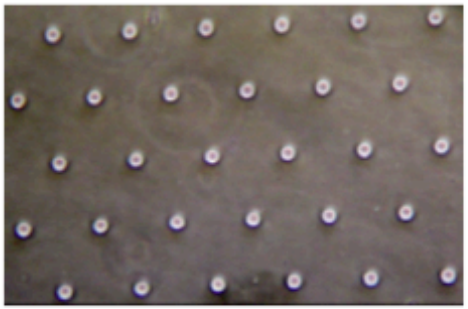
M1D5: Develop approach for sub-nuclear visualization of DNA damage

09/29/16

1. Pre-lab Discussion
2. ½ class to TC room
3. ½ class start data analysis

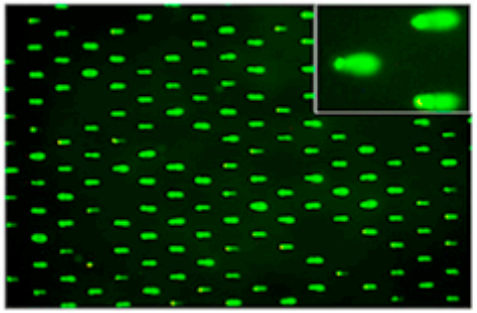
- Announcements: Photographer in lab Tuesday Oct. 4th 2:30pm

Overview of
"M1: Measuring Genomic Instability"



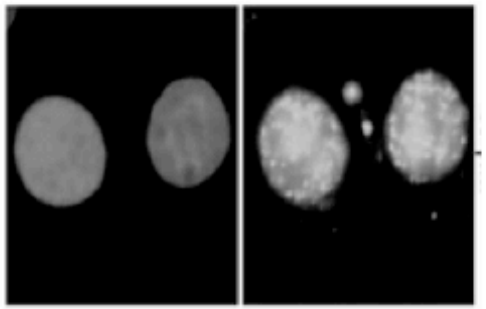
1. Optimize comet chip assay

- Test loading variables



2. Use comet chip assay to measure DNA damage / repair

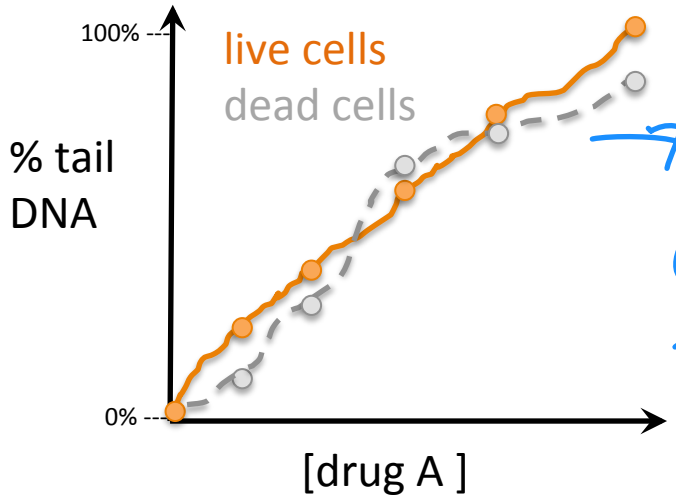
- Measure effects of MMS and H_2O_2 on BER
- Assess repair variability in healthy individuals

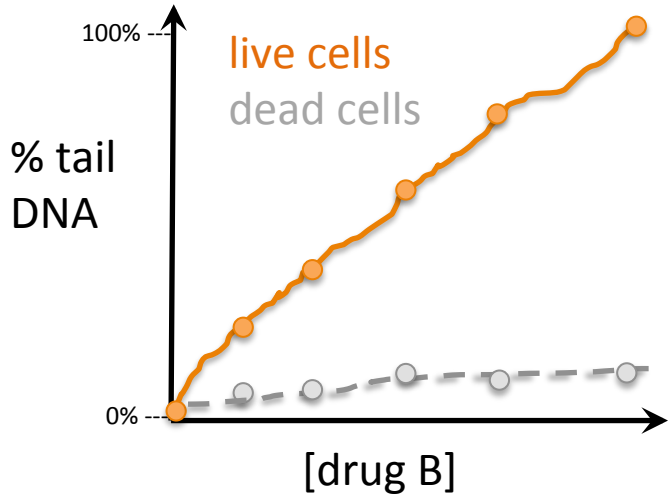


3. Use immuno-fluorescence assay to visualize DNA repair

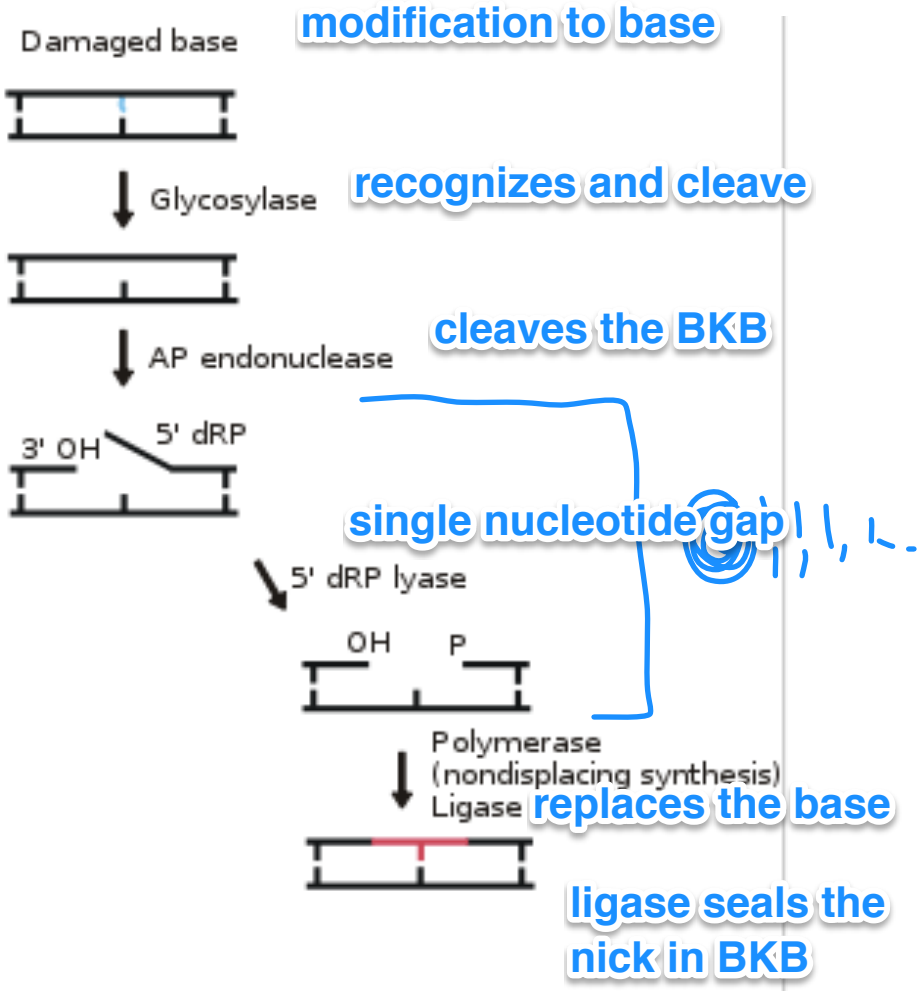
- Examine effect of H_2O_2 on DSB abundance

Both H_2O_2- and MMS-caused DNA damage is repaired by base excision repair (BER)





→ chemical damage to BKB



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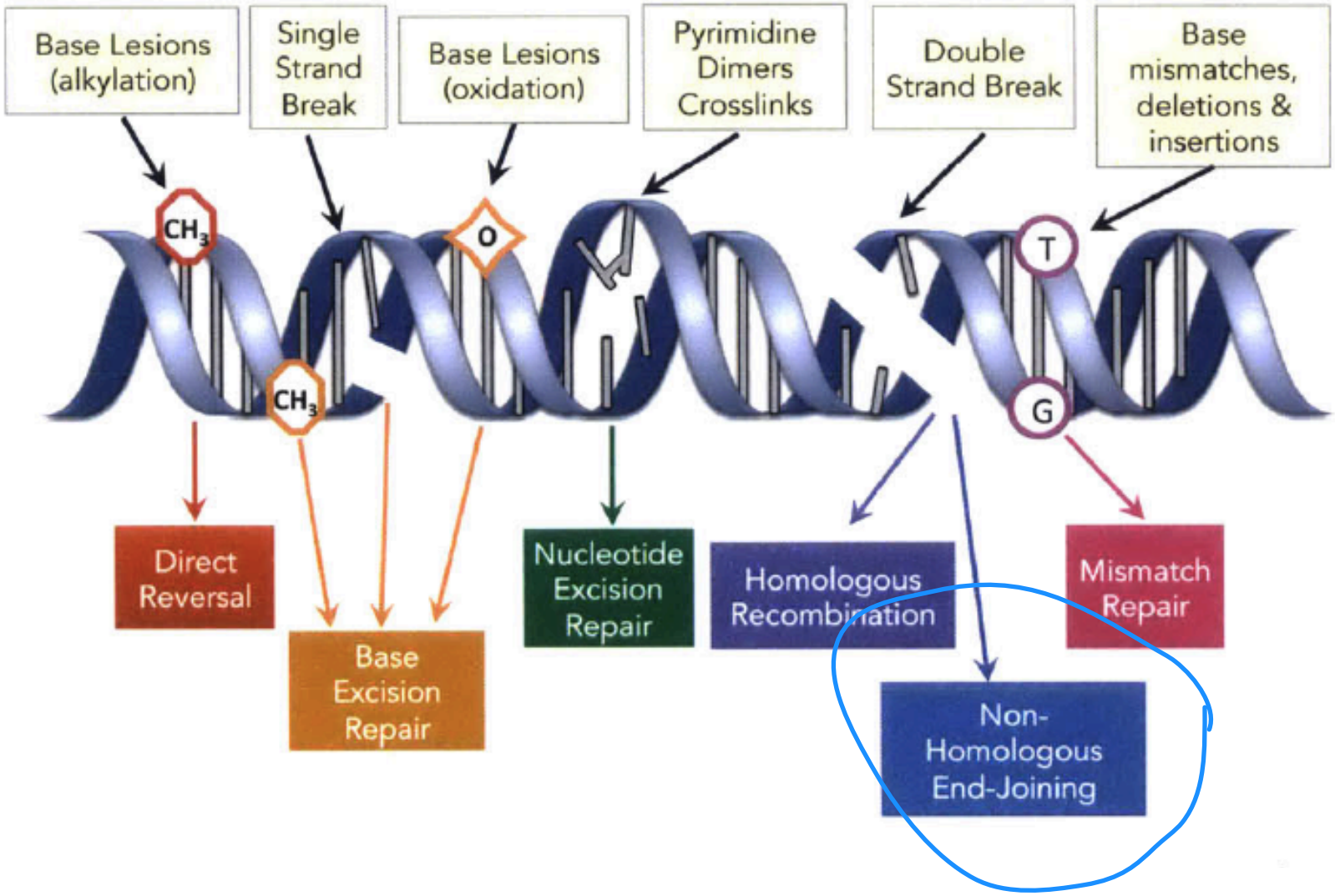
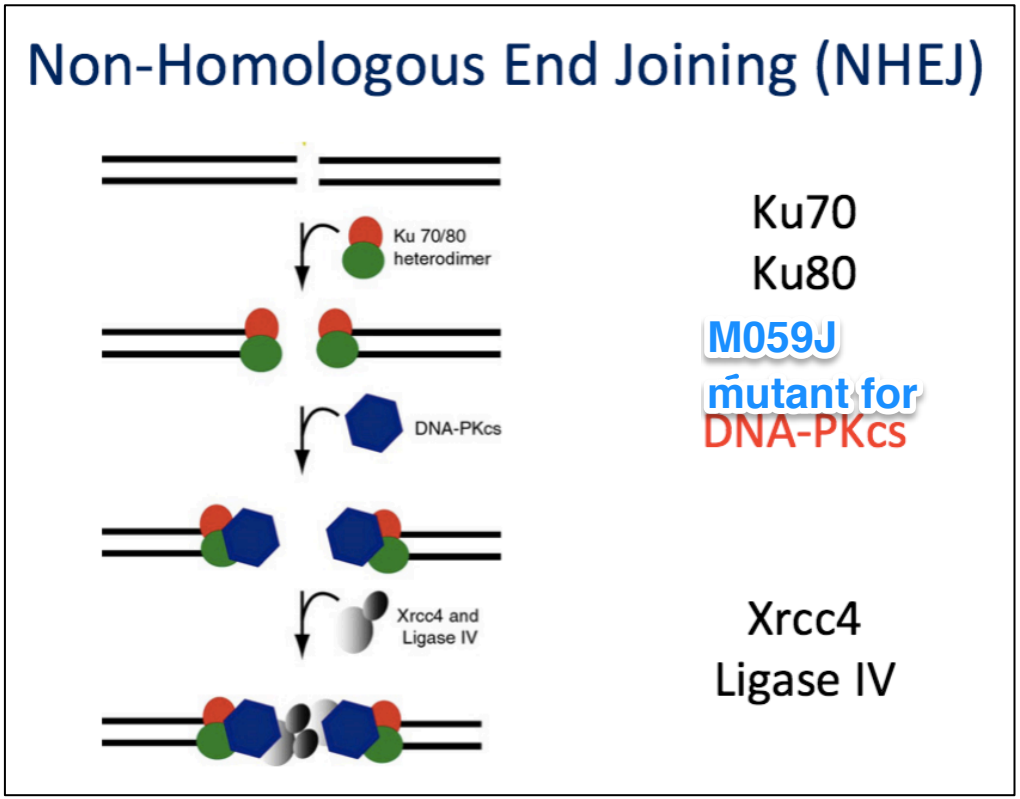
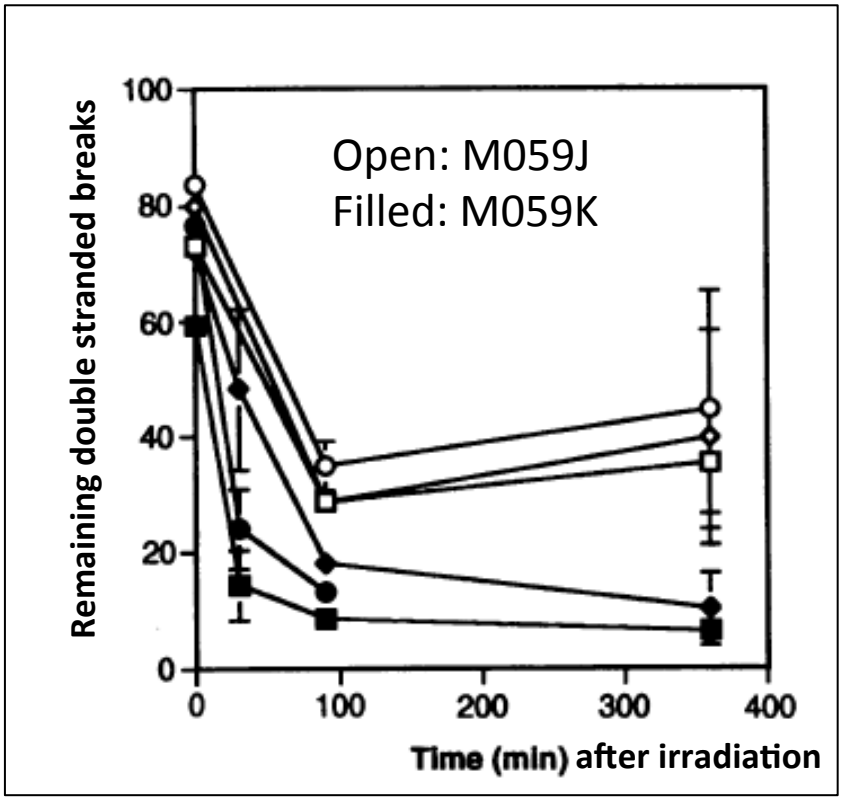


M059K and M059J cell lines

- M059K is wild type
- M059J is missing DNA-PKcs, deficient in NHEJ DNA repair
- human glioblastoma fibroblasts

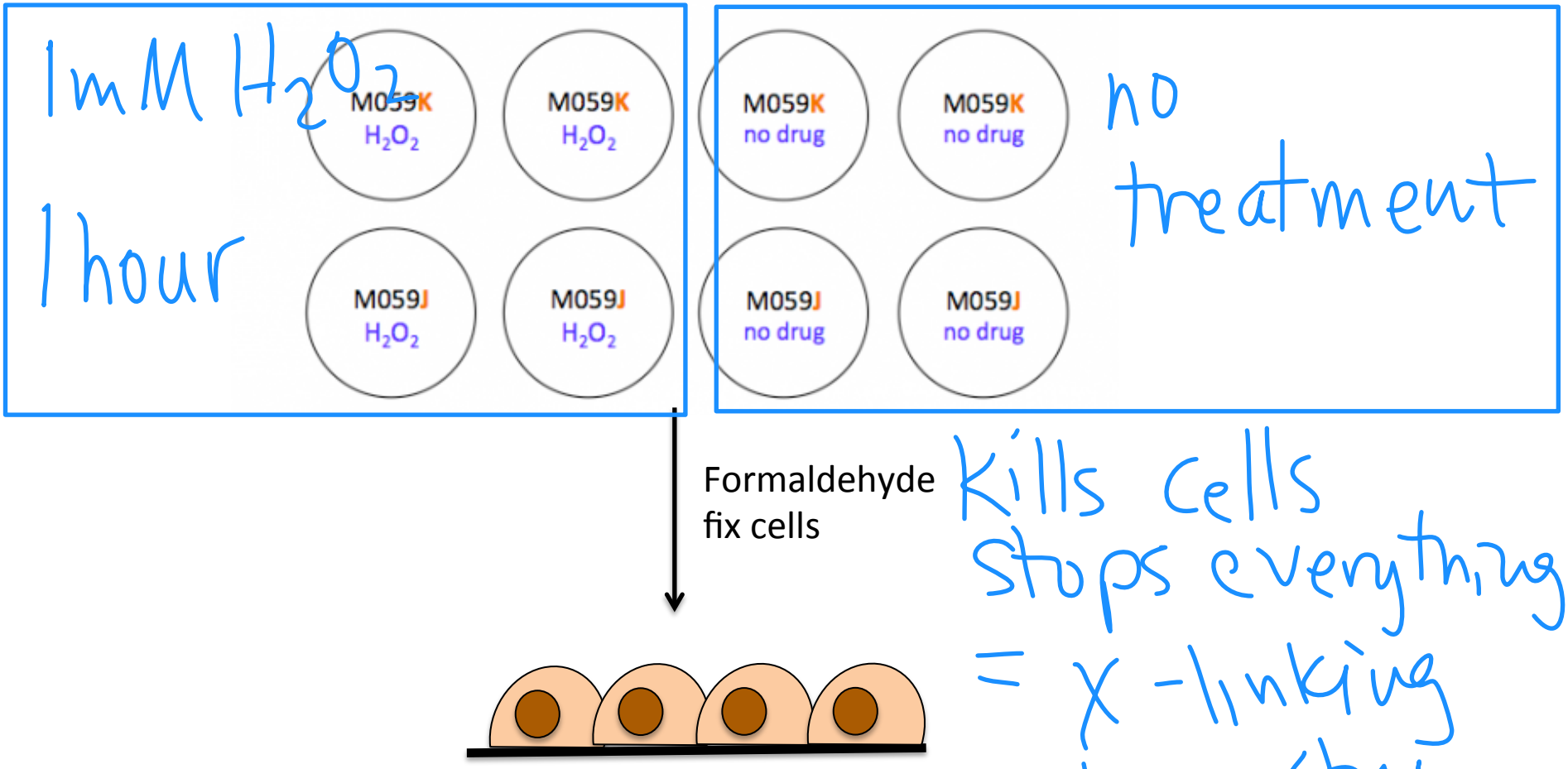




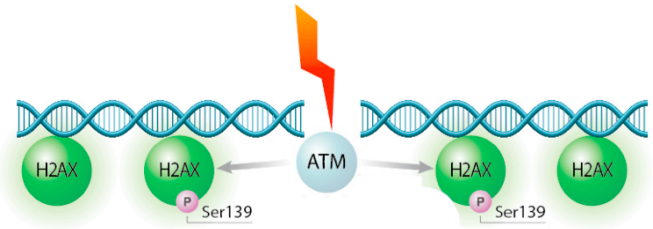
Prof. Leona Samson's M2D2 lecture, Spring 2016

Lees-Miller *et al.* (1995) *Science* **267**: 1183-1185

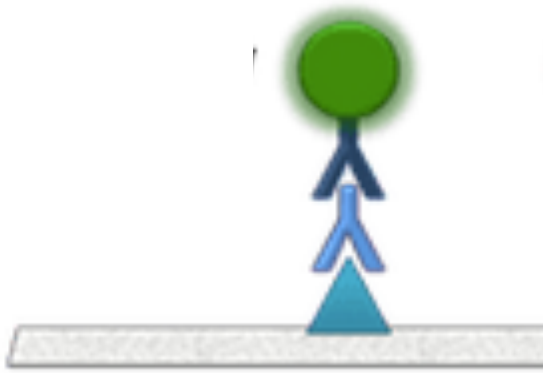
γH2AX assay to detect double-strand DNA breaks



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

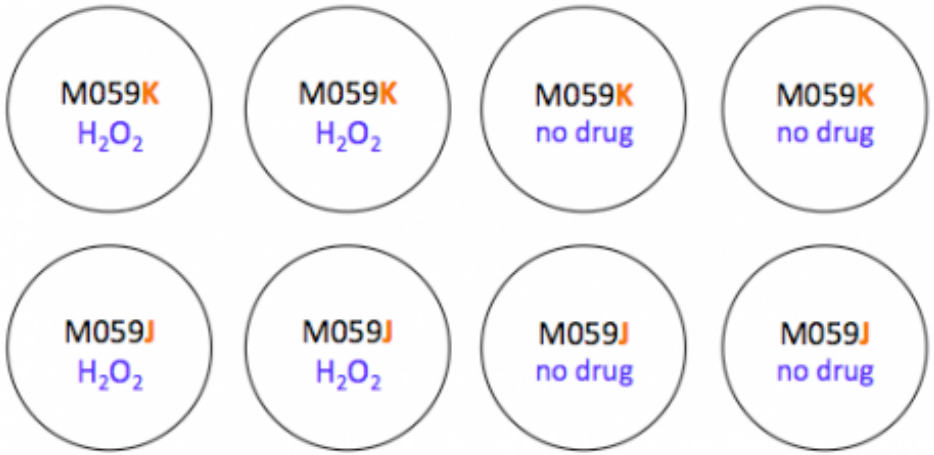


- histone H2AX phosphorylated at Ser139 if DSB
- use antibodies against γH2AX

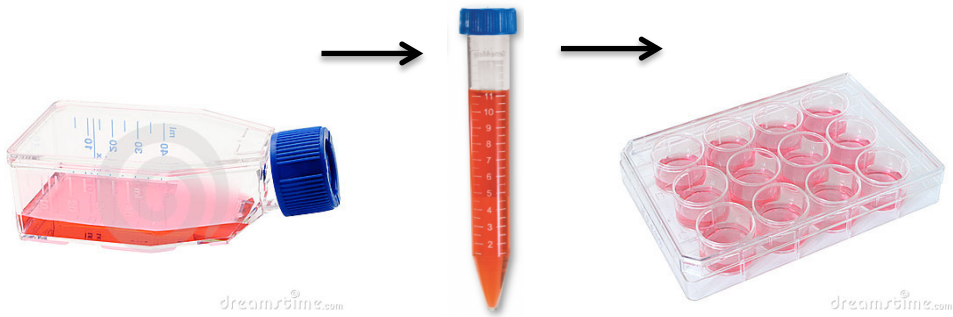


protein of interest	γH2AX
primary antibody	mouse anti-human anti-γH2AX
secondary antibody	goat anti-mouse
fluorescent dye exc./ em. wavelengths	488 / 520 nm

Seeding coverslips in the tissue culture hood



- on gelatin-coated coverslips
- 100,000 cells / well



- trysinize adherent cells to detach from flask

Mammalian cell culture medium

M059K/J

Food:

- DMEM : F12 Ham's
  - Dulbecco's Modified Eagle's Medium
  - nutrient mixture F12

    glucose, salts, amino amids, vitamins

    phenol red is a pH indicator

- FBS: fetal bovine serum

  BSA and other proteins

  growth factors, cytokines, lipids, cholesterol
- non-essential amino acids

  glucose primarily used for growth

Non-food:

- antibiotics:
  - penicillin
  - streptomycin

Seeding your M059J/K cells

1. Rinse with PBS

   remove extra proteins that block trypsin activity

2. Detach with trypsin

   cleave binds between cells and plastic

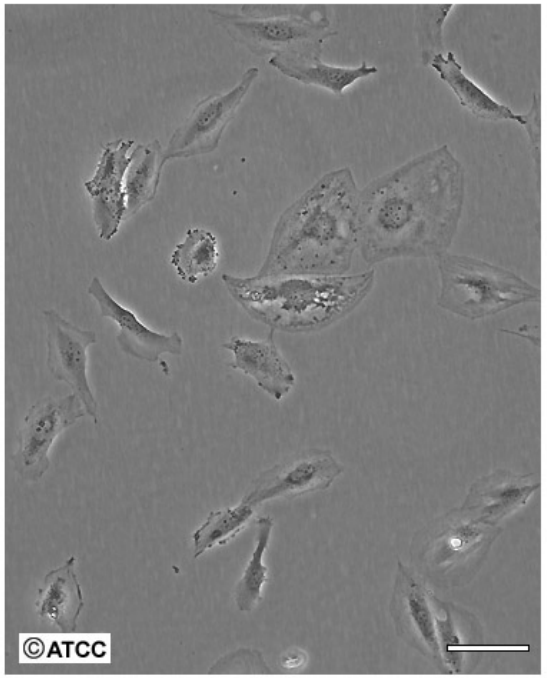
3. Calculate number of cells

   seed specific # on glass coverslips

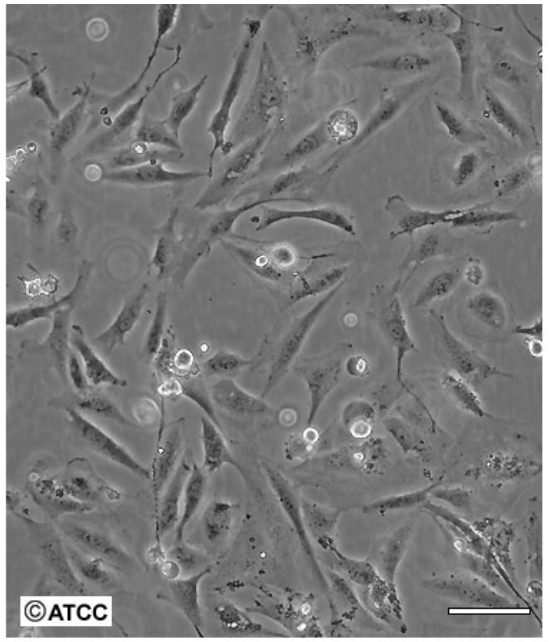
4. Seed coverslips

   -higher resolution to image through glass (over plastic)
   -ease of use; transferring coverslip rather than washing dish with primary, secondary, etc.

M059K



Low Density Scale Bar = 100µm



High Density Scale Bar = 100µm

Data analysis in ImageJ, MATLAB, and Python

1. ImageJ

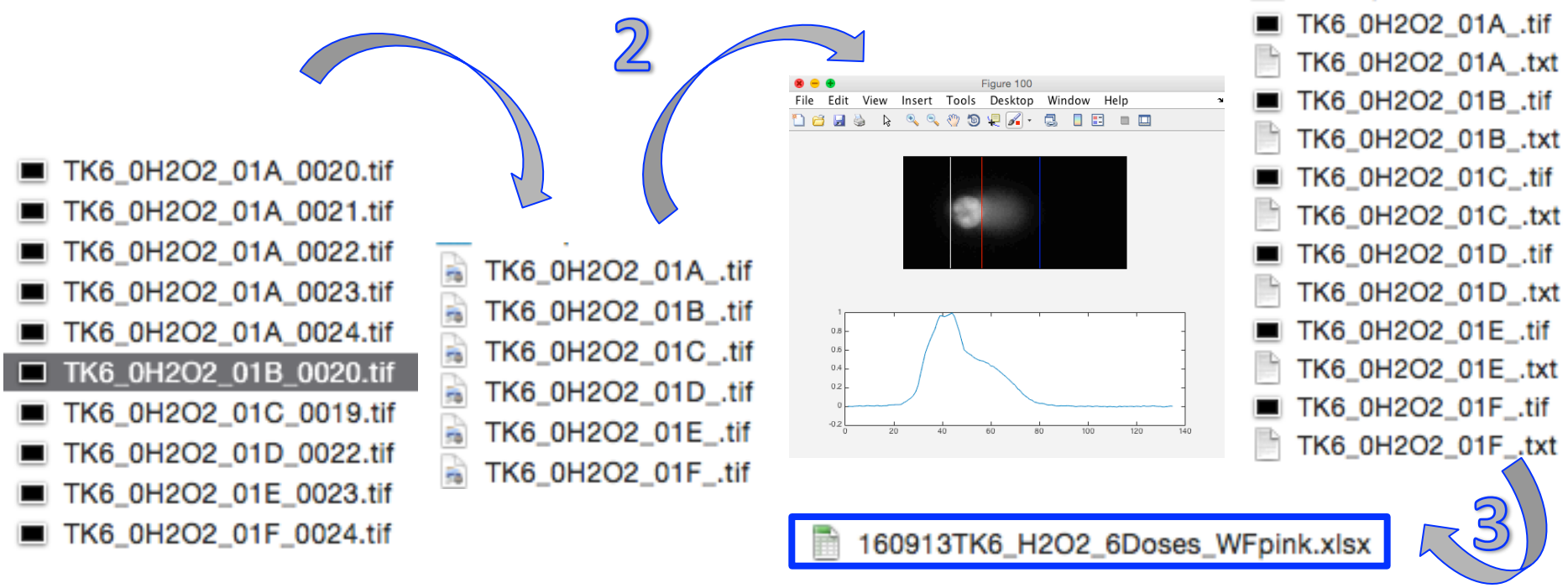
- from several images per well to one stack per well
- `GenImageStacks_singleimage.txt`

2. MATLAB

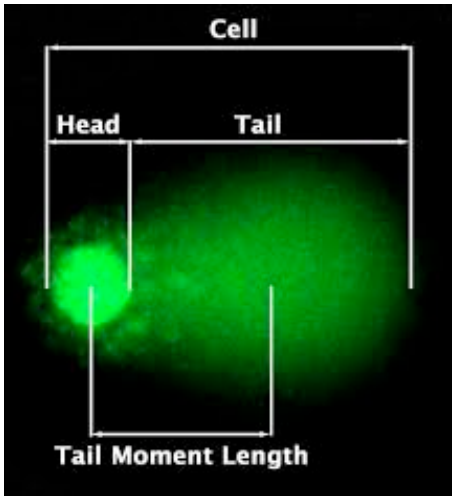
- for each comet in stack, calculates intensity of head and tail, as well as length of tail
- creates one .txt per comet
- `guicometanalyzer.m`

3. Python

- summarizes all MATLAB-created .txt files into one .xlsx 6-tab spreadsheet
- `comettoexcel_gui.command`



What's in the final Excel file?



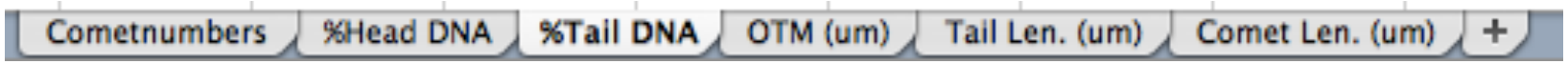
	01	02	03	04	05	06
A	7.45	7.68	11.33	16.49	34.06	29.43
B	8.59	7.33	10.03	14.49	26.58	37.04
C	6.86	8.73	11.94	18.78	34.69	37.87
D	10.37	11.93	10.77	12.14	9.68	11.71
E	14.10	10.54	9.76	10.79	11.85	10.32
F	15.28	10.51	9.53	10.36	11.67	9.29
[H2O2] (mM)	0	0.25	0.5	1	2	4
[MMS] (uM)	0	10	20	40	60	80

→ [drug]

triplicates

live

dead



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

Make strides on your statistical analysis!

- On **M1D6**, you'll continue creating Results figures:
  - Plot your data with 95% confidence intervals

$$\bar{x} \pm \frac{t_{table} * stdev}{\sqrt{n}}$$

$$t_{table} = TINV\left(0.05, n-1\right)$$

  - How certain are you that two populations are different?

$$p = TTEST(array1, array2, 2, 3)$$

2-tailed

unequal variance (heteroscedastic ☺)

✧ The Student's t-test only applies to two data sets.

Only compare two conditions at a time.

Assignments for M1

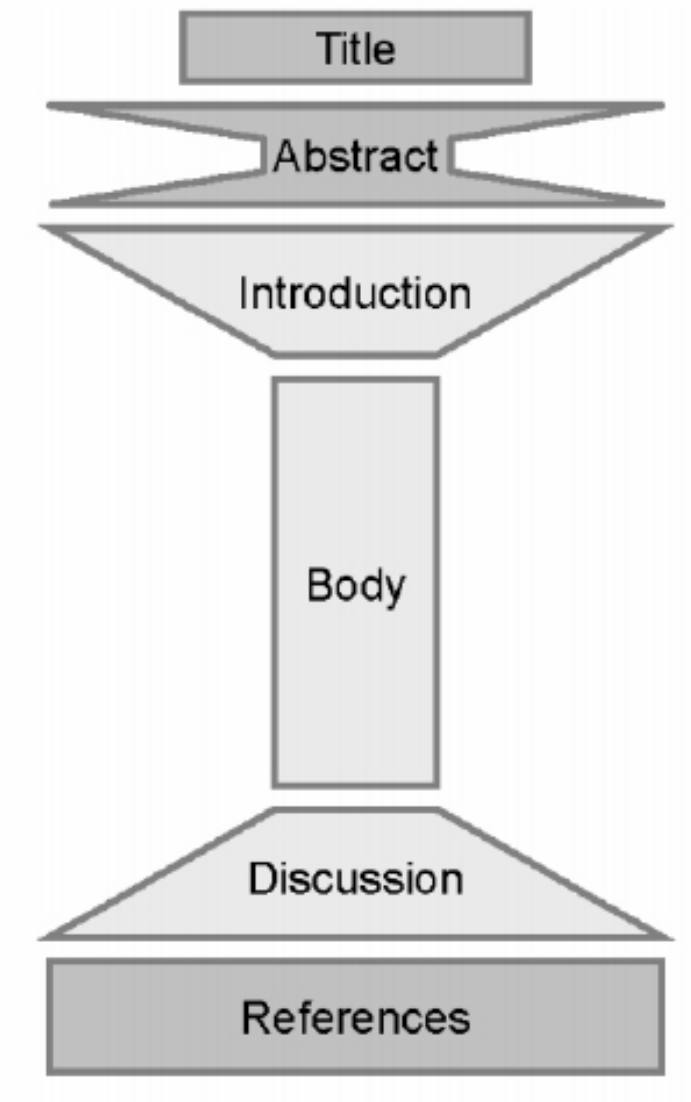
- Data summary draft
  - due by 5pm on Wed., October 12
  - revision due by 5pm on Mon., October 24

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 15
- Blog post for M1 due by 5pm on Tue., October 25

Manuscript architecture: Data summary



In paragraph form!!

Background and Motivation (bullets)

Results and Interpretation (bullets)
-Goal/Purpose
-what is your expected result?
-what are your actual results?
-what does this suggest/indicate?
-what does this motivate to do next?

Implication and Future Work (bullets)

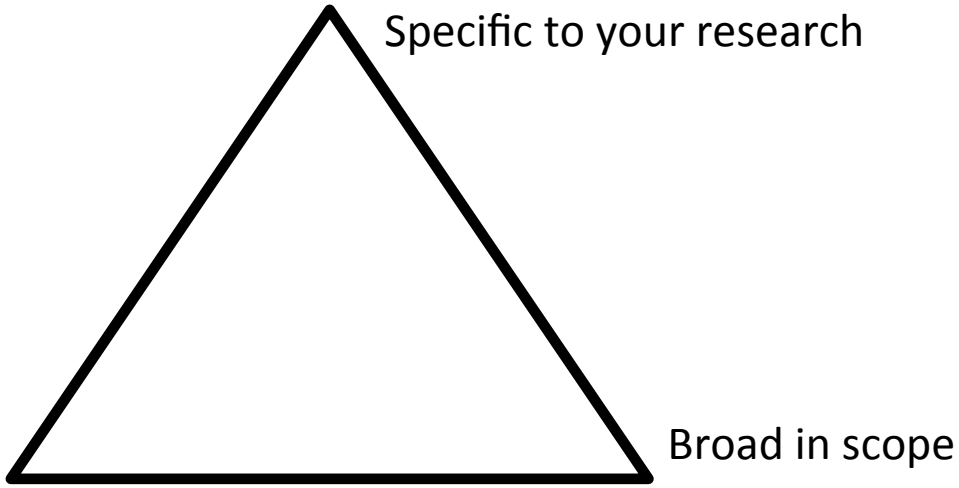
References from Intro

Implications and Future Works

M1D6 HW: Draft Implications and Future Work section

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?



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

Mini Presentation

- Follow time and content guidelines
- Introduce yourself and your research
- Clearly state your hypothesis to identify main question

-Use actual numbers (or fold changes) when discussing data

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?

In lab today…

1. 3 teams into tissue culture room to seed M059J/K onto coverslips (Yellow, Green and Blue)
2. Use this time for data analysis with our *new* macbooks; get ahead on your data summary!
   - you need to complete some analysis to draft your implications section for next week