M1D7: Complete sub-nuclear foci assay staining and data analysis

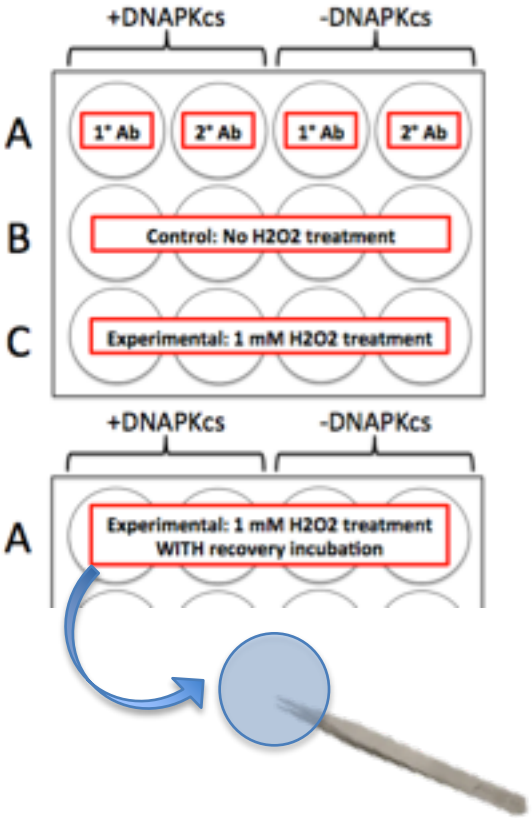
10/4/18

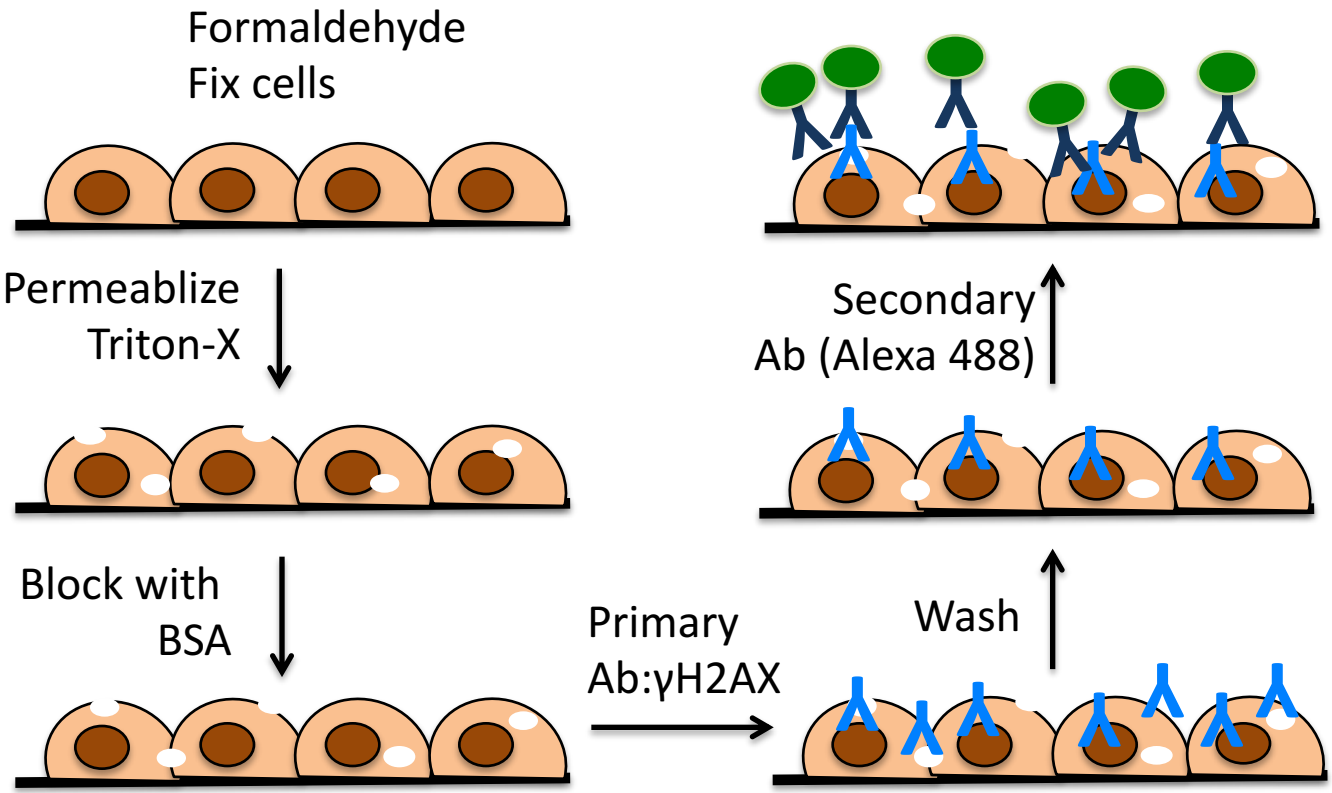
1. Stats
2. Finish staining γH2AX foci
3. Visit microscope
4. γH2AX image analysis
5. Data analysis—ask questions while you're here!

Announcements

- Notebook due 10/5, 10pm
  - Graded in detail: M1D5
- Regular office hours:
  - Fri. (10/5) 2-3pm, 56-341c
- Extra office hours:
  - Sat. (10/6) 10am-12pm, 56-302
  - Mon. (10/8) 12-5pm, 56-302
  - Prof Engelward: Wed. (10/10), 9am-12pm, 1-3pm
- Data Summary draft due 10pm, Wed. 10/10

Practically using immunofluorescence: γH2AX assay to detect double-strand DNA breaks





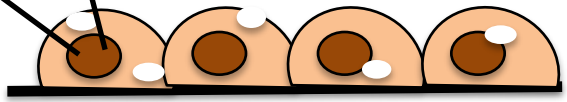
Complete γH2AX assay today

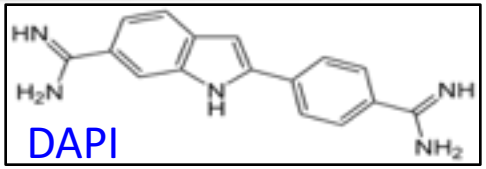
Phalloidin stain (F actin)

Together with Secondary Ab

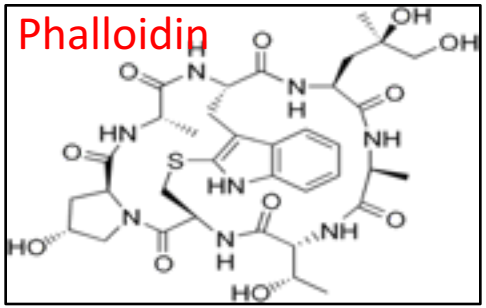
DAPI stain (DNA)

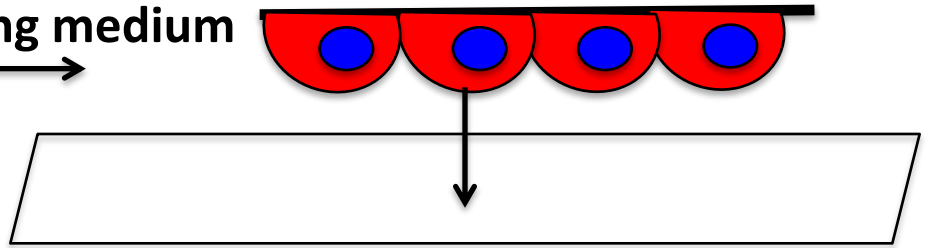
In mounting medium





DAPI

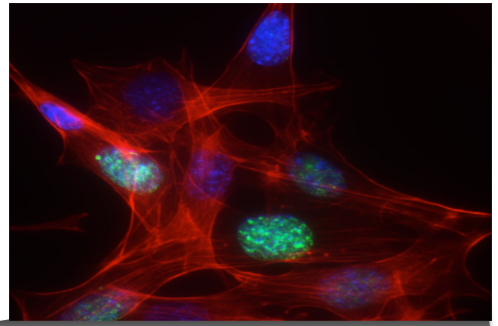




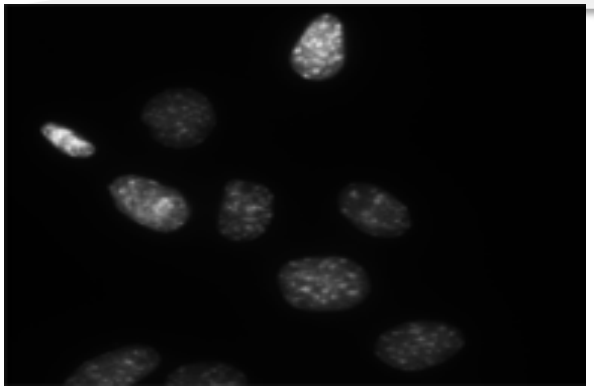
Mount on glass slide

γH2AX Data Analysis

What is the total amount of γH2AX signal of each nucleus?

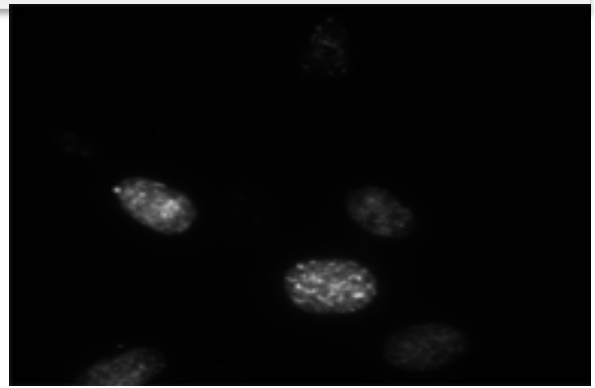


- Use DAPI channel to identify nuclei
- Use ImageJ to quantify total FITC (γH2AX) fluorescence in each nucleus
- Imaged using same exposure times per channel across all conditions



Nuclei (DAPI)

450 ms

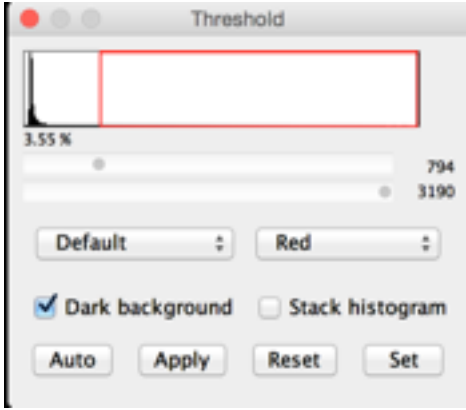


γH2AX (FITC)

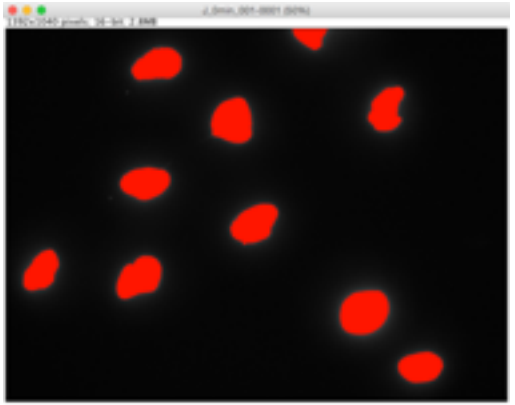
1 s (30 min recovery)

800 ms (60 min recovery)

γH2AX Data Analysis using ImageJ



- Pick a threhold value in the DAPI channel to identify nuclei—typically good to be consistent, use same threshold on all images.
- Identify nucleus location using DAPI channel, & quantify fluorescence in FITC channel.
- Normalize fluorescence intensity by area of nucleus



Perform analysis on resultant excel spreadsheet

#post on wiki

#of nuceli region identified.

filename

px^2

total fluorescence intensity in region

	A	B	C	D	E	F	G	H	I	J	K	L
1		Label	Area	Mean	Min	Max	Circ.	IntDen	RawIntDen	AR	Round	Solidity
2	1	J_H2O2_001-0002	10213	487.021	371	1062	0.768	4973943	4973943	1.382	0.723	0.951
3	2	J_H2O2_001-0002	10249	591.748	391	1005	0.862	6064824	6064824	1.077	0.929	0.979
4	3	J_H2O2_001-0002	8482	459.955	381	549	0.811	3901340	3901340	1.519	0.658	0.97
5	4	J_H2O2_001-0002	11661	496.568	380	865	0.838	5790485	5790485	1.386	0.721	0.978
6	5	J_H2O2_001-0002	10959	659.783	439	2451	0.729	7230565	7230565	2.062	0.485	0.96
7	6	J_H2O2_001-0002	38645	595.773	398	1937	0.456	23023658	23023658	2.432	0.411	0.837

$\frac{\text{RawIntDens}}{\text{area}}$

$(a.u./px^2)$

arbitrary units

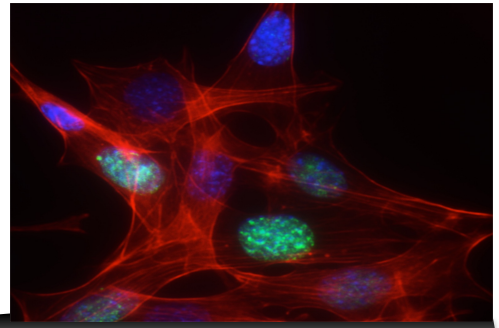
Compare:

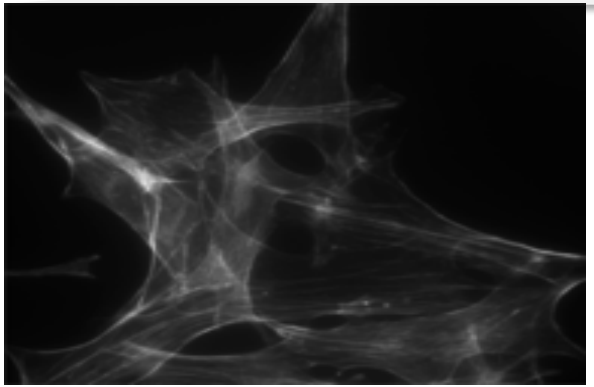
- timepoints
- cell lines
- H_2O_2 treatment

- Comet-chip vs. γH2AX kinetics

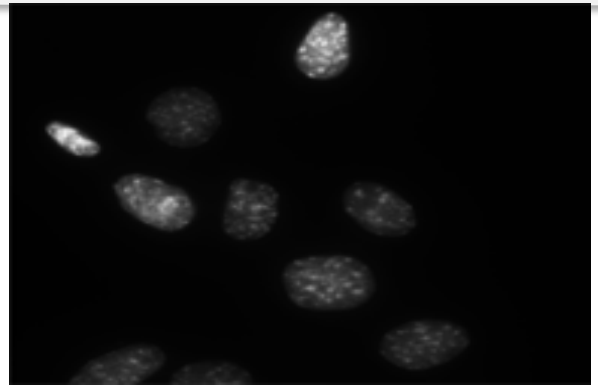
Make stacked images

- Use ImageJ to overlay and color the images
- Don’t forget a scale bar: 0.17 um/px

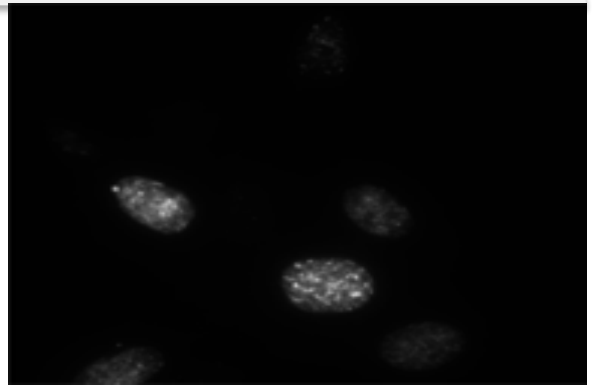




Actin (TxRed)



Nuclei (DAPI)



ϒH2AX (FITC)

Major assignments for Mod1

- **Data summary** draft
  - due by 10pm on Wed., October 10
  - revision due by 10pm on Sat., October 20

Summary content

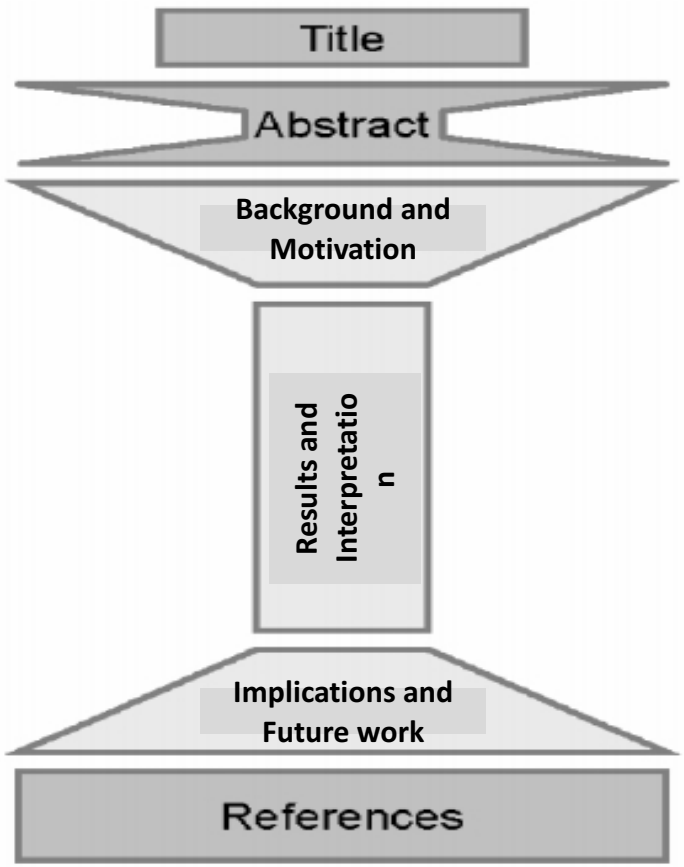
1. Title
2. Abstract
3. Background & Motivation
4. Figures, Results & Interpretation
5. Implications & Future Work

- **Mini presentation** due by 10pm on Sat., October 13
- Lab notebook for Mod 1 due by 10pm on Fri. October 5
- Blog post for M1 due by 10pm on Thurs., October 11

20.109 Blog Post

- You will receive an invitation to join the class blog
- Possible topics listed on the blog
- Details about use:
  - Do not publish MIT logo
  - Do not post photographs with names tagged
  - Do not write malicious comments
  - Do not plagiarize

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 repair data (Engelward lab)
- H2AX assay (team & class data, images)
- 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 (e.g. BER, DNAPKcs, CometChip)
  - 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

cite PNAS paper Wood et al. 2010

← Be specific to your experiments

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)

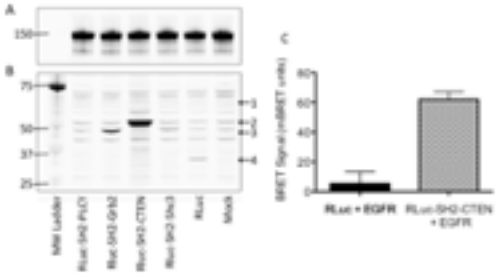


Figure 1: Development of BRET assay to monitor EGFR and SH2 domain interactions. CHO-K1 cells were transfected with Citrine-EGFR (A) and renilla luciferase (RLuc)-tagged SH2 domains from PLCg, Grb2, CTEN, and Shc3 (B). Western blots of CHO-K1 lysates were probed with anti-EGFR (A) or anti-RLuc (B) antibodies.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

* include putative substrate for DNAPK (refer to lecture slides)

- 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

In lab today

1. Finish staining γH2AX coverslips
2. Stats exercise
3. γH2AX image analysis
4. Finish data analysis
5. Continue working on Data Summary