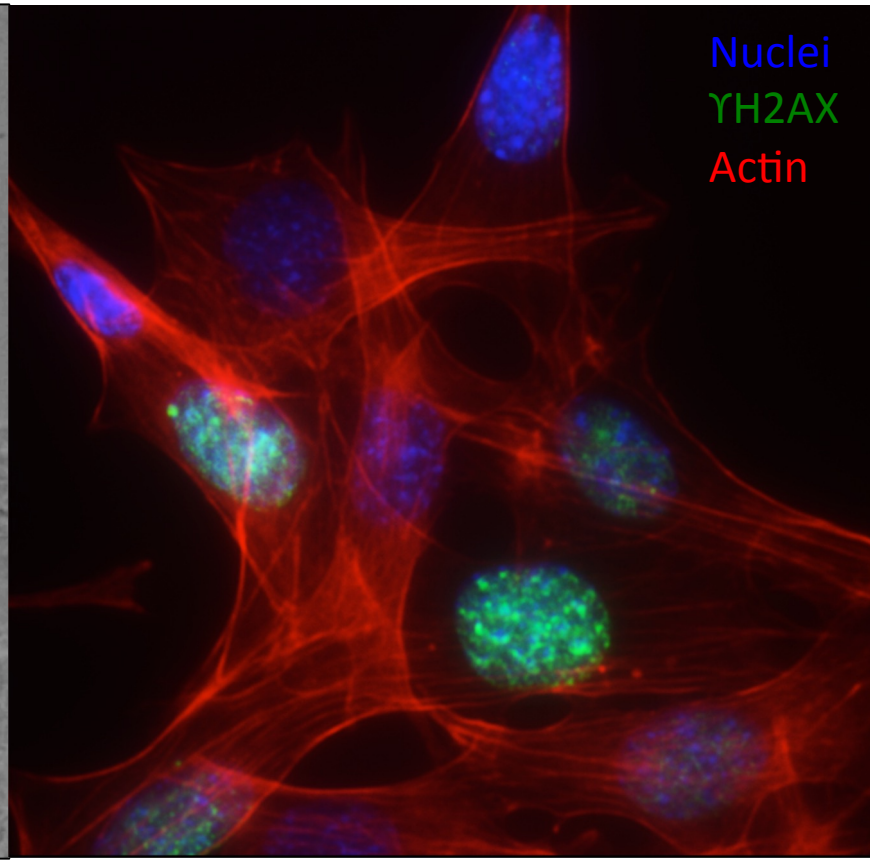
- Josephine, Thursday 2pm-3pm in 56-341c
- Leslie, Friday 9am-10am and 3pm-4pm in 56-341c
- All regular OHs posted to wiki announcements tab

Come work in office hours even if you don't have specific questions!
You might hear good questions you hadn't thought of ☺

Why is fluorescence imaging so widely used in biology?

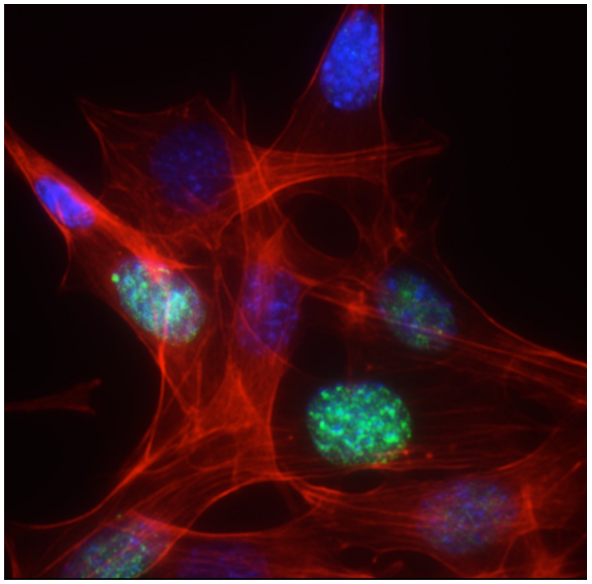


Bright-field



Fluorescence

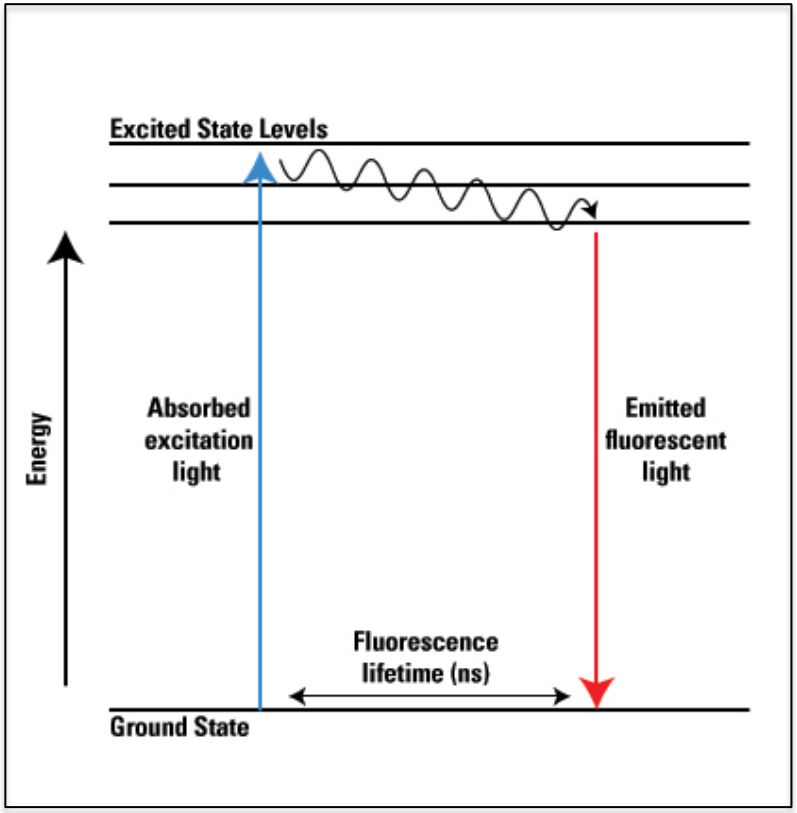
Considerations for fluorescence imaging



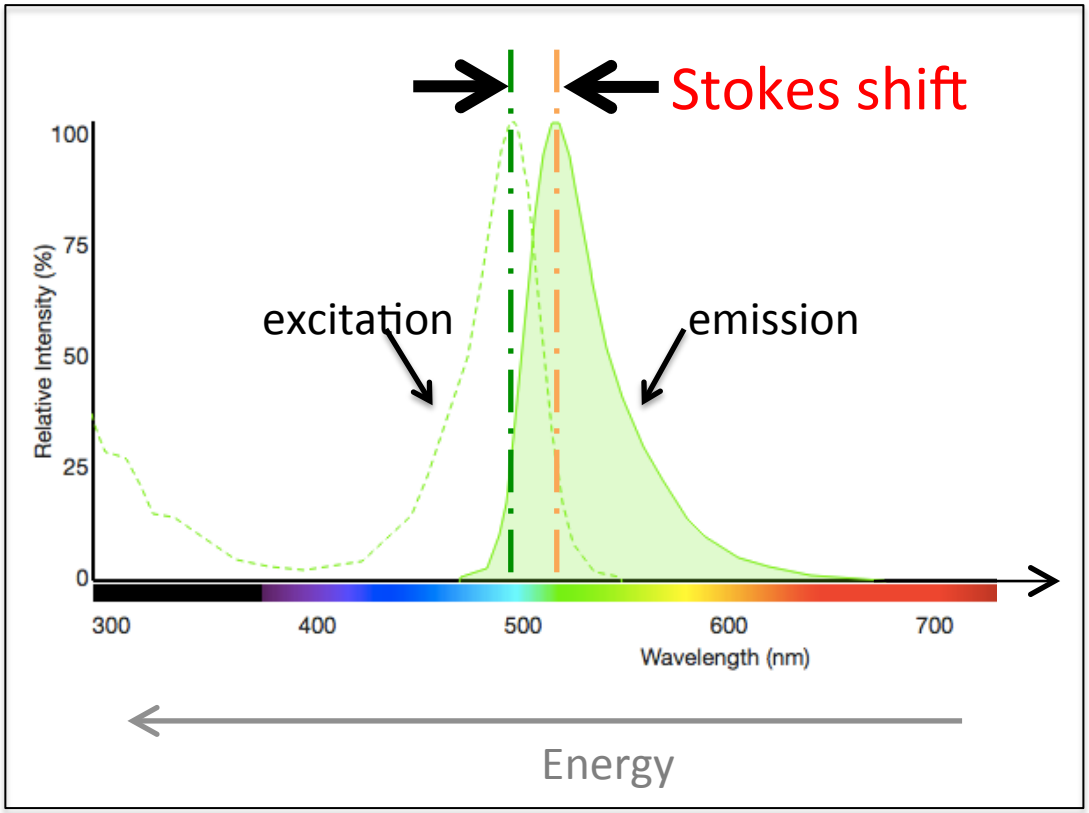
- pros:
  - low background
  - excellent contrast
  - multiple colors
  - molecular and structural specificity
  - biochemical sensitivity for functional imaging (Ca^{2+}, pH)
  - genetic expression
  - specialized techniques for 3D and high-resolution imaging
- cons:
  - expensive equipment: laser, filters, sensitive cameras, …
  - toxicity to cells
  - need for fixing or gene manipulation
  - does the added fluorophore moiety impair biological function?

Physical principles of fluorescence

Jablonski diagram

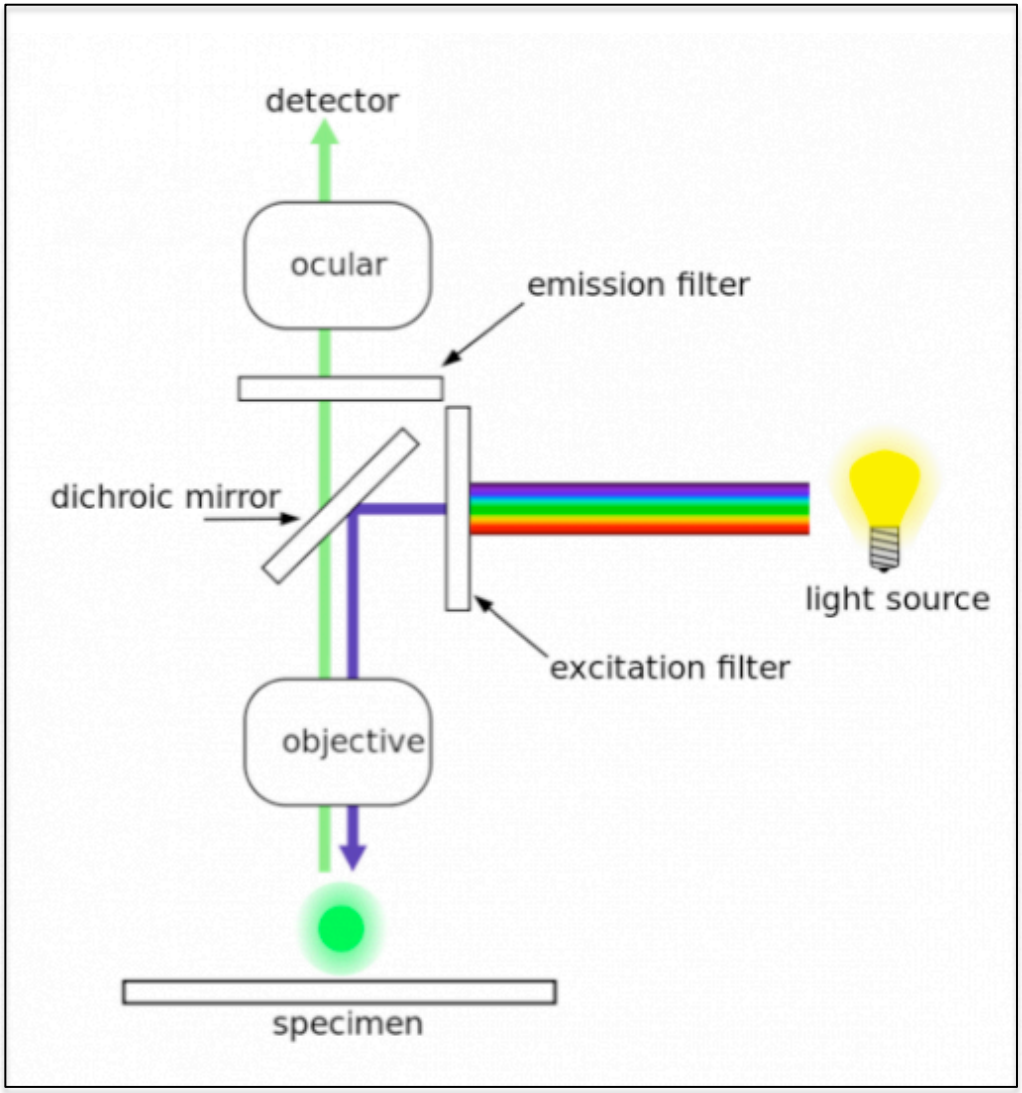


Stokes (red) shift of emission wavelength



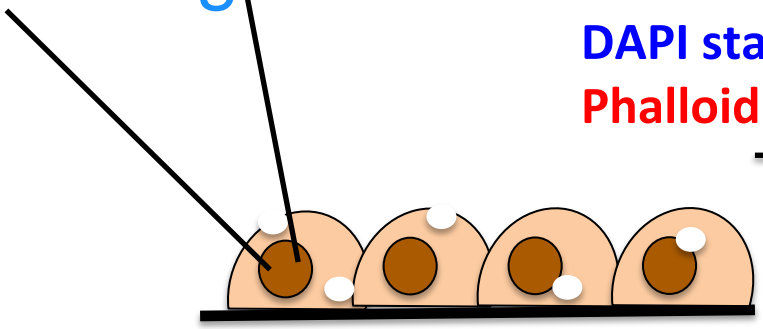
lower energy = longer wavelength

Epi-fluorescence microscope



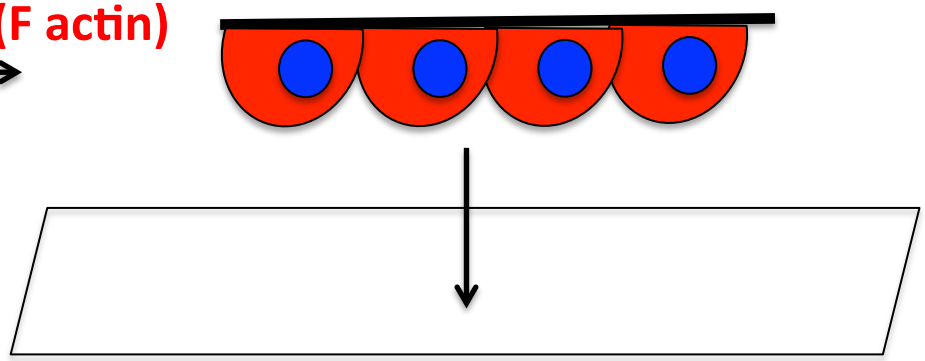
- Our secondary antibody
  - Excitation max 488 nm
  - Emission max 525 nm
- Filter set (cube) FITC
  - Selects/reflects blue light
  - Transmits green light
- Emission filter
  - Allows ~90% of emitted green light to pass through
  - Attenuates excitation light by a factor of ~10^6

Completing γH2AX assay

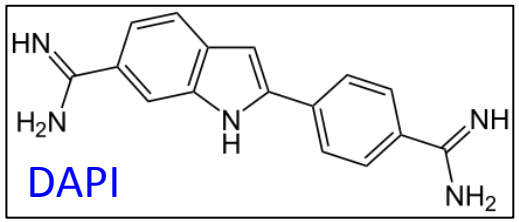


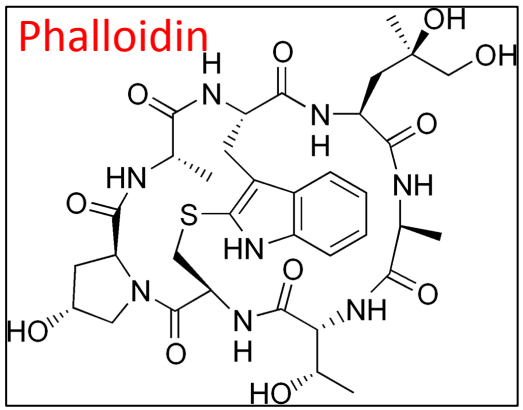
DAPI stain (DNA)

Phalloidin stain (F actin)



Mount on glass slide





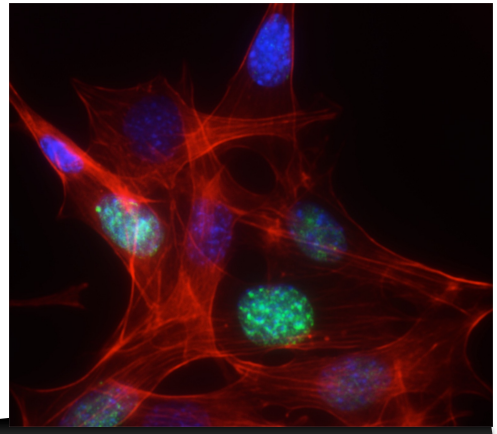
small

bind tightly

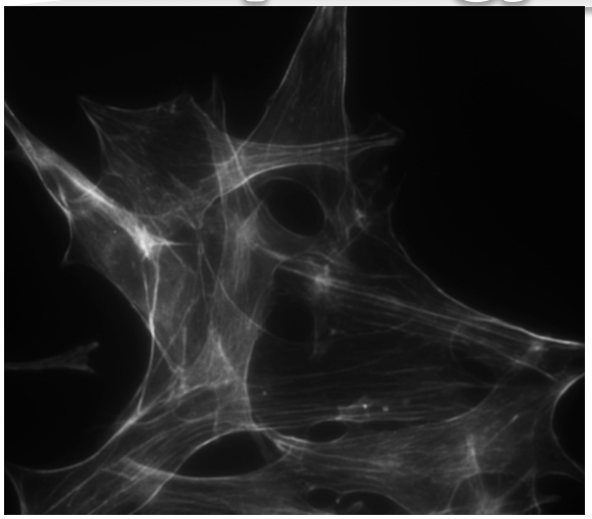
membrane permeable

γH2AX Data Analysis

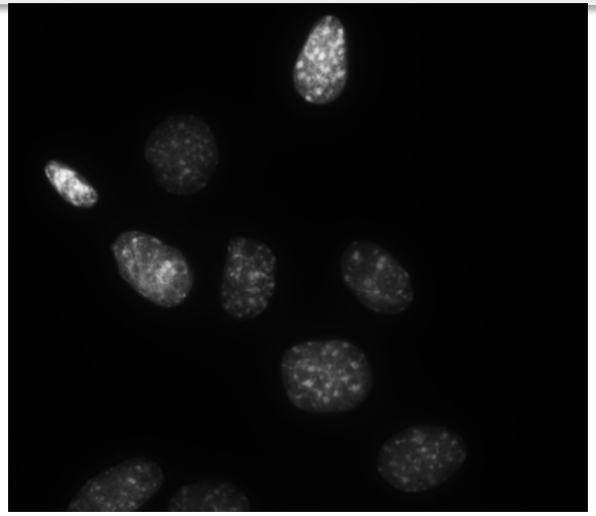
What fraction of cells have a high amount of double strand breaks?



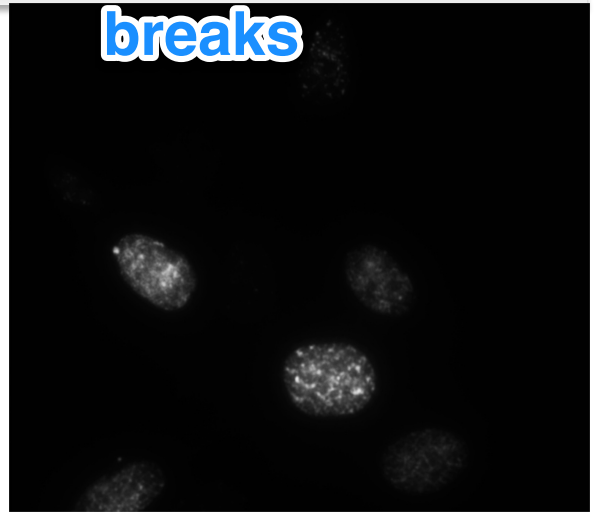
morphology **count # cells** **double strand breaks**



Actin (TxRed)



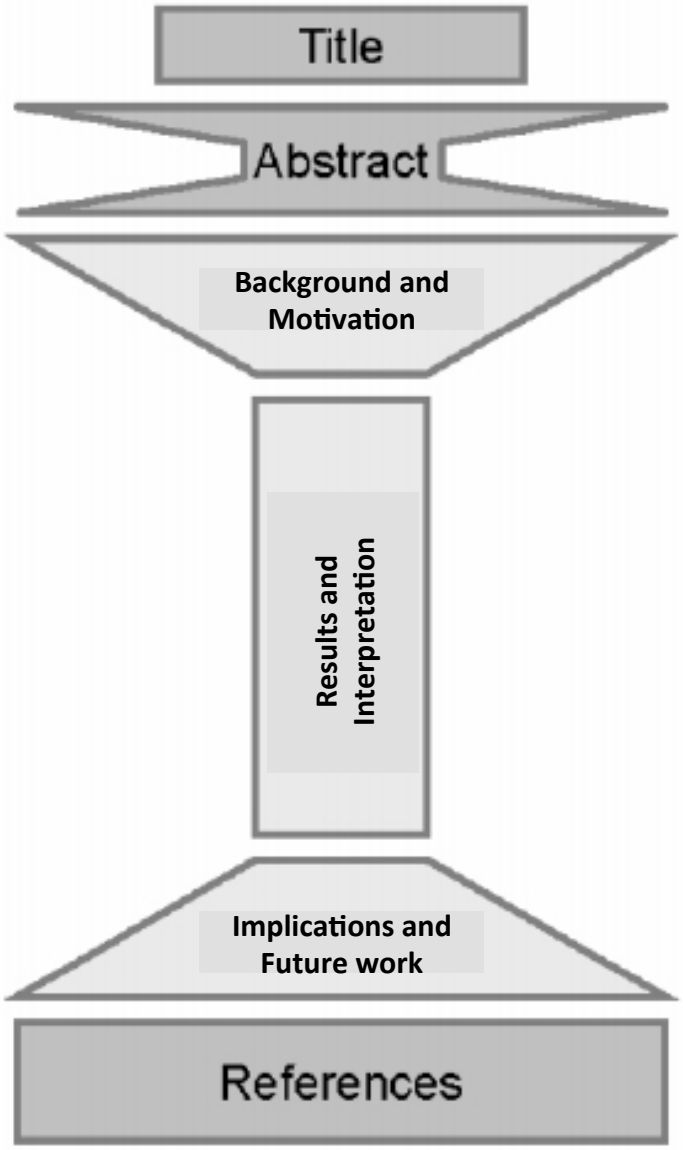
Nuclei (DAPI)



ϒH2AX (FITC)

MMS/H2O2 doses, recovery time, wild type/mutant, comet chip

M1 Data Summary



Title: take-home message

Abstract: the only page *not* in bullet points

ALL BULLET POINTS:

-background and motivation (include references)

- schematics

-Results and interpretation

- Cell loading
- Comet Chip analysis (team expt.)
- Comet Chip class data (MMS and H2O2)
- H2AX assay (team expt.)
- schematics

Implications and future work

References (*see wiki for format suggestions*)

Background & Motivation

- Impact statement
  - general background
  - describe previous work in the field
- Specific background
  - introduce topics, pathways and specific technologies necessary to understand the experiment
  - narrow focus to the specific question addressed in your study
- Knowledge gap/statement of problem
  - what is unknown, therefore motivating your study
- Hypothesis
  - what do you propose will be the outcome of your study
- A brief preview of your findings
  - Here we show…
  - end with broad implications of the study

The meat of your paper: Results & Interpretation

- Figures and captions
  - **Decide on these first**
  - Use subpanels
  - Text: limited on figure, explicit in caption
  - reasonable size
  - descriptive title
  - intro sentence in caption
  - caption descriptive of image, very light on methods
- Result bullets
  - Goal / intent / purpose of experiment = intro topic sentence
  - What you did: experiments and expectations, including controls
  - What you found: quantitatively describe your result, referring to the figure
  - What does this indicate: interpret your result, what does it mean?
  - What does this motivate you to do next: transition to next experiment

Example Results slide (from Wiki)

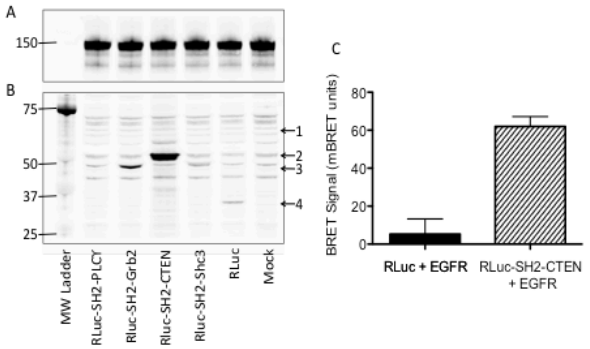


Figure 1: Development of BRET assay to monitor EGFR and SH2 domain interactions. CHO-K1 cells were transfected with Citrine-EGFR (A) and renilla luciferase (RLuc)-tagged SH2 domains from PLCg, Grb2, CTEN, and Shc3 (B). Western blots of CHO-K1 lysates were probed with anti-EGFR (A) or anti-RLuc (B) antibodies.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

Implications & Future Work

- Start with a very similar paragraph to the last paragraph in your Background/Motivation (restate major results and broad implications)
- Follow same order as in Figures/Results
  - Describe your conclusions from your data
  - Describe caveats and suggest remedy
  - Identify unknowns and speculate within reason
  - Don't make huge generalizations or overreach
- Propose future experiments, identify new questions that arise
- Come back to (the same) big picture topic introduced in background

Today in lab

1. Complete the final washes of your staining and mount coverslips on slides
   – slides and DAPI mounting media on front bench
2. Teams will be taken to the microscope as they finish
3. Download your team's H2AX images and complete data analysis of your images
4. Complete your in-class results section and turn in for feedback
5. Continue to analyze CometChip results and draft sections of your Data summary