M1D5: Analyze CometChip data and treat cells for sub-nuclear foci assay

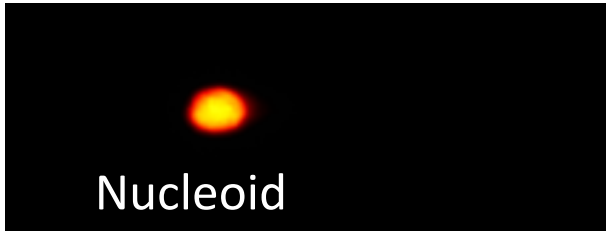
09/27/18

1. Treat cells for γH2AX assay
2. CometChip data analysis—post on wiki
3. Get ahead on reading journal article and homework for next time

Announcements

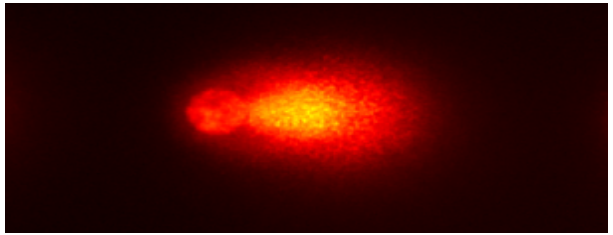
- Extra office hours coming soon (probably Saturday 10/6)
- Data Summary draft due Monday 10/8 (11 days away!)

Clarifications—Output of Alkaline CometChip Assay



No Damage

- Supercoiled nucleoid
- Little or no migration



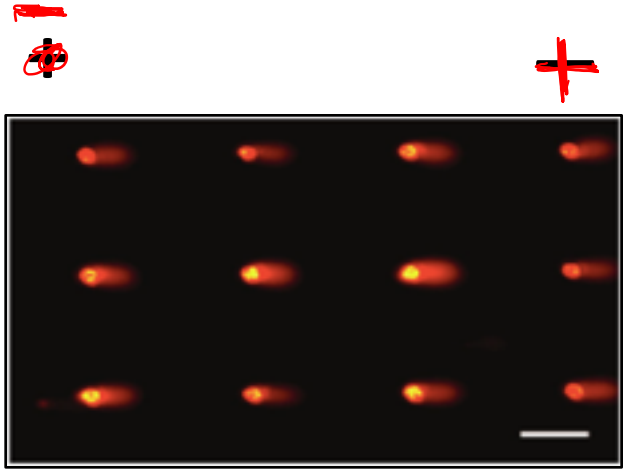
High Damage

- SSBs, DSBs, abasic sites, alkali labile sites
- forms a “Comet tail”

Genomic damage from direct strand breaks and repair intermediates

Updated slide—Buffers used for CometChips

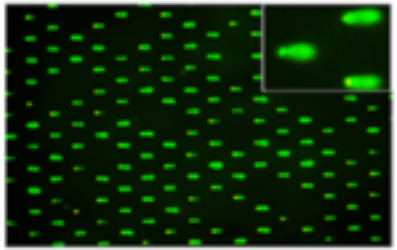
- Alkaline lysis solution
  - 2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris
  - pH 10
  - Triton X-100
- Unwinding & electrophoresis buffer
  - 0.3 M NaOH
  - 1 mM Na_2EDTA
  - pH 13.5
- Neutralization buffer
  - 0.4M Tris
  - pH 7.5
- SYBR Gold DNA stain in PBS



Overview of Mod 1: Measuring Genomic Instability

1. Optimize comet chip assay

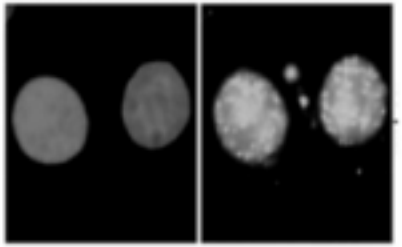
- Test loading variables



2. Use comet chip assay to measure DNA damage

- Measure effects of H_2O_2 on +/- DNA-PK cell lines

Today

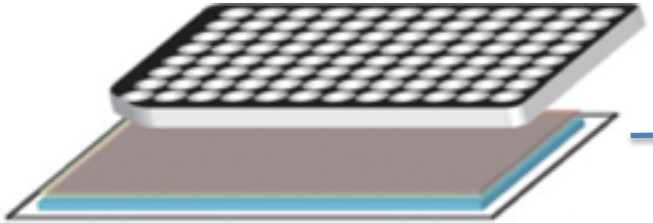


3. Use immuno-fluorescence assay to measure DNA damage

- Examine effect of H_2O_2 on γH2AX foci formation

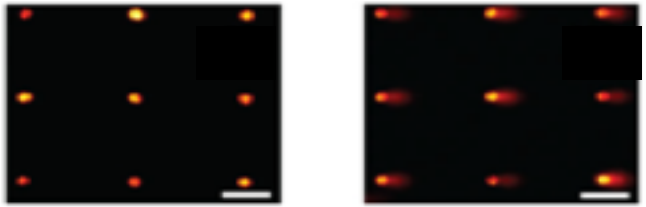
Assess DNA damage in tumor cells with & without DNAPKcs

Treat captured cells in comet chip with H_2O_2 (oxidative damage)

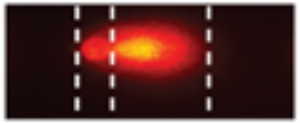


Lyse cells & unwind DNA (DNA still captured agarose in overlay)

Agarose Electrophoresis

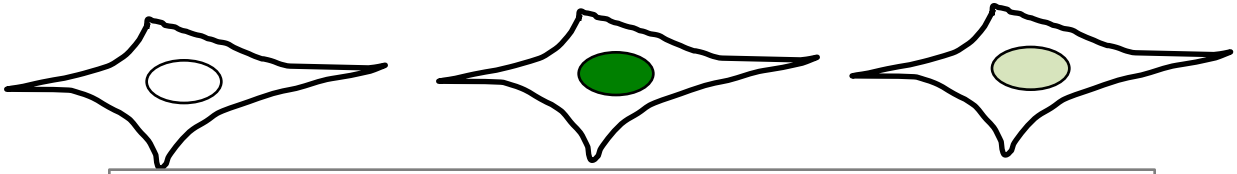


Stain DNA and image via fluorescence microscopy



Analysis via Matlab

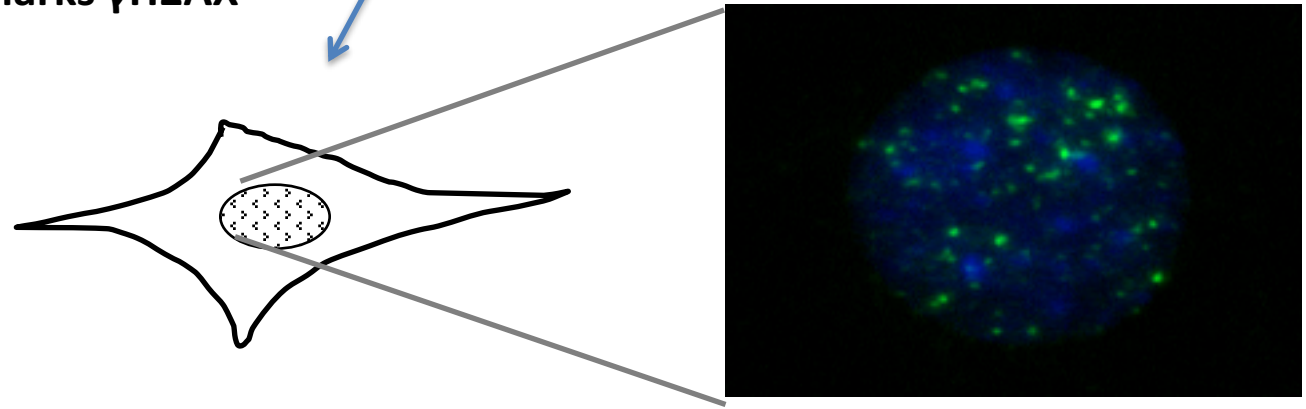
Measuring DNA damage via γH2AX Assay



no treatment, H_2O_2 treatment, recovery condition

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

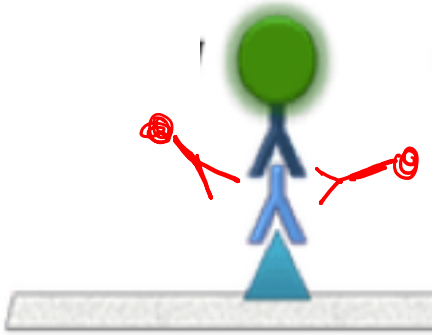
Fix cells and stain with antibody that marks γH2AX



Blue: DNA
Green: γH2AX staining

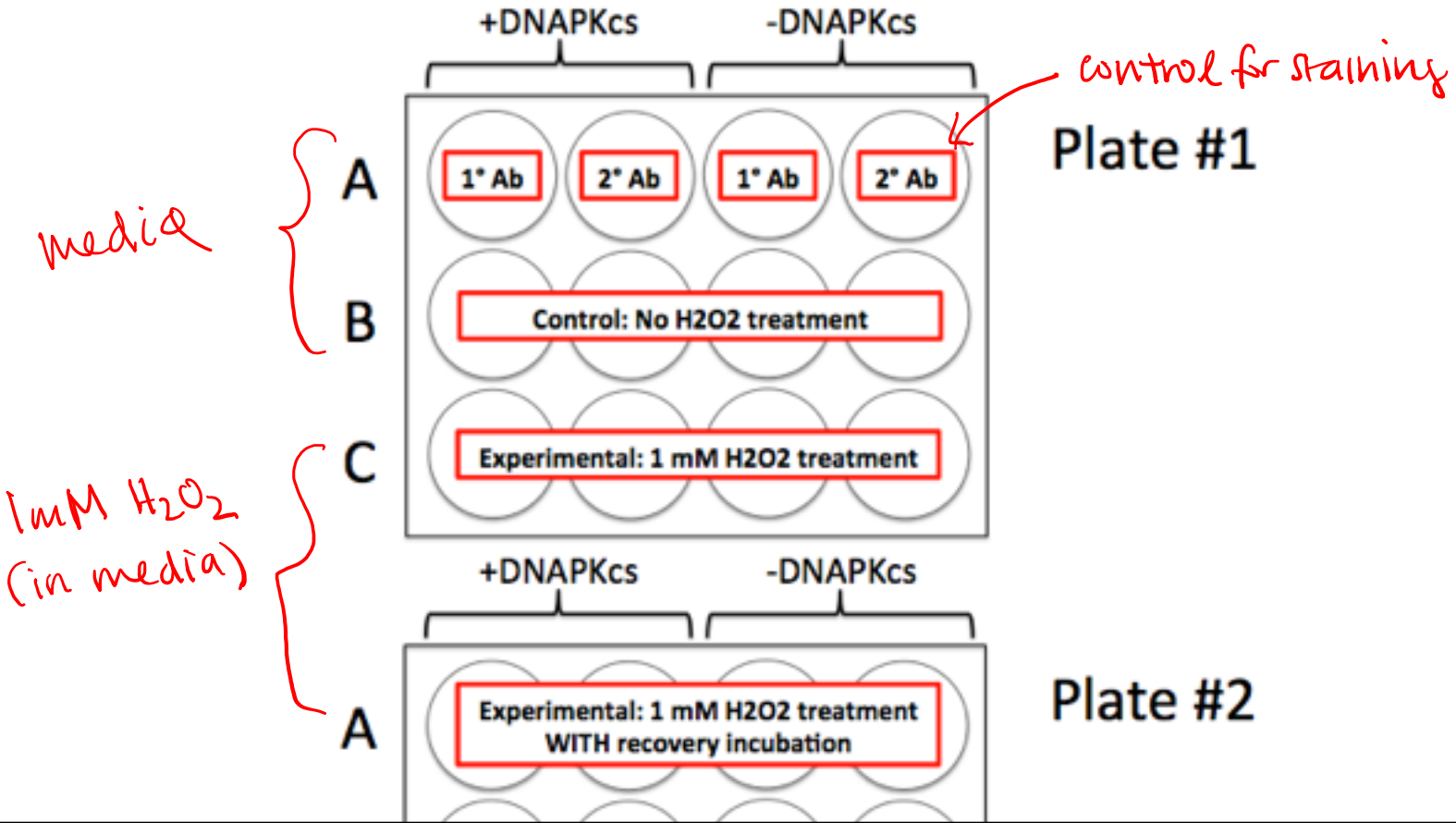
Using immunofluorescence: γH2AX assay to detect double-strand DNA breaks

- Histone H2AX phosphorylated at Ser139 if DSB
- Use antibodies against γH2AX

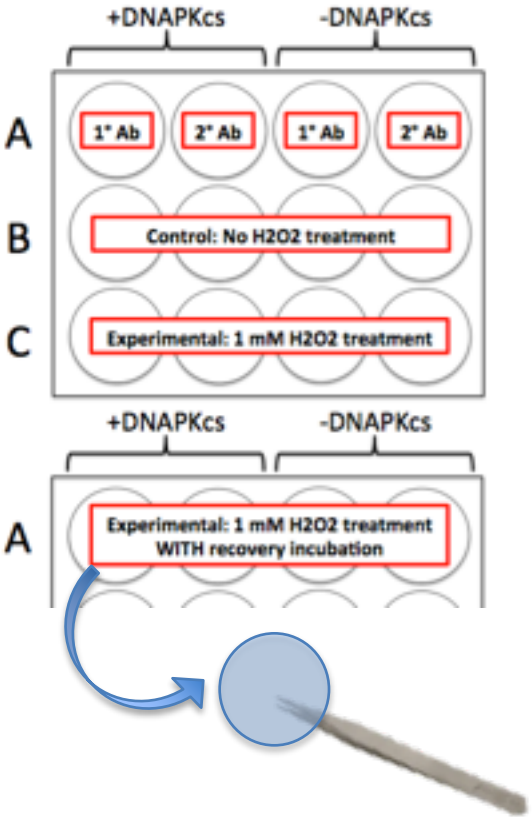


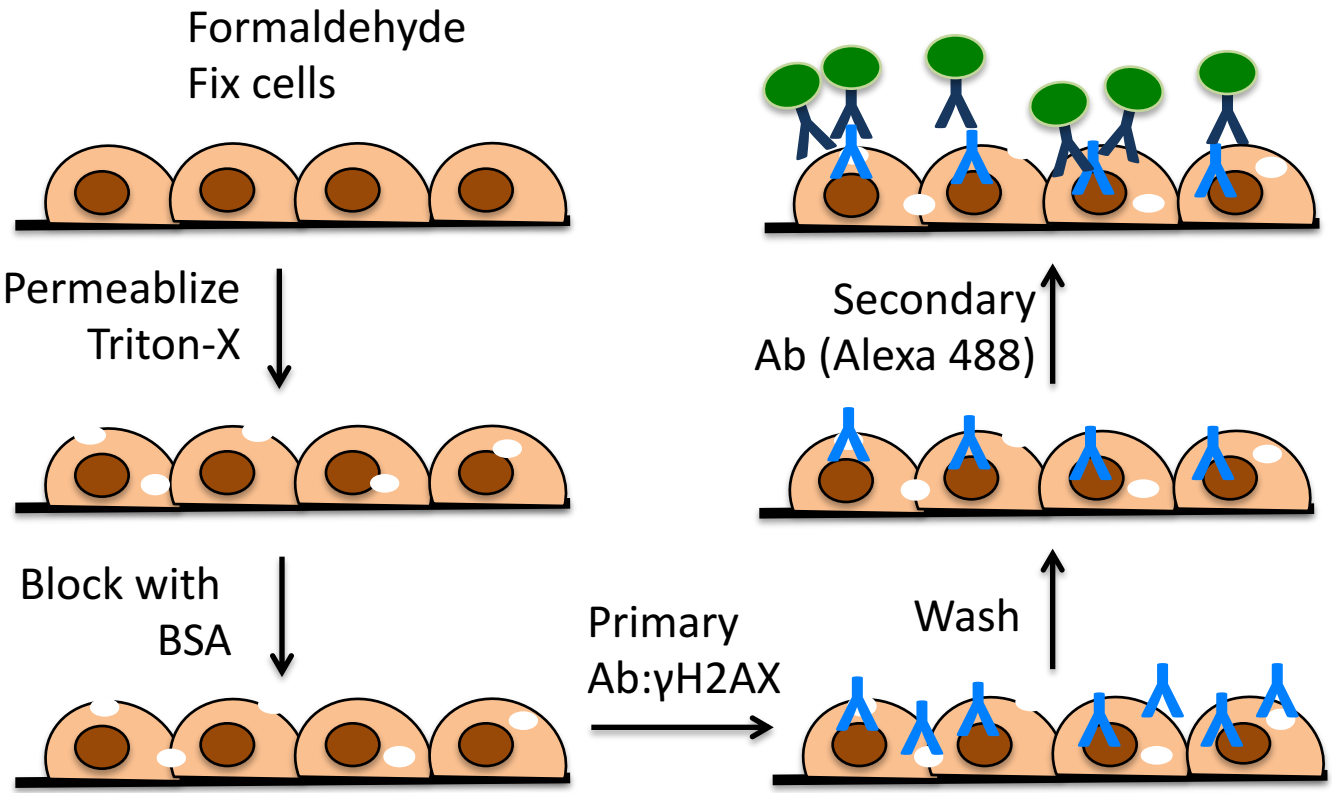
protein of interest	γH2AX
primary antibody	mouse anti-human anti-γH2AX
secondary antibody	goat anti-mouse
Fluorophore (conjugated to secondary antibody) exc./ em. wavelengths	488 / 520 nm

Treating cells with H_2O_2 for γH2AX assay



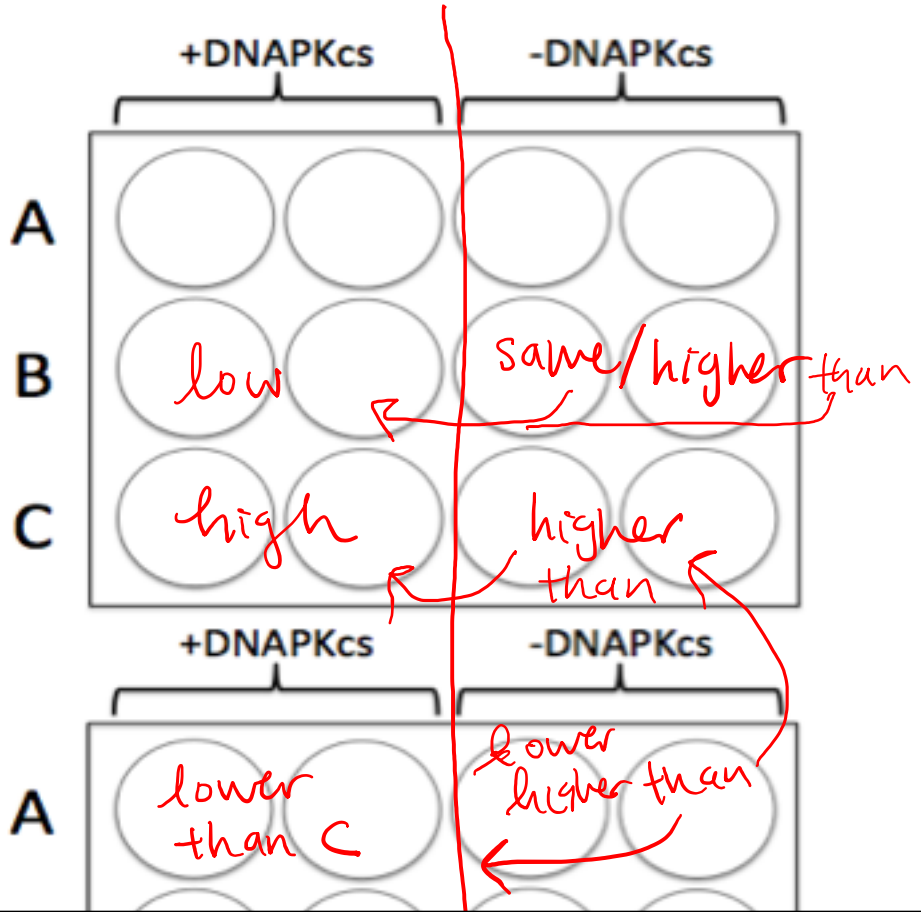
Practically using immunofluorescence: γH2AX assay to detect double-strand DNA breaks





What do we hypothesize will happen…

γH2AX signal



No H_2O_2

H_2O_2 for 20min
Fixed immediately

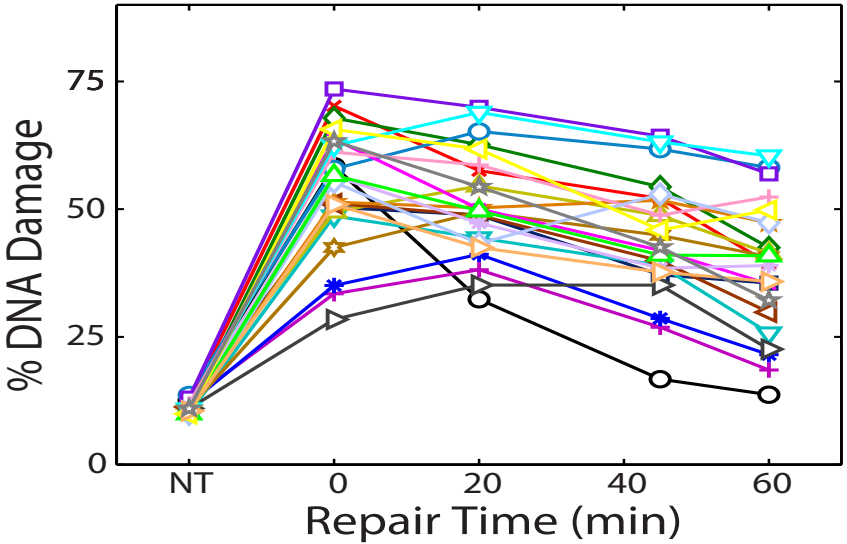
H_2O_2 for 20min
Recovery in fresh media

30min or 1hr

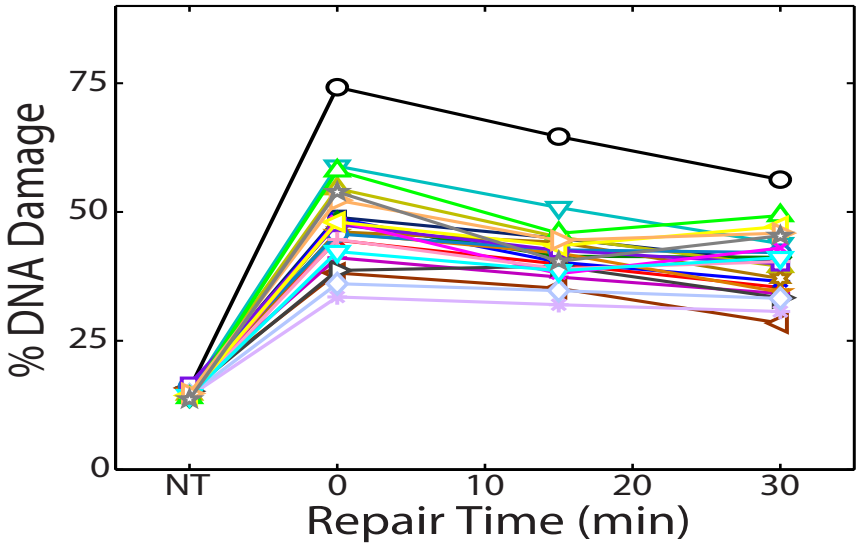
Detecting Repair over time in Human Cells

Each color represents a different human cell line's response to mutagen after an initial exposure followed by recovery time

Oxidative Damage



Alkylation Damage



- Consider difference in kinetics of seeing repair in CometChip vs γH2AX assays
- Will have a paper discussion next week with repair kinetic data

from Prof. Engelward's lecture slides

CometChip Data analysis in ImageJ and MATLAB

1. ImageJ

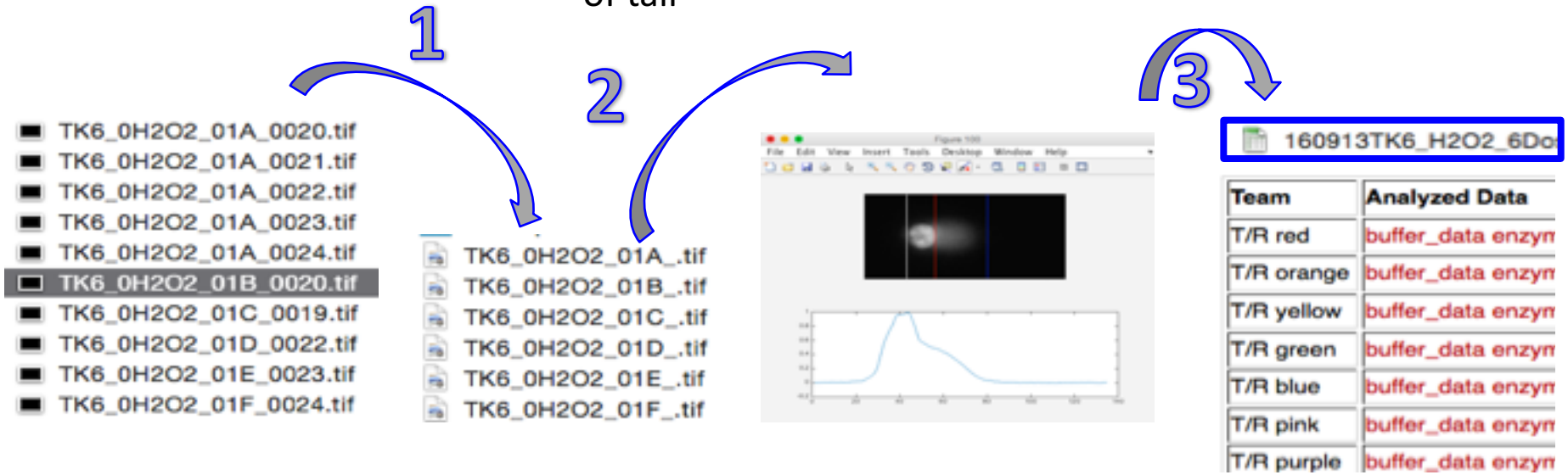
- from several images per well to one stack per well
- `GenImageStacks_single image.txt`

2. MATLAB

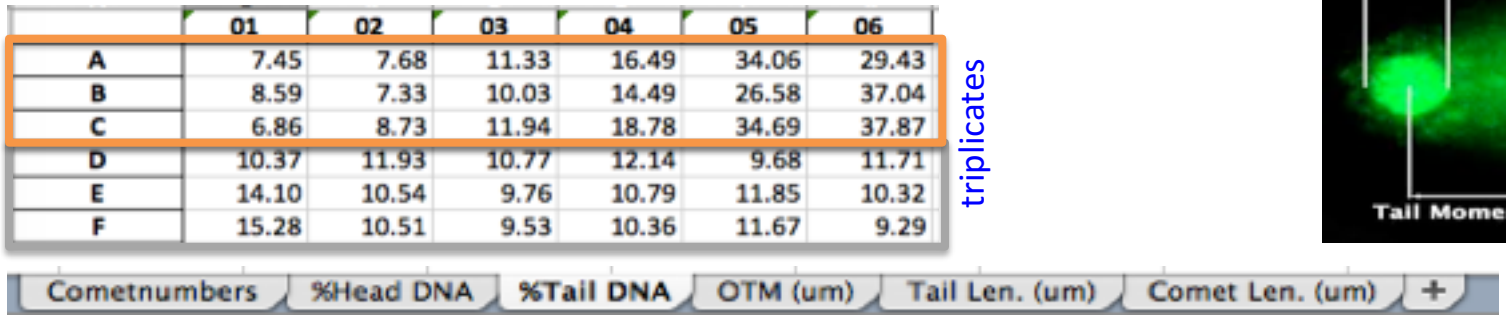
- for each comet in stack, calculates intensity of head and tail, as well as length of tail

3. Excel

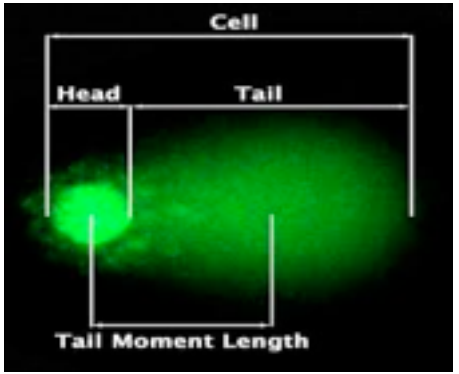
- export data from Matlab and compile
- post data to the wiki



What's in the final Excel file?



	01	02	03	04	05	06
A	7.45	7.68	11.33	16.49	34.06	29.43
B	8.59	7.33	10.03	14.49	26.58	37.04
C	6.86	8.73	11.94	18.78	34.69	37.87
D	10.37	11.93	10.77	12.14	9.68	11.71
E	14.10	10.54	9.76	10.79	11.85	10.32
F	15.28	10.51	9.53	10.36	11.67	9.29



- Cometnumbers: how many comets were used for calculation in each well (= stack)
- %Head DNA = 100 * HeadFluorescence / (HeadFluorescence + TailFluorescence)
- **%Tail DNA = 100 * TailFluorescence / (HeadFluorescence + TailFluorescence)**
- Olive tail moment (OTM) = (%TailDNA / 100) * (TailCenterOfMass – HeadCenterOfMass)
- Tail length
- Comet length

Major assignments for Mod1

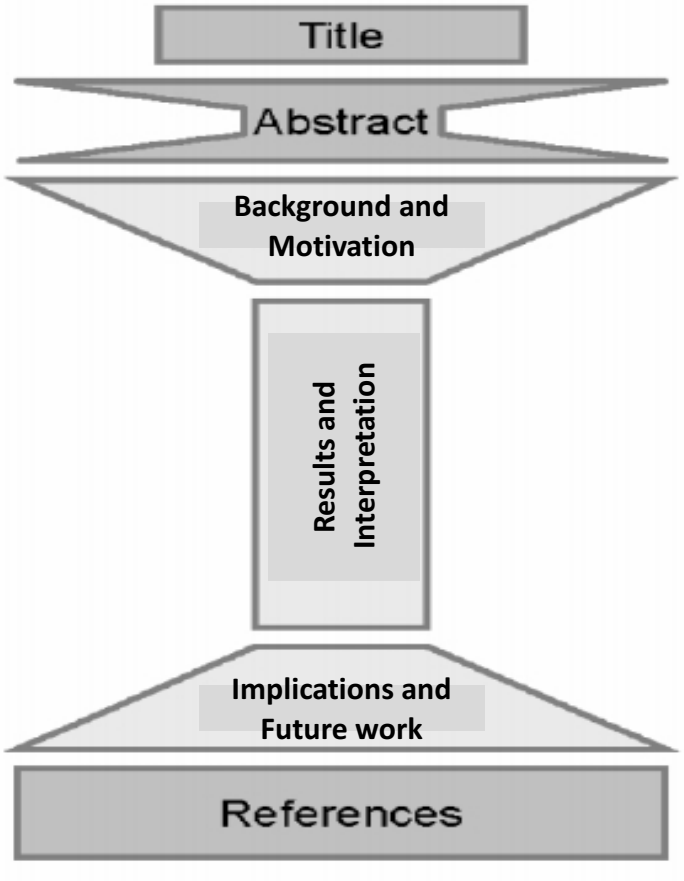
- Data summary draft
  - due by 10pm on Mon., October 8
  - revision due by 10pm on Sat., October 20

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

M1 Data summary Architecture



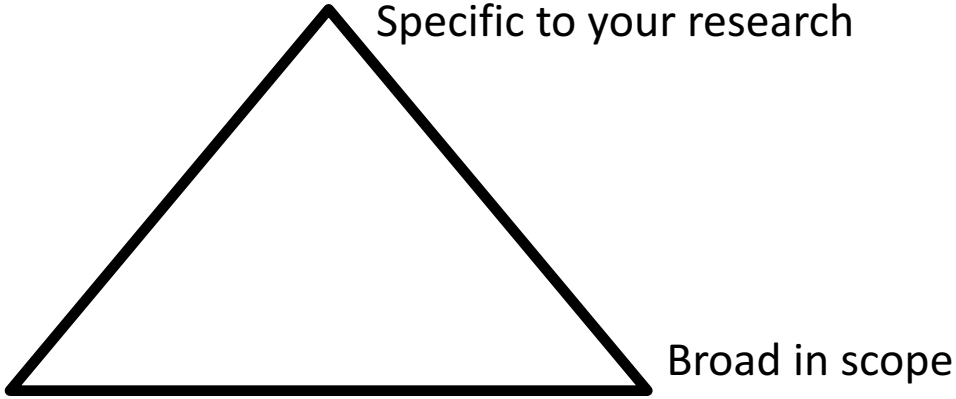
HW M1D6: ~~Implications & Future Works~~

Implications and Future Work: potential topics [edit]

Revised methods

- ***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?

Check for email from Noreen tomorrow!



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

HW M1D6: Mini Presentation **Outline**

- Follow time and content guidelines
- Introduce yourself and your research
- Clearly state your hypothesis to identify main question
- Be quantitative when stating your findings (NOT “This was more/less than…”)
- For this HW assignment put placeholder statements for key findings

Category	Approximate worth	Elements of a strong presentation
Content	50%	• Did you introduce your research? • Did you include the key findings (and the techniques used to gather these results, if necessary)? • Was the importance of your project clear?
Organization	25%	• Is the presentation logical and easy-to-follow? • Are the main points emphasized? • Did you include transition statements such that the presentation 'flows' and is easily followed/understood?
Delivery	25%	• Do you show confidence and enthusiasm? • Did you use appropriate language (technical or informal, as appropriate)? • Is your speech clear?

HW M1D6: Prepare for 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,*]

In lab today

1. Choose recovery time—check in with me
2. Treat cells for γH2AX and set timer for recovery—fix cells at appropriate time.
3. Obtain CometChip data from me and finish analysis today—post results on wiki.
4. With extra time, consider reading journal article and doing homework for next time.