M1D6: Complete sub-nuclear foci assay staining

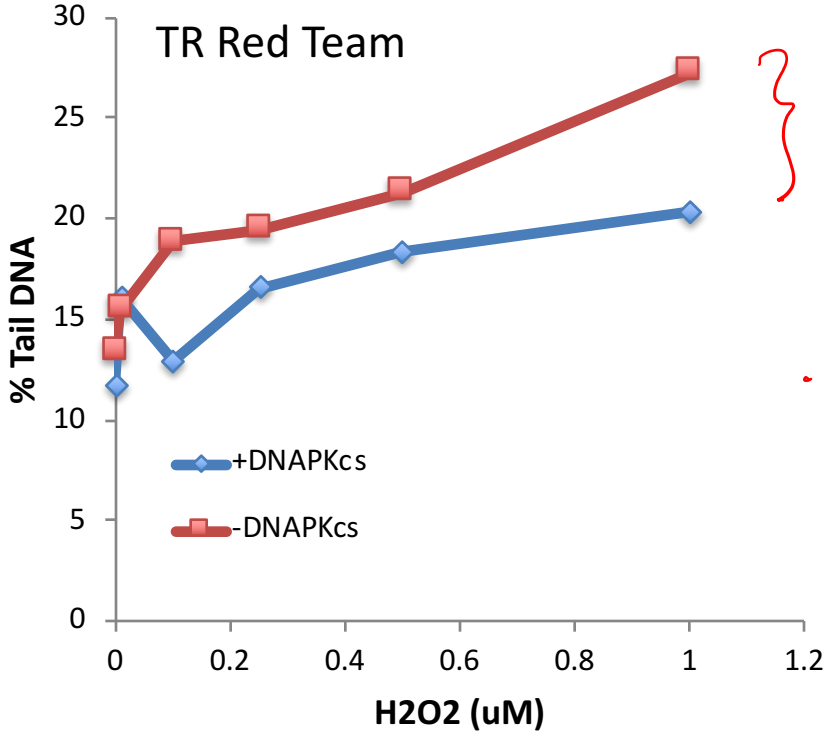
10/2/18

1. Stain γH2AX foci
2. Work on Data Summary in down time
3. Paper discussion

Announcements

- Quiz Thursday (10/4)
- Extra office hours – Friday 2-3p, Sat/Mon? Tues 9 7
- Data Summary draft due ~~Monday~~ 10/8 (~~6~~ days away!)
- You already have over half the data you need to complete your Data Summary!

What did your CometChip data look like?



talk about diff. in Data summary

WT-Yellow team for dose response

- Be quantitative! stats

You will need to plot 95% confidence intervals—explanation coming in Thursday's prelab!

CometChip recovery data to include in Data Summary

- Data from Jing Ge, Engelward Lab
- 50 uM H_2O_2

replicate CometChip experiments

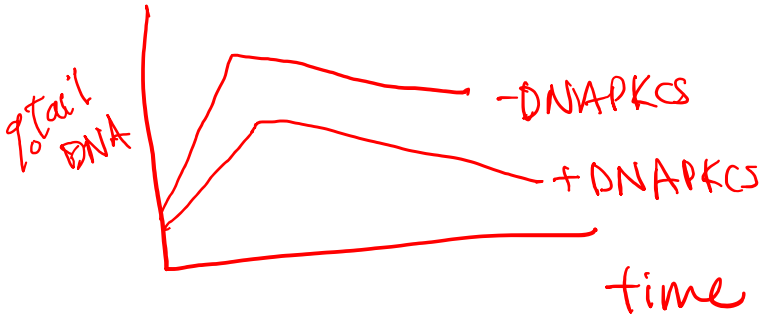
1						
2	M059K (+DNAPKcs)					
3	Time (min)	17-Oct	21-Oct	23-Oct	7-Nov	8-Nov	15-Nov
4	-20	8.8307	9.3469	15.1883	6.4421	10.1519	8.3265
5	0	65.5986	73.7173	11.5915	13.6661	22.1336	33.9372
6	20	32.3809	30.3926	25.659	8.9514	11.4634	16.667
7	40	11.3853	23.8967		8.6218	16.3445	10.4776
8	60	13.4105	14.8082	15.1418	8.3472	9.8262	12.8872
9							
10							
11	M059J (-DNAPKcs)					
12	Time (min)	17-Oct	21-Oct	23-Oct	7-Nov	8-Nov	15-Nov
13	-20	9.784	20.5153	13.7129	8.134	14.466	10.6093
14	0	76.2265	74.9286	70.3199	70.8689	50.5001	63.5164
15	20	76.7371	77.3443	55.0552	69.212	43.9916	37.2402
16	40	61.5771	72.2957		51.5209	32.7484	30.8764
17	60	40.9693	53.4273	44.9221	29.9882	14.2085	44.325
18							

Time

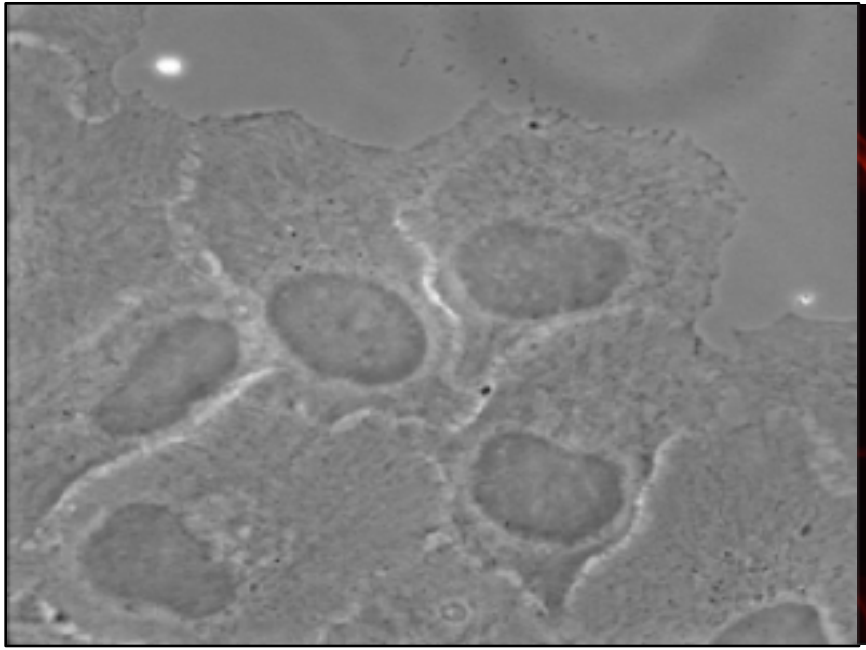
-20 min : untreated cells (control)

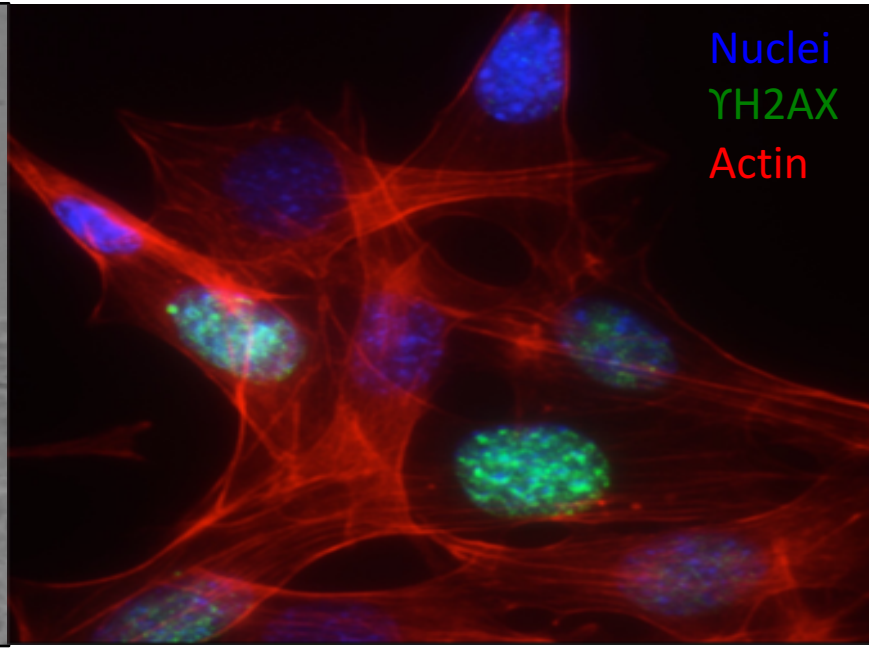
0 min: right after 20 min H_2O_2 treatment

20-60 min: time after treatment, recovering in media



Why is fluorescence imaging so widely used in biology?

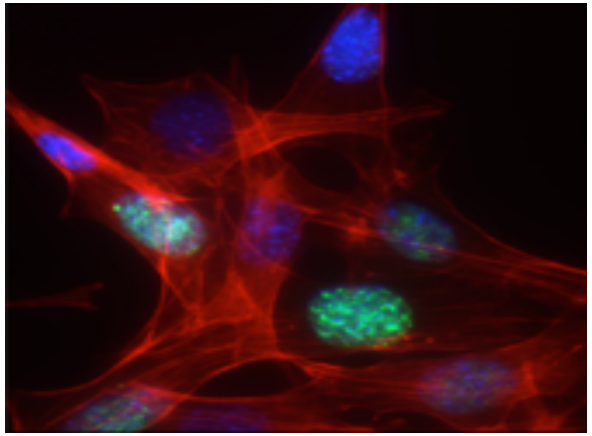




Bright-field

Fluorescence

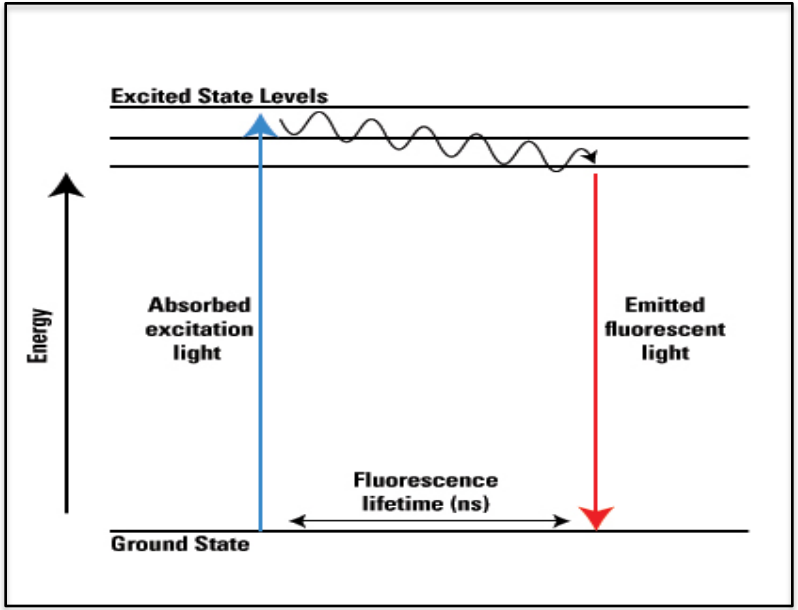
Considerations for fluorescence imaging



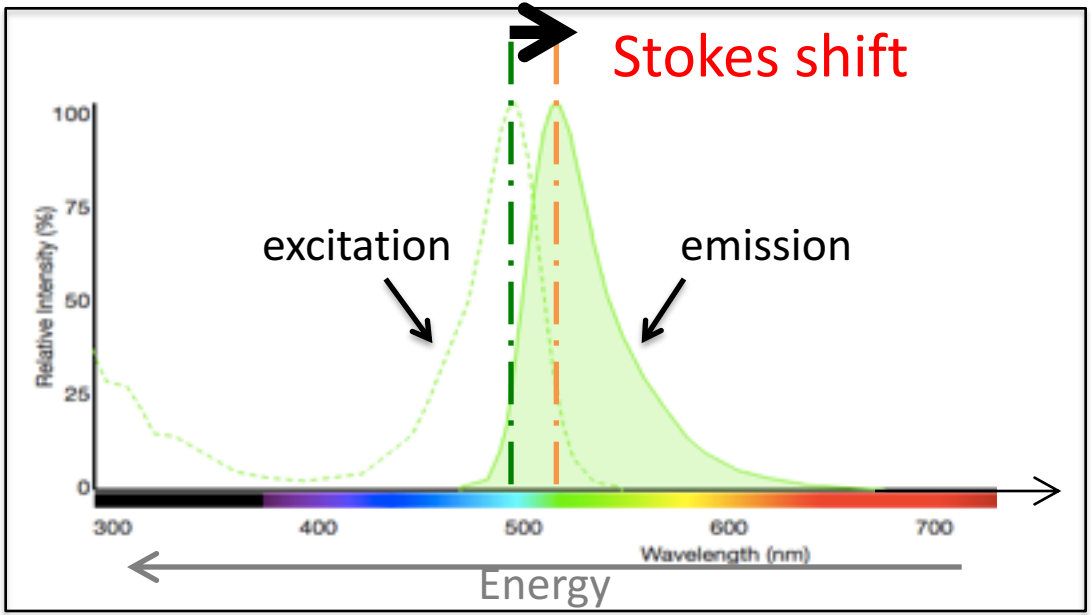
- Pros:
  - Low background
  - Excellent contrast
  - Multiple colors
  - Molecular and structural specificity
  - Biochemical sensitivity for functional imaging (Ca^{2+}, pH)
  - Genetic expression
  - Specialized techniques for 3D and high-resolution imaging
- Cons:
  - Expensive equipment: laser, 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

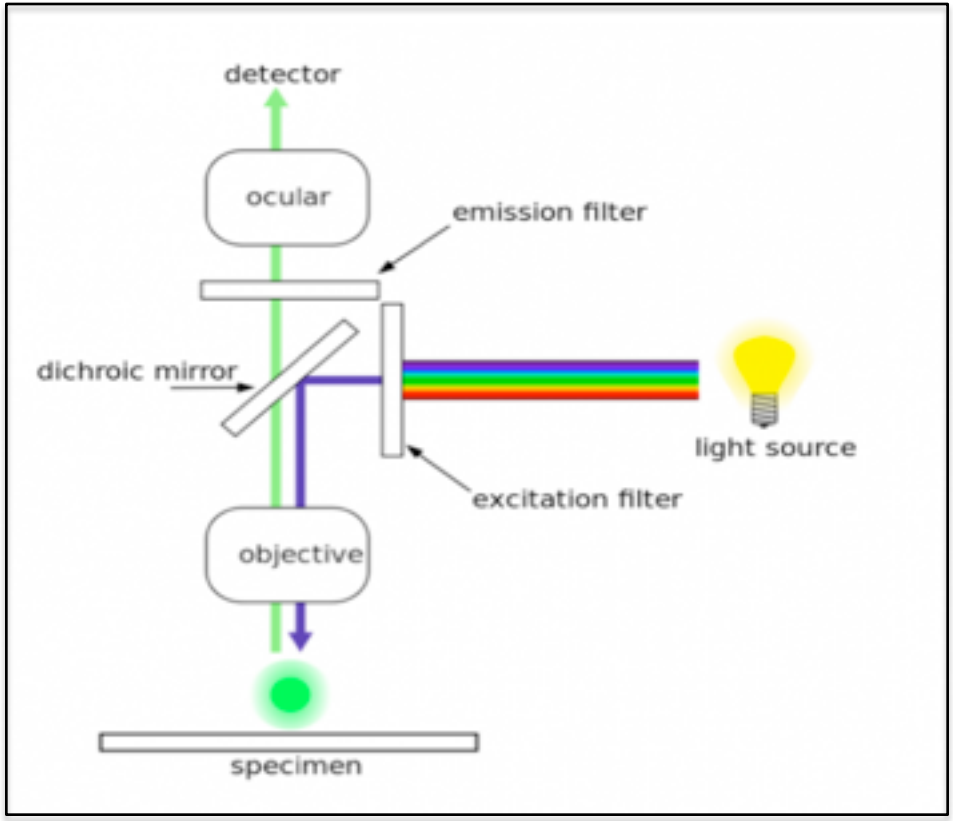


Stokes (red) shift of emission wavelength



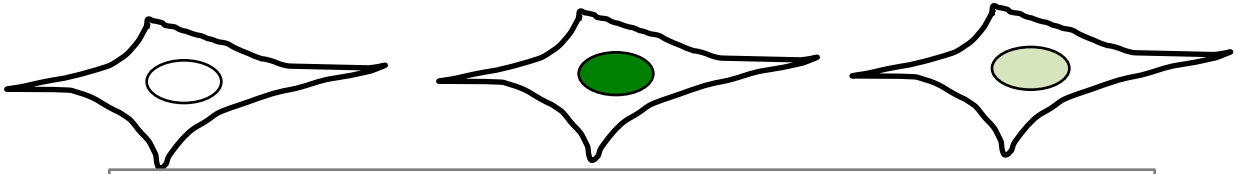
longer wavelength = lower energy

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

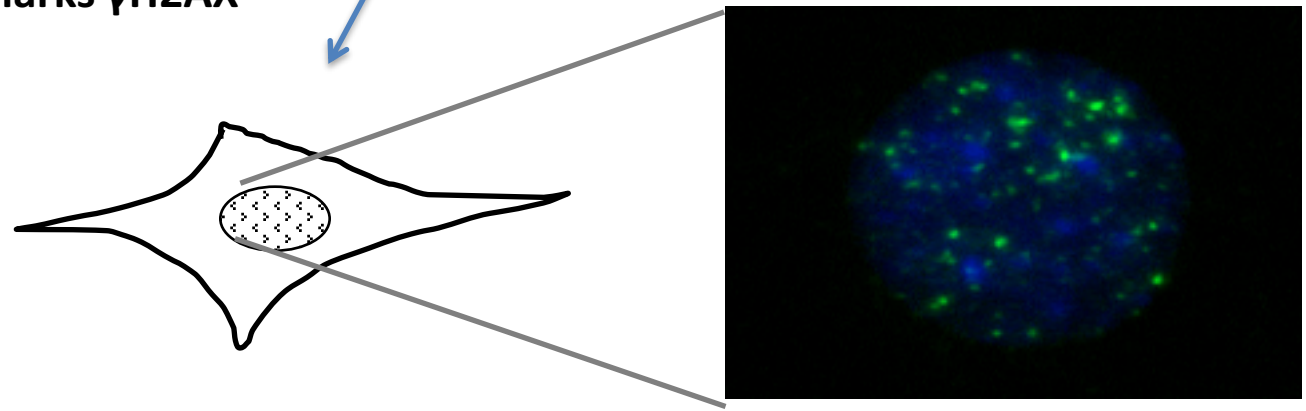
Measuring DNA damage via γH2AX Assay



no treatment, H_2O_2 treatment, recovery condition

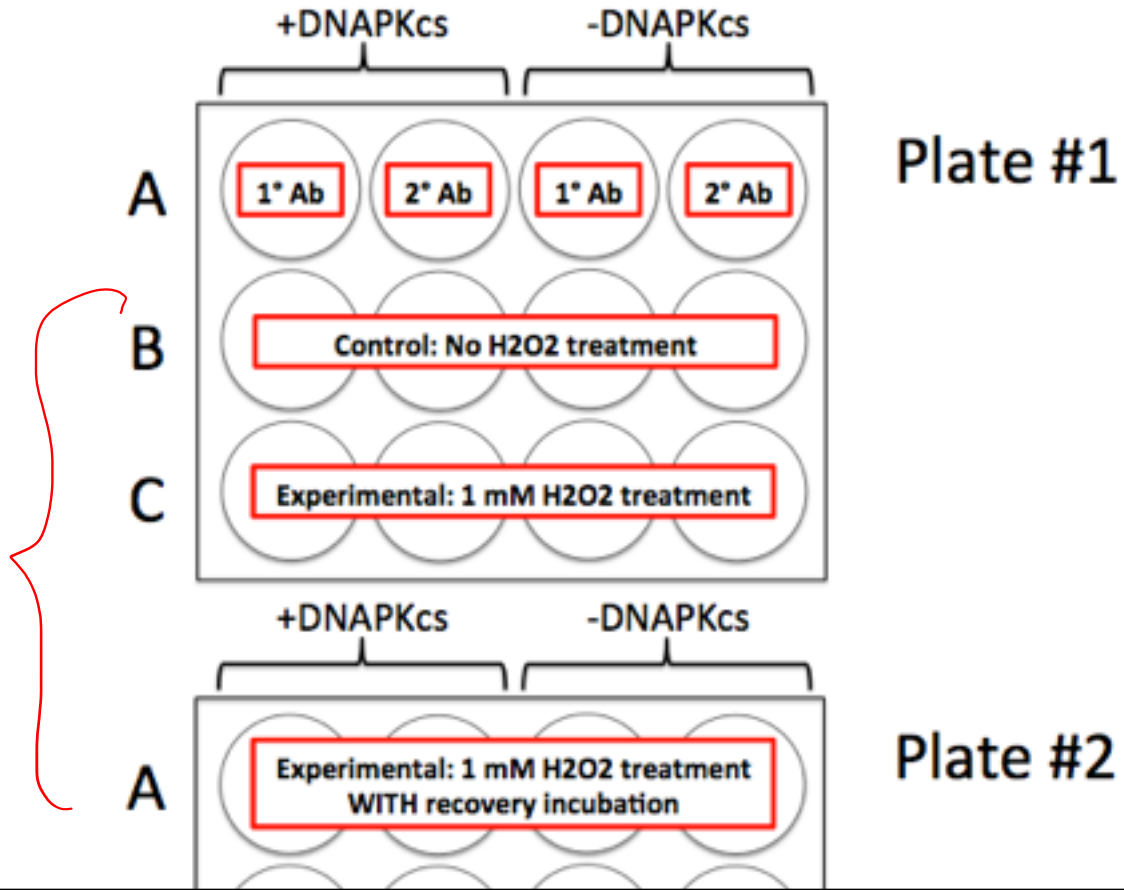
γH2AX = phosphorylated H2AX histone, indicative of DSBs (and potentially other types of DNA damage)

Fix cells and stain with antibody that marks γH2AX



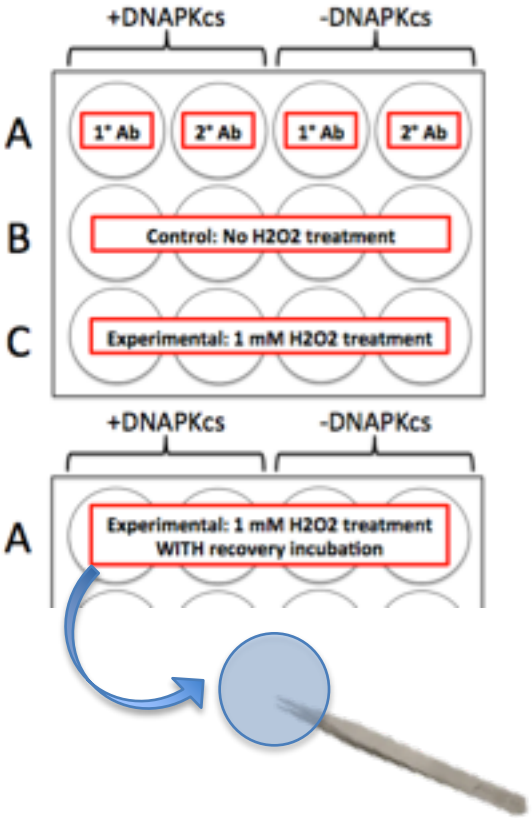
Blue: DNA
Green: γH2AX staining

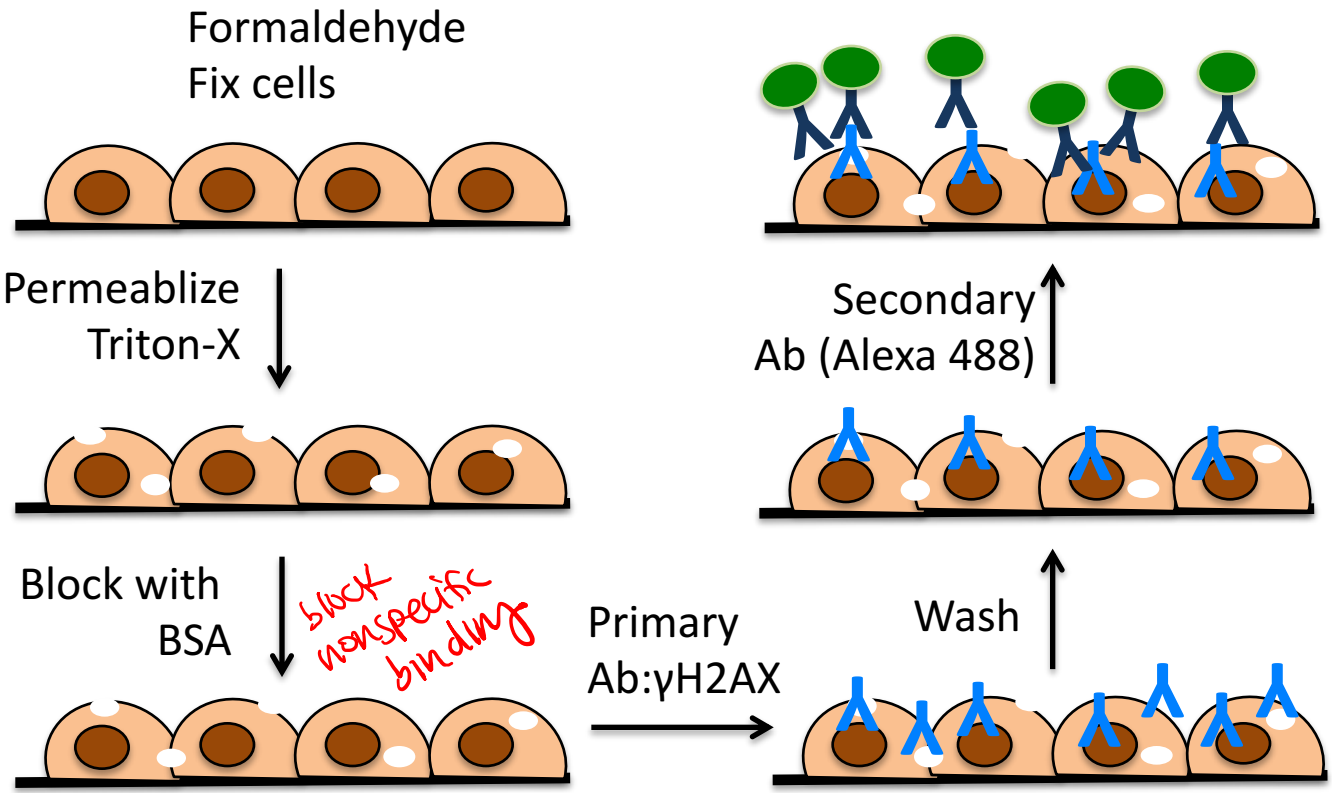
Conditions in γH2AX assay



stain 6 coverslips today

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





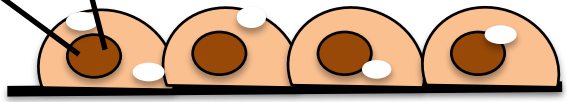
Complete γH2AX assay next time

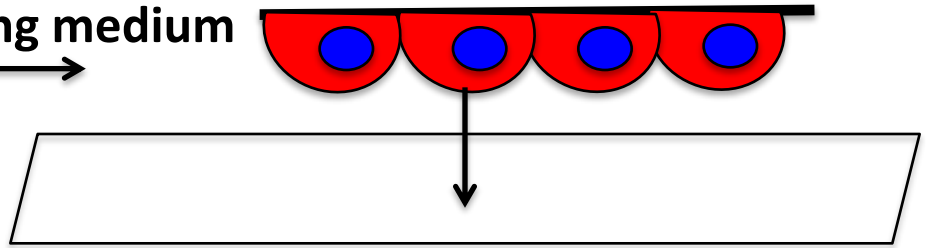
Phalloidin stain (F actin)

Together with Secondary Ab

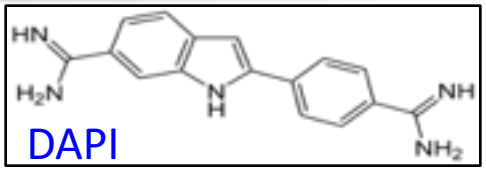
DAPI stain (DNA)

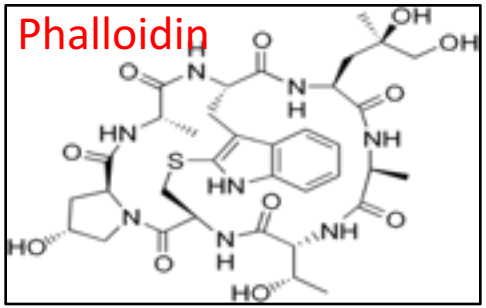
In mounting medium





Mount on glass slide





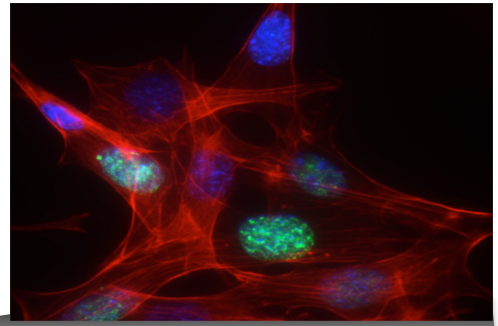
small

bind tightly

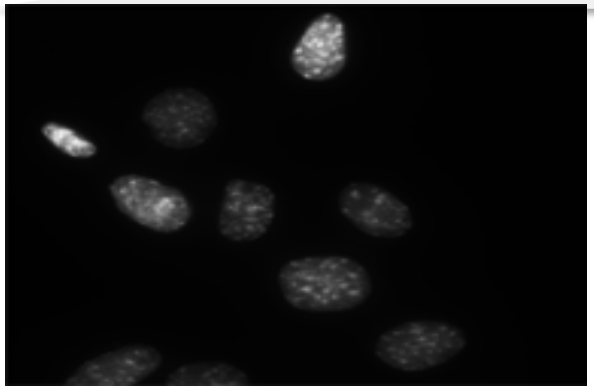
membrane permeable

γH2AX Data Analysis

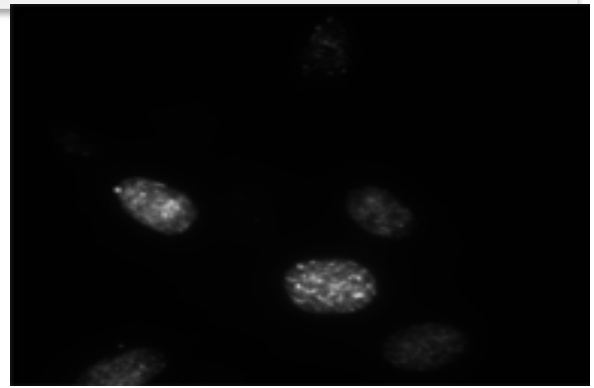
What is the total amount of γH2AX signal of each nucleus?



- Use DAPI channel to identify nucleus
- Use ImageJ to quantify total FITC (γH2AX) fluorescence in each nucleus
- Normalize fluorescence intensity by area of nucleus



Nuclei (DAPI)



ϒH2AX (FITC)

In-class paper discussion

- Consider discussion guidelines on wiki while reading the paper
- Contributing to the discussion is impt. for your participation score

REPORT

Cell Cycle 12:6, 907–915; March 15, 2013; © 2013 Landes Bioscience

Single-cell microarray enables high-throughput evaluation of DNA double-strand breaks and DNA repair inhibitors

David M. Weingeist,[1,†] Jing Ge,[1,†] David K. Wood,[2] James T. Mutamba,[1] Qiuying Huang,[3] Elizabeth A. Rowland,[1] Michael B. Yaffe,[1,3,4,5] Scott Floyd[4,6] and Bevin P. Engelward[1,*]

Major assignments for Mod1

- Data summary draft
  - due by 10pm on ~~Mon~~ Tues., October ~~8~~ 9 on Stellar (filename: TeamColor_LabSection_DS.pptx) with lab partner(s)
  - revision due by 10pm on Sat., October 20

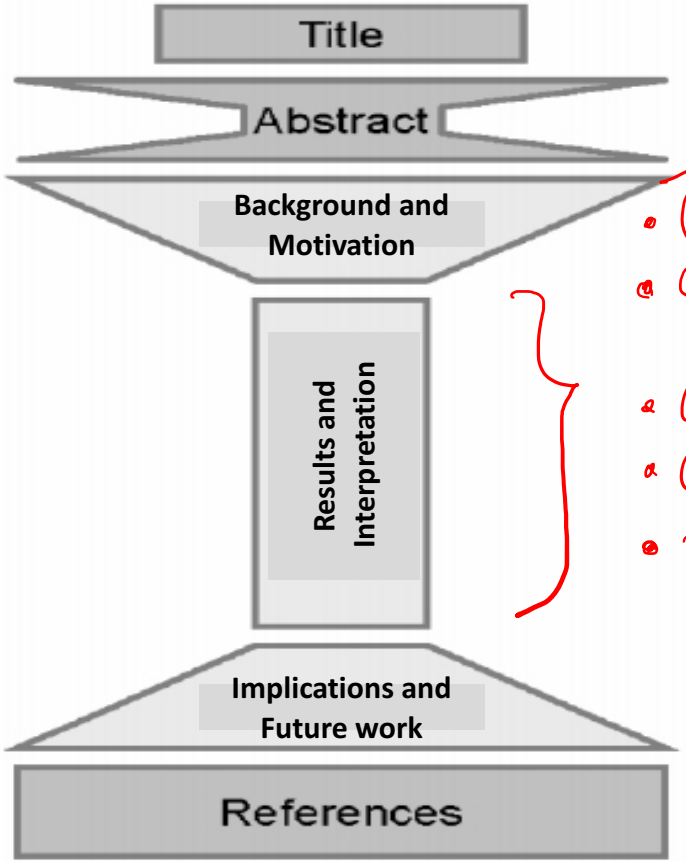
Summary content

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

- Mini presentation due by 10pm on Sat., October 13
- Blog post for M1 due by 10pm on Tues., October ~~9~~ 10

M1 Data summary Architecture

You already have most of the information you need. Start working on it now!



Figures:

- BER pathway (schematic?)
- Cell loading optimization (cell line difference)
- CometChip dose response
- CometChip repair (Engelward lab)
- γH2AX data / representative images

Example Results slide (from Wiki)

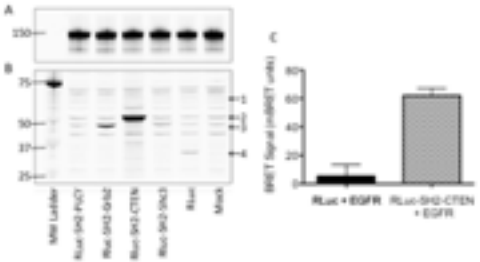


Figure 1: Development of BRET assay to monitor EGFR and SH2 domain interactions. CHO-K1 cells were transfected with Citrine-EGFR (A) and renilla luciferase (RLuc)-tagged SH2 domains from PLCg, Grb2, CTEN, and Shc3 (B). Western blots of CHO-K1 lysates were probed with anti-EGFR (A) or anti-RLuc (B) antibodies.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

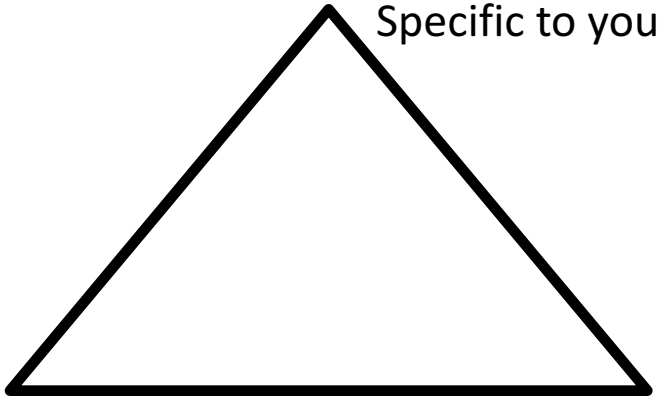
- To determine if the BRET system could be used to monitor EGFR activation, CHO-K1 cells were transfected with fluorescent EGFR and luciferase-tagged SH2 domains and a BRET assay was performed after growth factor stimulation.
- CHO-K1 were transfected with Citrine-EGFR in all conditions as indicated 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.

- In PowerPoint
- Limit figure size
- Bullet points

HW M1D7: Implications & Future Works

Implications and Future Work: potential topics [edit]

- ***Topic***: Did your results match your expectations?
  - If no, provide a putative explanation. If yes, how can you further test if your hypothesis is correct?
- ***Topic***: Based on the results, whether they matched your expectations or not, what experiments might you recommend next?
  - Follow-up experiments could distinguish between competing explanations of a given outcome or broaden the sample set for a question you already asked, to give just two examples.
- ***Topic***: How might this assay be improved?
- ***Topic***: How might this assay be used as a research tool? in the clinic? in industry?



- What BER protein is possibly phosphorylated by DNAPKcs?
  - check phosphorylation sites
  - Suggest experiments

In your Data summary tie together (and mirror) your background and motivation, and implications and future work

Tips on writing 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

In lab today

- Obtain aliquots and staining chamber from front bench
- Begin staining coverslips
- Paper discussion with Noreen
- Work on Data Summary in down time