M1D6:
Examine sub-nuclear foci abundance to measure DNA damage

1. Prelab discussion
2. Benchwork
   - Begin γH2AX staining
   - Process CometChip Images
   - Visualize CometChip results
3. Paper discussion

Keep track of the due dates!

Assignments for M1

- Data summary draft
  - due by 10 pm on Wed., October 11
  - revision due by 10 pm 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 10 pm on Sat., October 14
- Blog post for M1 due by 10 pm on Mon., October 23

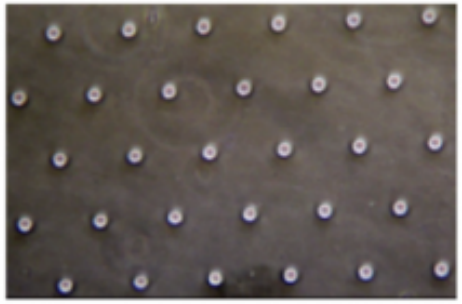
Extra office hours

- **56-302**
- Saturday, 10/7, 10am-2pm
- Wednesday, 10/11, ~~10~~11am-3pm

Regular office hours

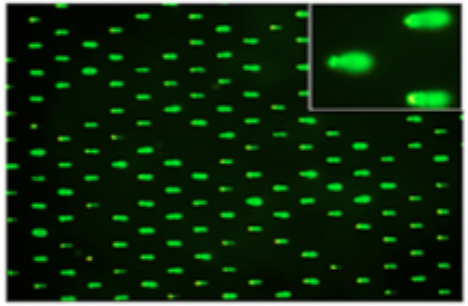
- **Next week Monday OH canceled**
- Josephine: Thursday 2-3 pm in 56-341c
- Leslie: Friday 9-10 am and 3-4 pm in 56-341c

Overview of Mod1 experiments



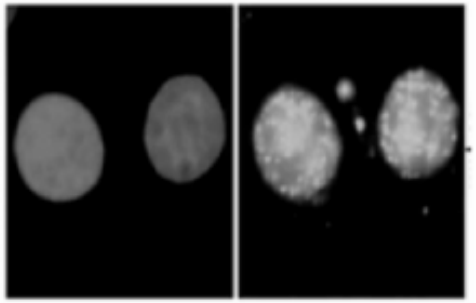
1. Optimize comet chip assay

- Test loading variables



2. Use comet chip assay to measure DNA repair

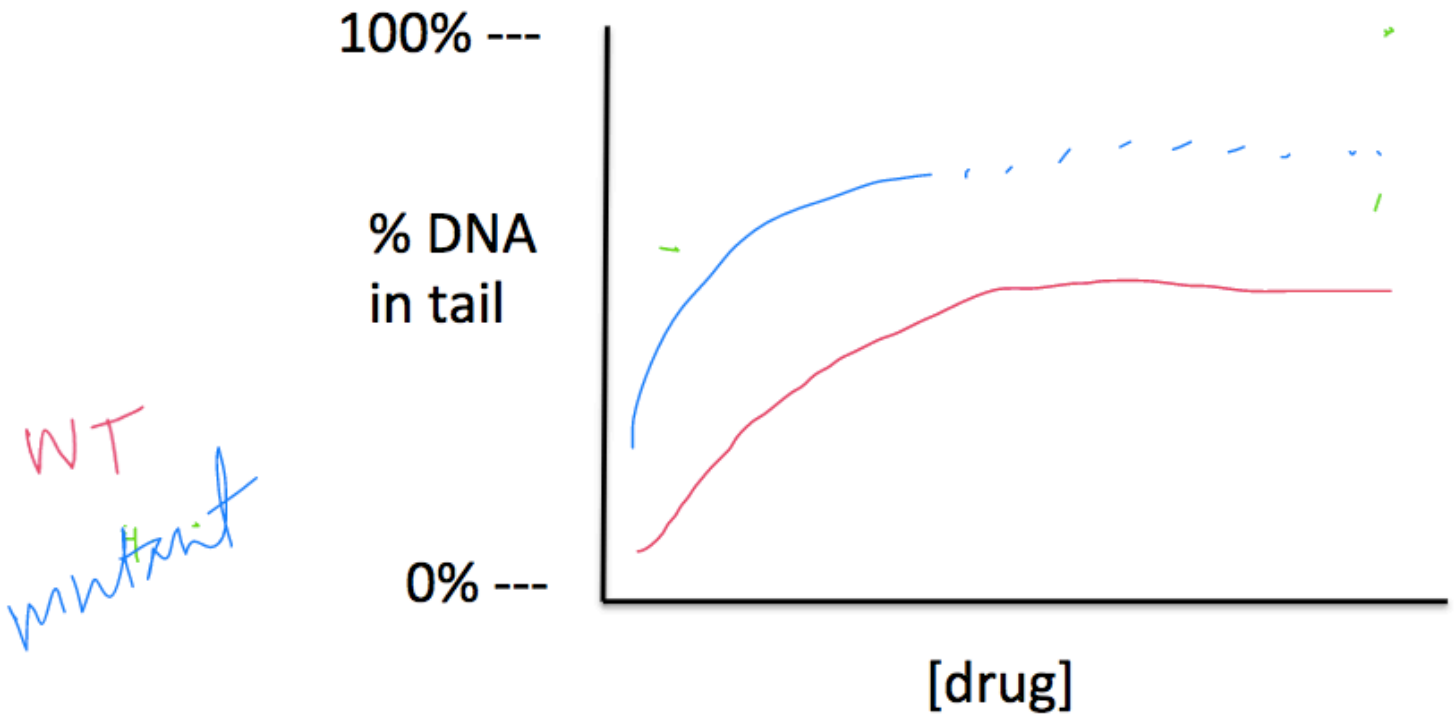
- Measure effects of MMS and H_2O_2 on BER



3. Use immuno-fluorescence assay to measure DNA repair

- Examine effect of MMS and H_2O_2 on DSB abundance

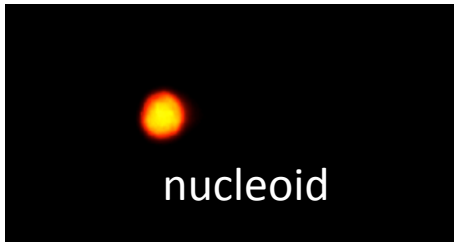
Remember your hypothesis



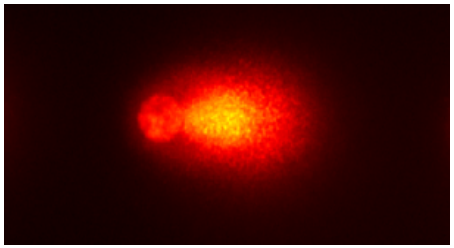
Incubating the Comet Chip with purified enzymes (OGG1, AAG) ***REVEALS*** damage not quantified otherwise (buffer control chip)

Exp2: Biochemical testing using CometChip

M1D6 and D7:



No damage:
supercoiled



Damage:
SSBs, DSBs, abasic sites, alkali labile sites

1. ImageJ

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

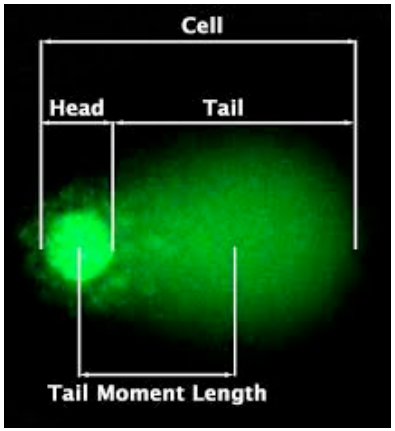
2. MATLAB

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

3. Excel

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

What are the data?



	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

triplicates

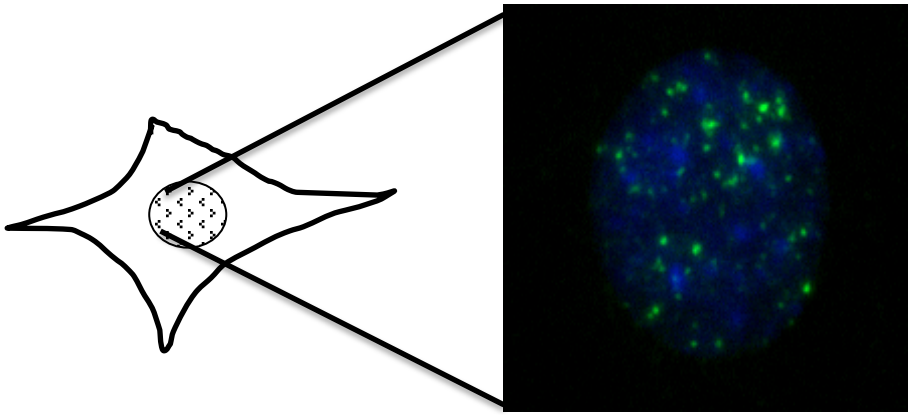
Cometnumbers | %Head DNA | %Tail DNA | OTM (um) | Tail Len. (um) | Comet Len. (um) | +

- 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

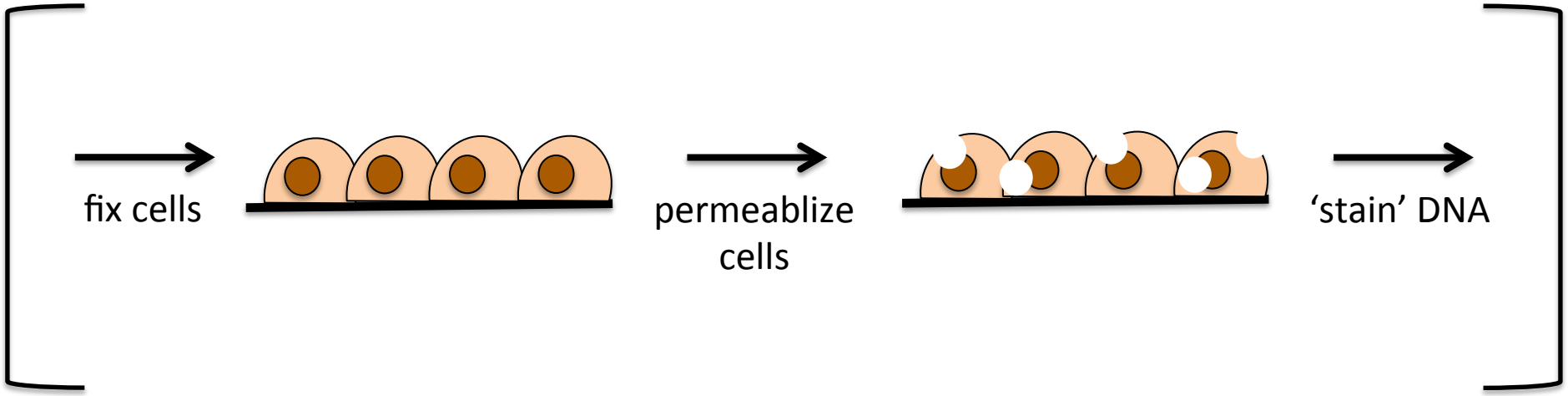
Exp3: DSB abundance using H2AX

M1D6 and D7:

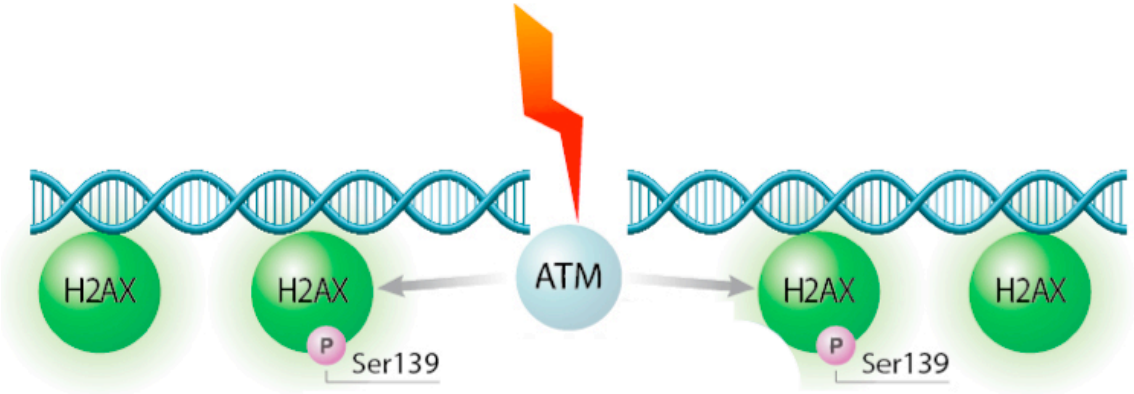
stain DNA



Blue: DNA
Green: γH2AX foci (DSB)



Antibodies used to detect 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

Remember your hypothesis

How will the treated / not treated cells differ?

How will the recovery / no recovery cells differ?

Notes on homework due M1D7

- Complete assignment with partner(s)
- First sentence of each subsection should be a brief introductory sentence motivating the method / procedure
- Use final concentrations, not stock concentrations
- Don't include '109' specific language
- Be concise while still providing enough information for the reader to repeat the experiment
  - Example: buffer recipes and details in parentheses

Today in lab…

- Image CometChips
  - Demonstration only, use images on wiki for image processing
  - Email results to Noreen (will post to wiki)
- Complete primary staining for γH2AX assay
- Paper discussion