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

10/03/17

1. Pre-lab Discussion
2. Start H2AX staining of fixed cells
3. Process CometChip microscopy images and analyze via Matlab
4. Rotate groups to Engelward microscope to image CometChip
5. 4:15pm start paper discussion

Assignments for M1

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

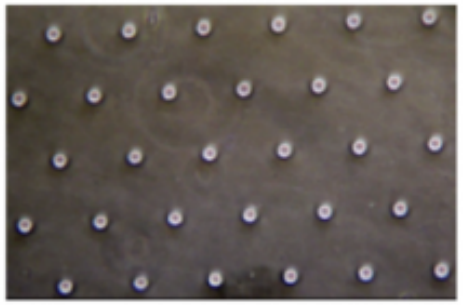
Extra office hours

- **56-302**
- Saturday, 10/7, 10am-2pm
- Wednesday, 10/9, 10am-3pm

Regular office hours

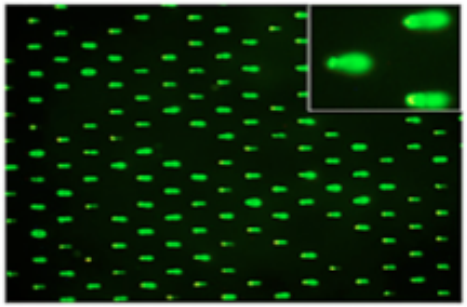
- **Next week Monday OH canceled**
- Josephine, Thursday 2pm-3pm in 56-341c
- Leslie, Friday 9am-10am and 3pm-4pm in 56-341c

Overview of
Module 1: Measuring Genomic Instability



1. Optimize comet chip assay

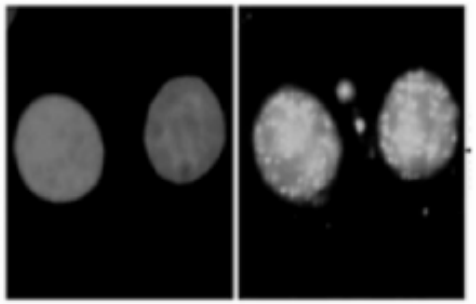
- Test loading variables



2. Use comet chip assay to measure DNA repair

- Measure effects of MMS and H_2O_2 on BER

Measure (Quantify) effect of DNA repair activity in response to alkylation damage and oxidative damage.



3. Use immuno-fluorescence assay to measure DNA repair

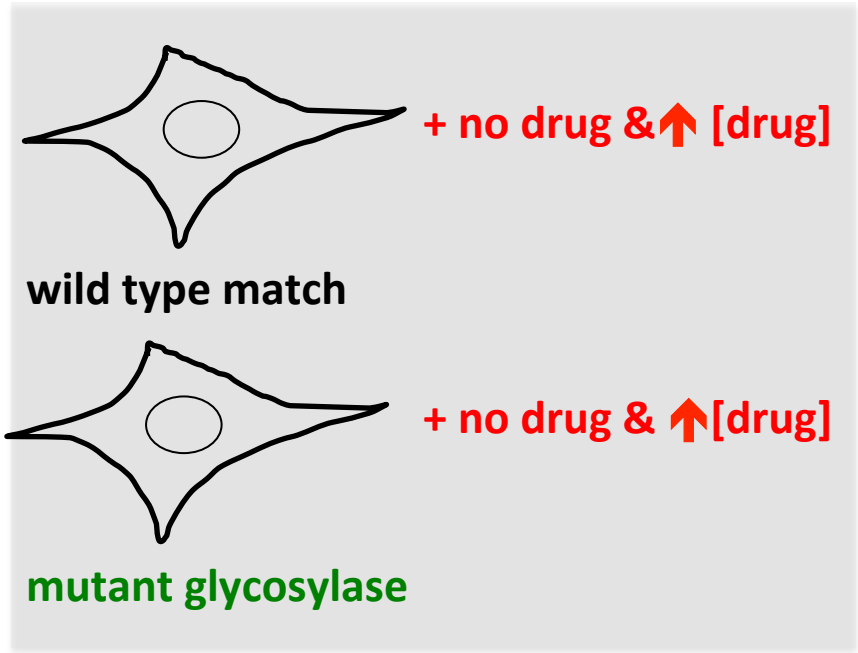
- Examine effect of MMS and H_2O_2 on DSB abundance

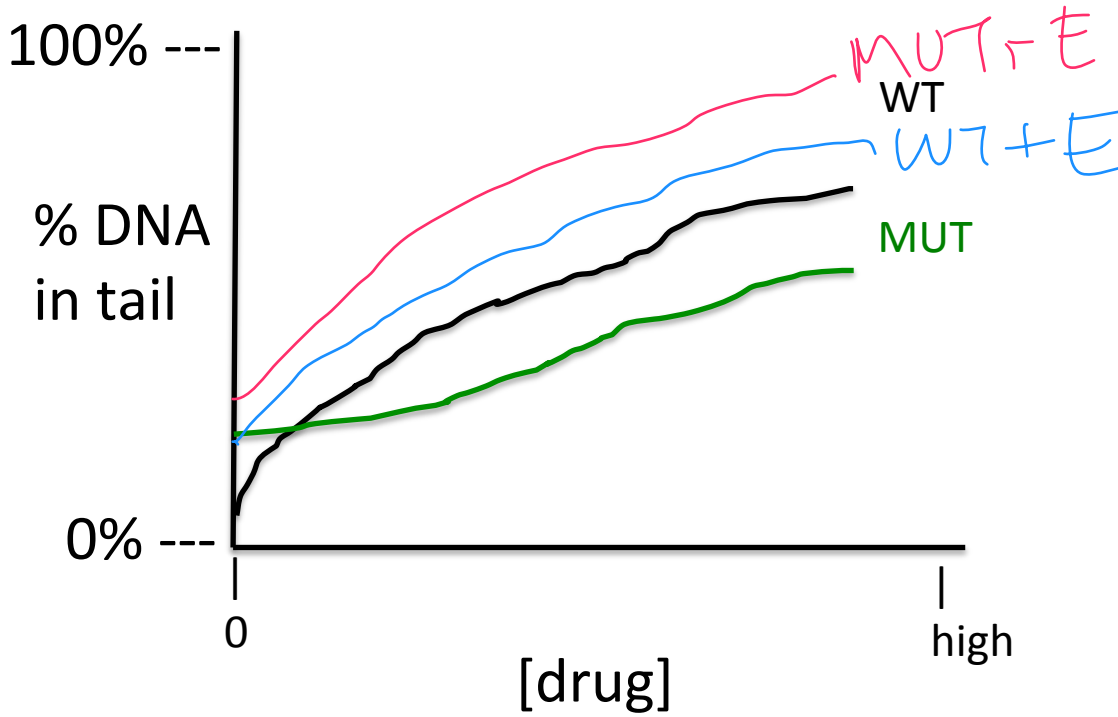
Quantify effect of DNA damage response (DNA repair and recovery) in response to alkylation damage and oxidative damage induced DSBs.

recovery= continued repair w/o presence of DNA damaging agents

enzyme treatment minus the non enzyme treatment= actual (unrepaired) damage from MMS/H2O2

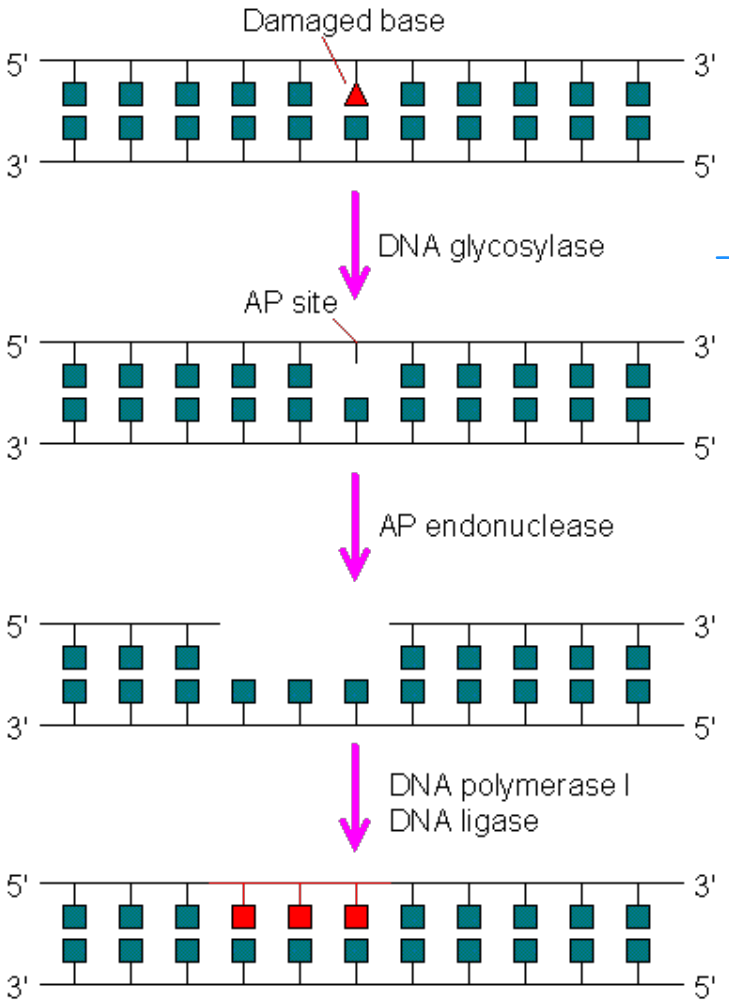
What result do we expect from our assay so far…





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

Base Excision Repair (BER) Pathway



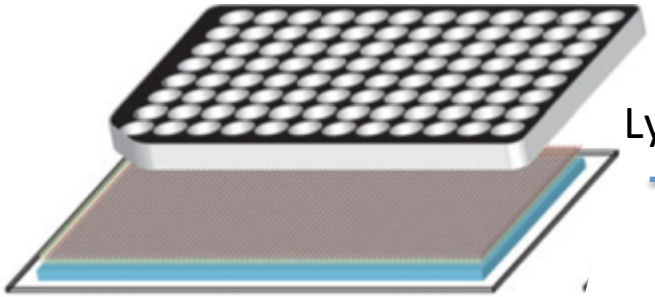
not necessarily visualized in comet tail

strand breaks visualized via Comet tail

http://www.web-books.com/MoBio/Free/Ch7G.htm

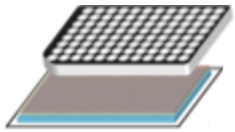
Complete experiment to test role of biochemical factors (MMS or H2O2) in genomic stability (DNA damage)

Load and capture cells in 2 CometChips, then treat with MMS or H2O2

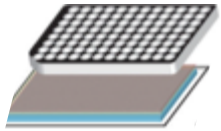


Lyse cells

Chip 1: No enzyme treatment (buffer control)

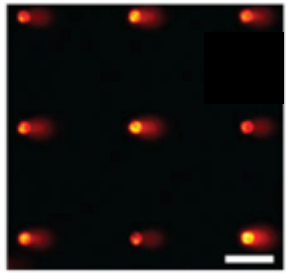


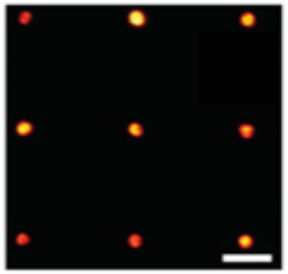
Chip 2: Treat with FPG (*E. coli* Ogg1) OR human AAG

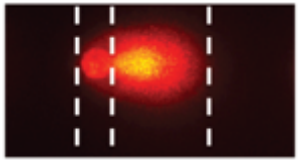


Alkaline unwinding

Electrophoresis



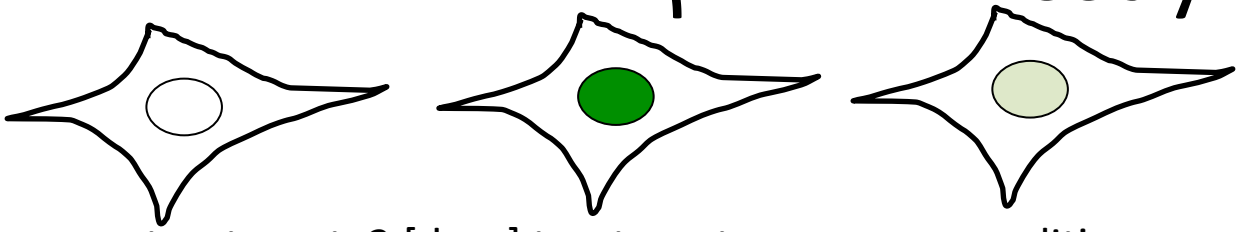




stain DNA and image via fluorescence microscopy

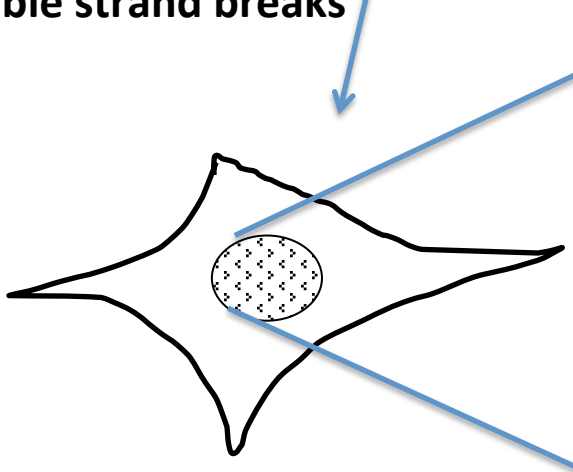
analysis via Matlab

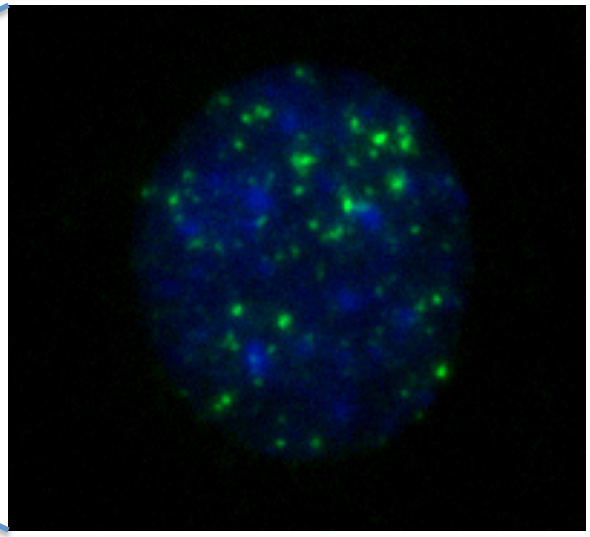
Measuring Double strand breaks via γH2AX Assay



no treatment, 2 [drug] treatments, recovery conditions

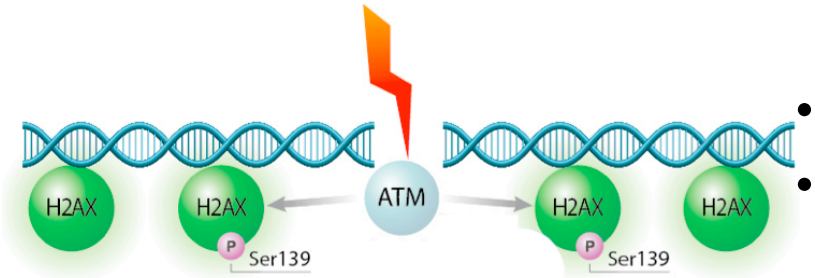
Fix cells and stain with antibody that marks double strand breaks



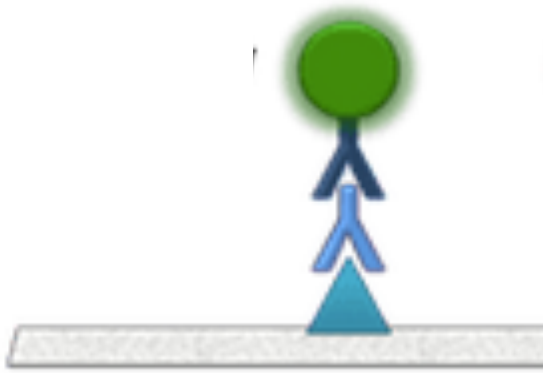


Blue: DNA
Green: γH2AX staining= double strand breaks

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

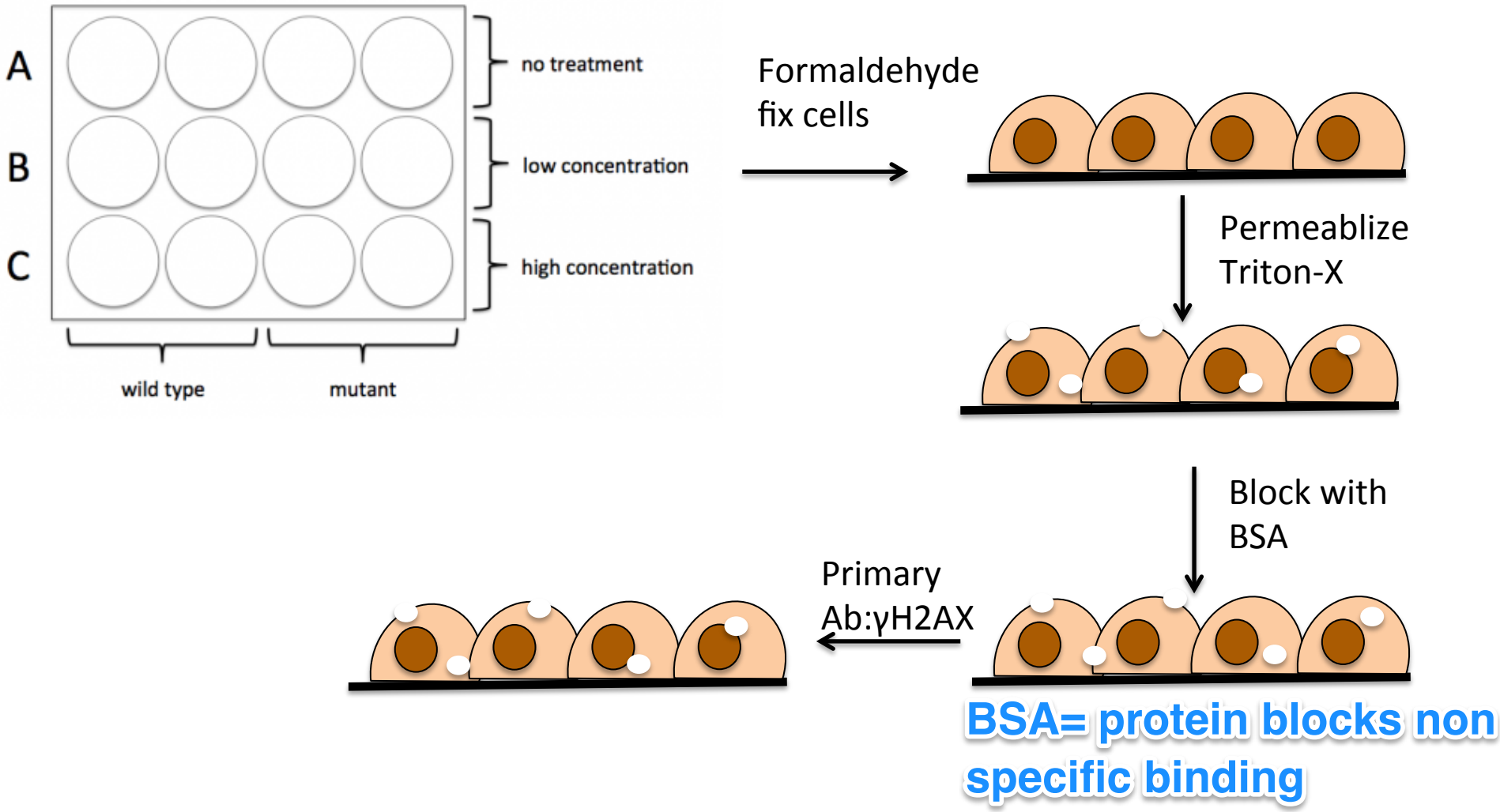


- histone H2AX phosphorylated at Ser139 if DSB
- use antibodies against γH2AX

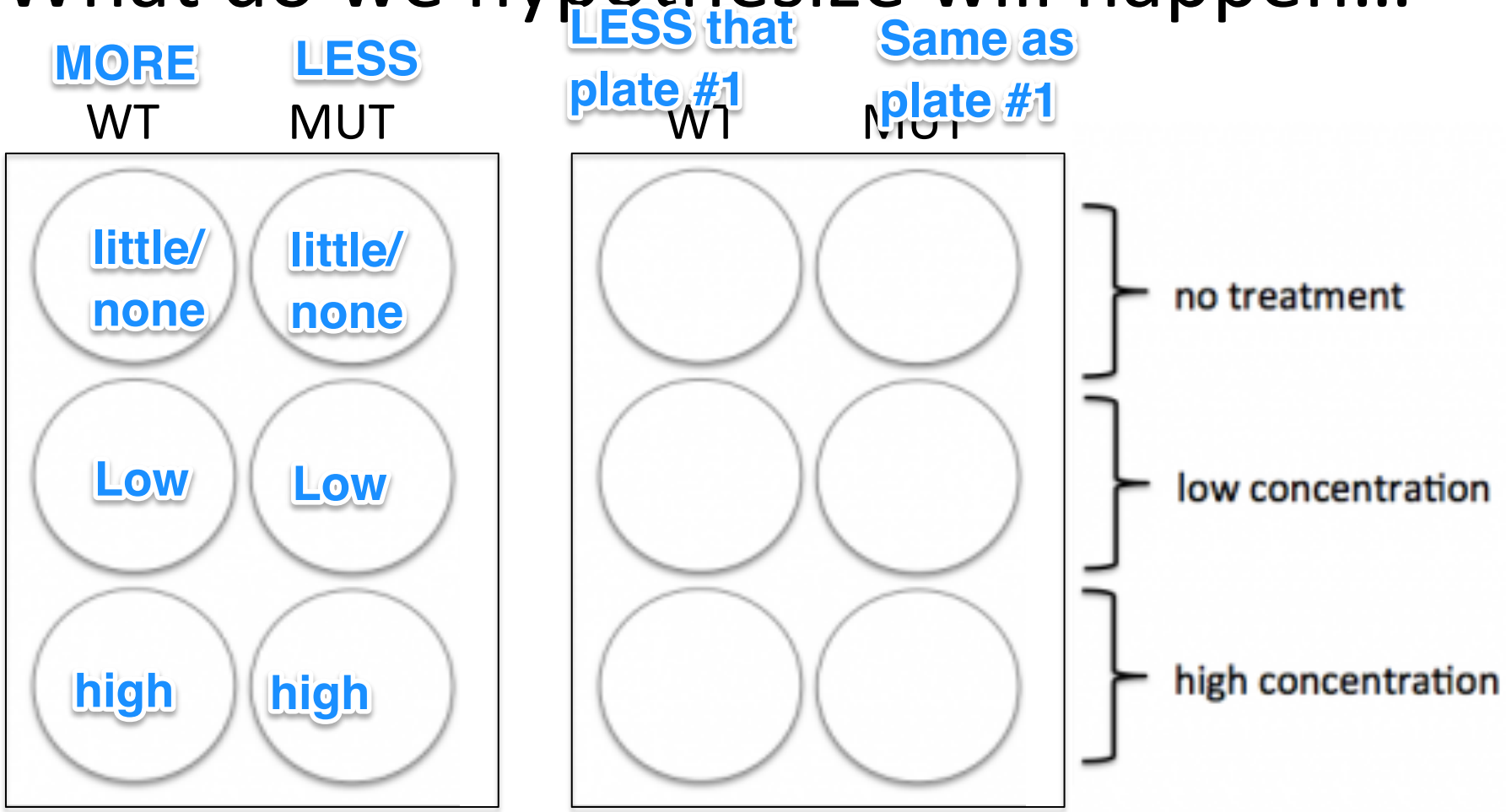


protein of interest	γH2AX
primary antibody	mouse anti-human anti-γH2AX
secondary antibody	goat anti-mouse
fluorescent dye exc./ em. wavelengths	488 / 520 nm

M1D6, practically using immunofluorescence: γH2AX assay to detect double-strand DNA breaks



What do we hypothesize will happen…



Cells fixed @30min

No recovery #1

Cells treated for 30min

Recovery in fresh media #2

CometChip Data analysis in ImageJ and MATLAB

1. ImageJ

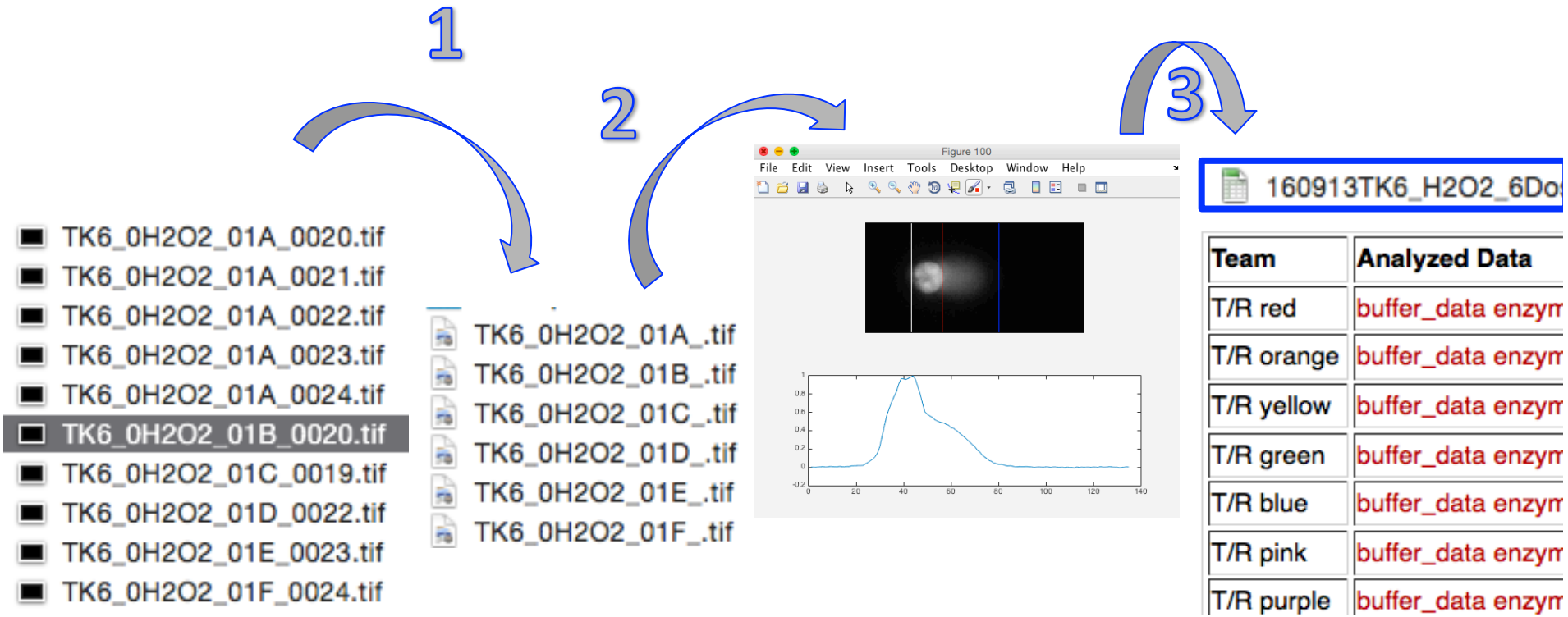
- from several images per well to one stack per well
- `GenImageStacks_singleimage.txt`

2. MATLAB

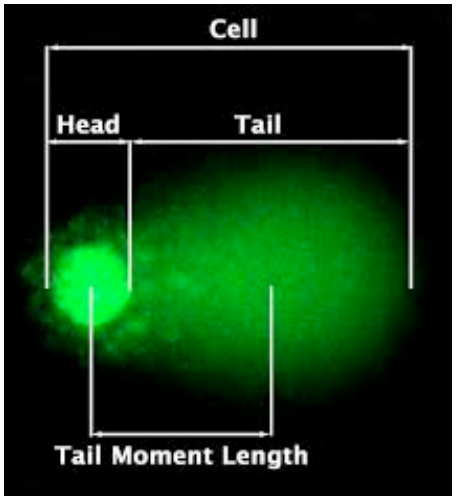
- for each comet in stack, calculates intensity of head and tail, as well as length of tail

3. Excel

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

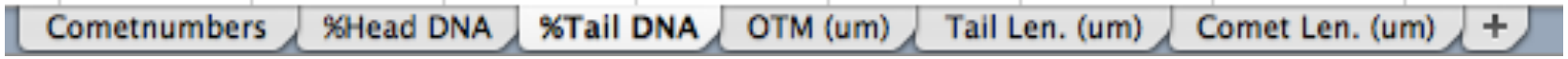


What's in the final Excel file?



	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

triplicates



- 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

Don't forget reported data must have 95% C.I. and include statistical analysis

- 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.

In lab today

1. Retrieve your 12 well plates and choose 6 conditions to for your immunofluorescence experiment; start staining protocol.
2. During incubations begin Matlab analysis.
3. Groups will go one at a time to the Engelward microscope to see your CometChip.
4. Paper discussion will start at 4:15pm in the lab.

HWM1D7 Methods M1D1-M1D5

- Complete assignment as team
- First sentence in each subsection should be a brief introductory sentence motivating the method
- Use final concentrations
- Don't '109 specific language
- Be concise while still providing enough information for the reader to repeat the experiment
  - Example: buffer recipes and wash times/number in parenthesizes