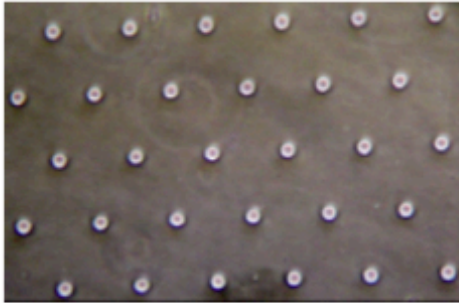


# M1D5: Develop approach for sub-nuclear visualization of DNA damage

09/29/16

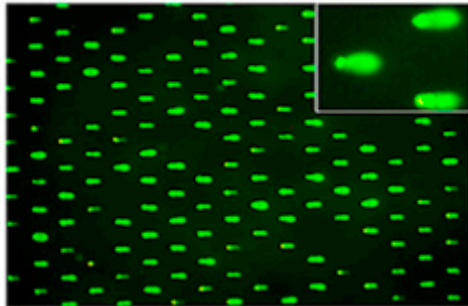
1. Pre-lab Discussion
  2.  $\frac{1}{2}$  class to TC room
  3.  $\frac{1}{2}$  class start data analysis
- Announcements: Photographer in lab Tuesday Oct. 4<sup>th</sup> 2:30pm

# Overview of “M1: Measuring Genomic Instability”



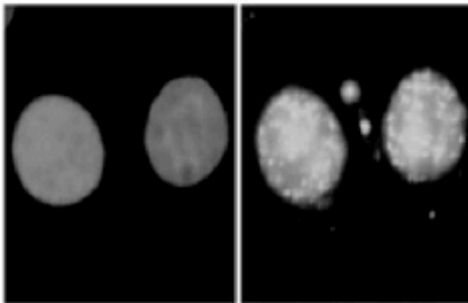
## 1. Optimize comet chip assay

- Test loading variables



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

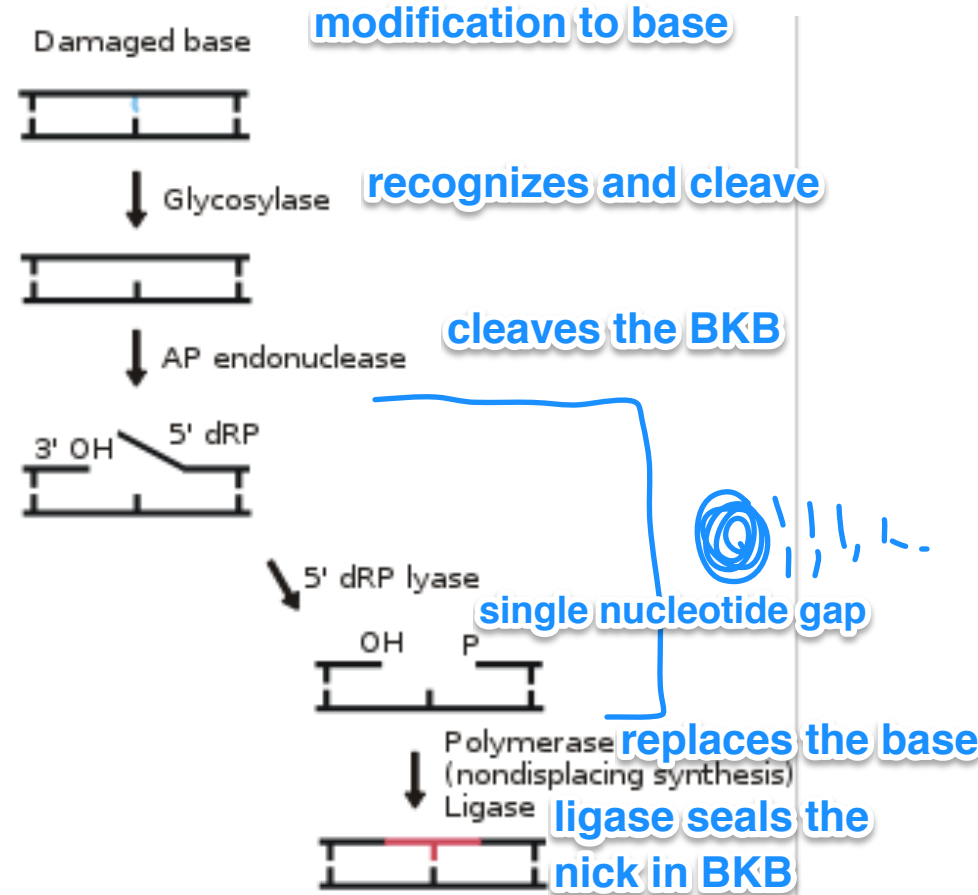
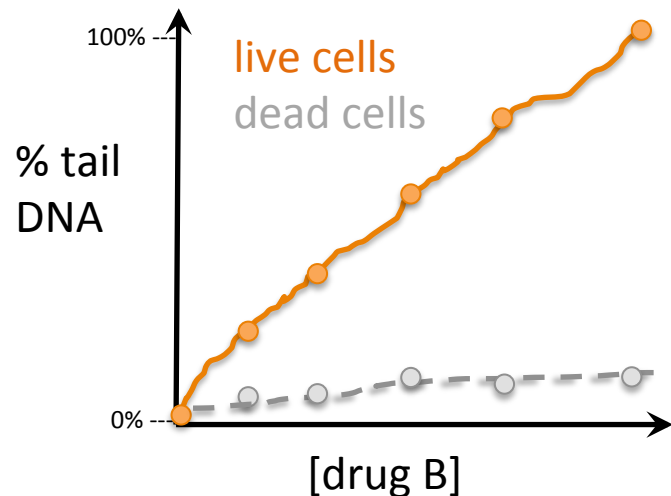
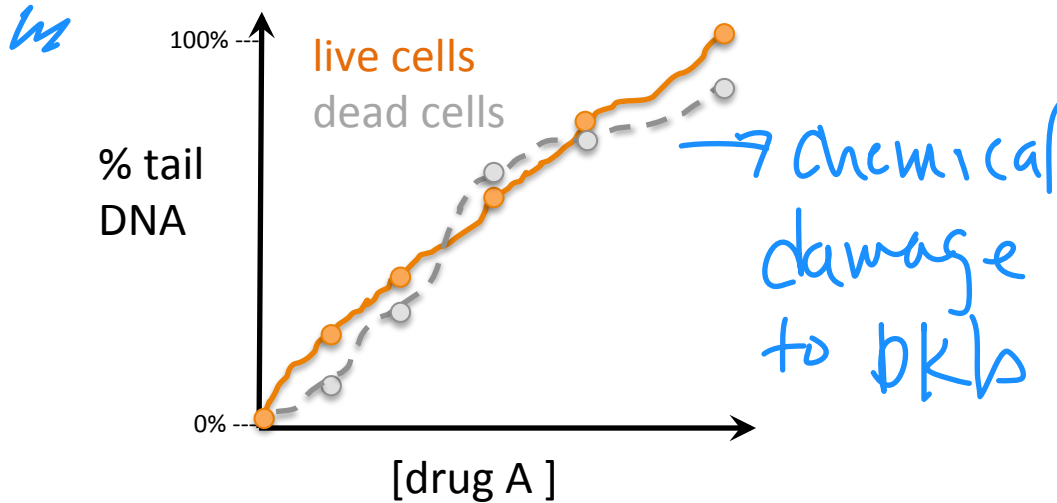
- Measure effects of MMS and  $H_2O_2$  on BER
- Assess repair variability in healthy individuals



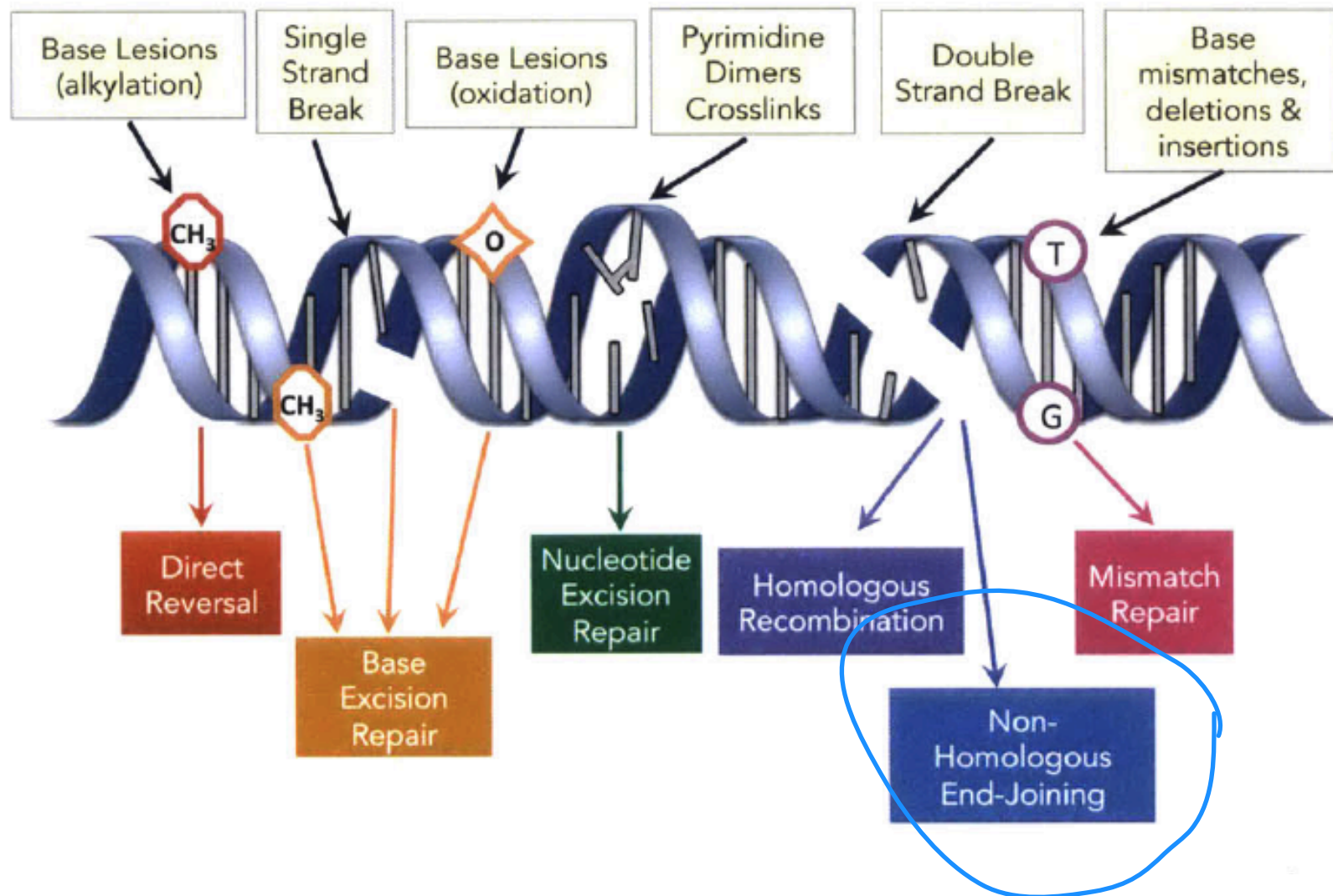
## 3. Use immuno-fluorescence assay to visualize DNA repair

- Examine effect of  $H_2O_2$  on DSB abundance

# Both H<sub>2</sub>O<sub>2</sub>- and MMS-caused DNA damage is repaired by base excision repair (BER)

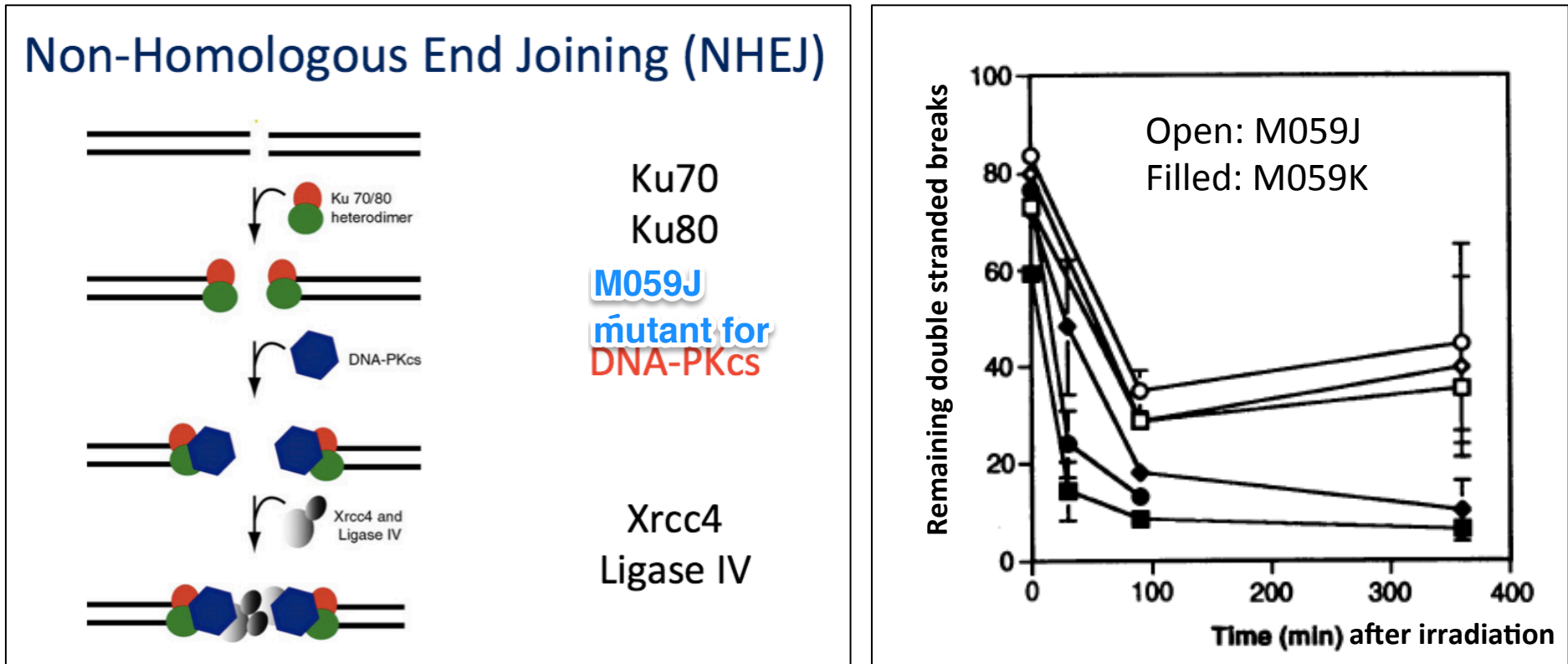


Both H<sub>2</sub>O<sub>2</sub>- and MMS-caused DNA damage is repaired by base excision repair (BER)



# M059K and M059J cell lines

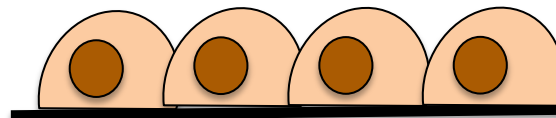
- M059K is wild type
- M059J is missing DNA-PKcs, deficient in NHEJ DNA repair
- human glioblastoma fibroblasts



# $\gamma$ H2AX assay to detect double-strand DNA breaks

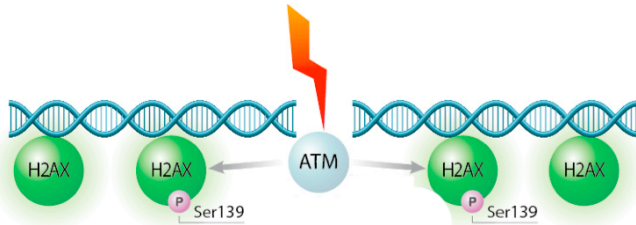


Formaldehyde  
fix cells

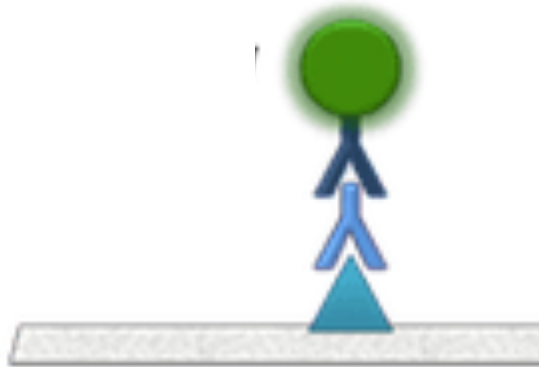






Kills cells  
Stops everything  
= X-linking  
chemistry

# Using immunofluorescence: $\gamma$ H2AX assay to detect double-strand DNA breaks

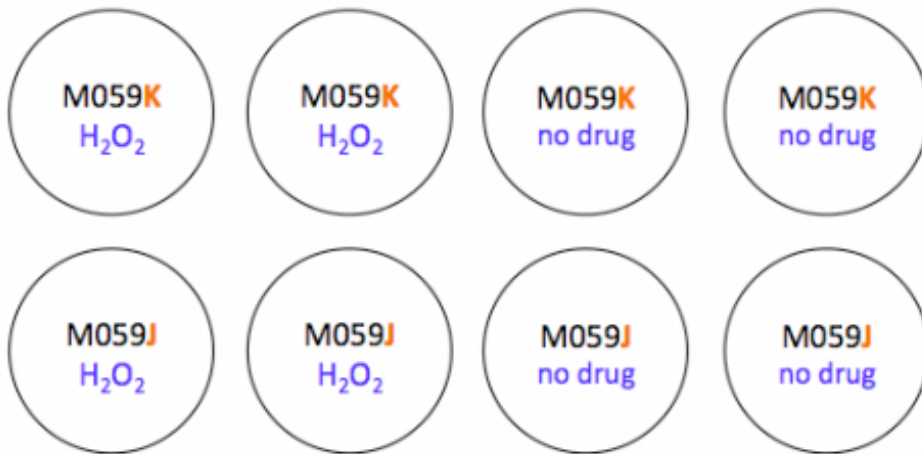


- histone H2AX phosphorylated at Ser139 if DSB
- use antibodies against  $\gamma$ H2AX

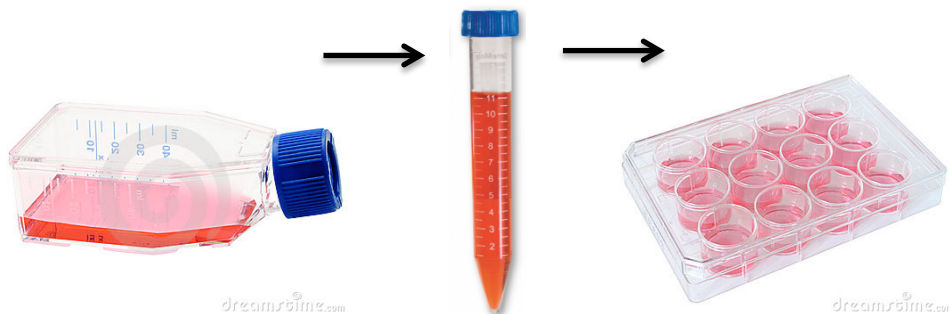


|  |  |
|--|--|
| protein of interest                      |  $\gamma$ H2AX                        |
| primary antibody                         |  mouse anti-human anti- $\gamma$ H2AX |
| secondary antibody                       |  goat anti-mouse                    |
| fluorescent dye<br>exc./ em. wavelengths |  488 / 520 nm                       |

# Seeding coverslips in the tissue culture hood



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



- **trypsinize** 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 acids, 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**

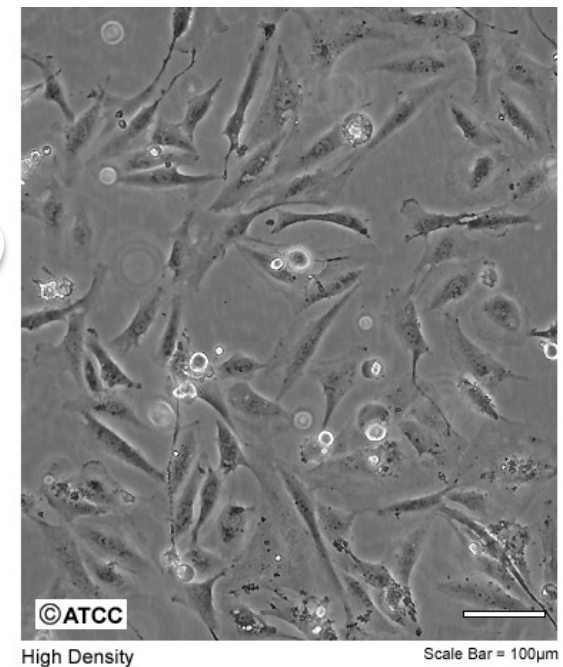
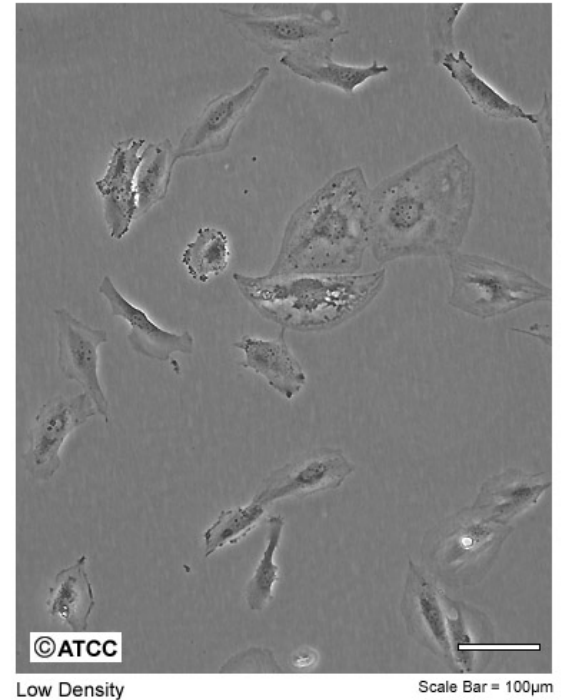
4. Seed coverslips

**-higher resolution to image through glass (over plastic)**

**-ease of use; transferring coverslip rather than**

**washing dish with primary, secondary, etc.**

M059K



# Data analysis in ImageJ, MATLAB, and Python

## 1. ImageJ

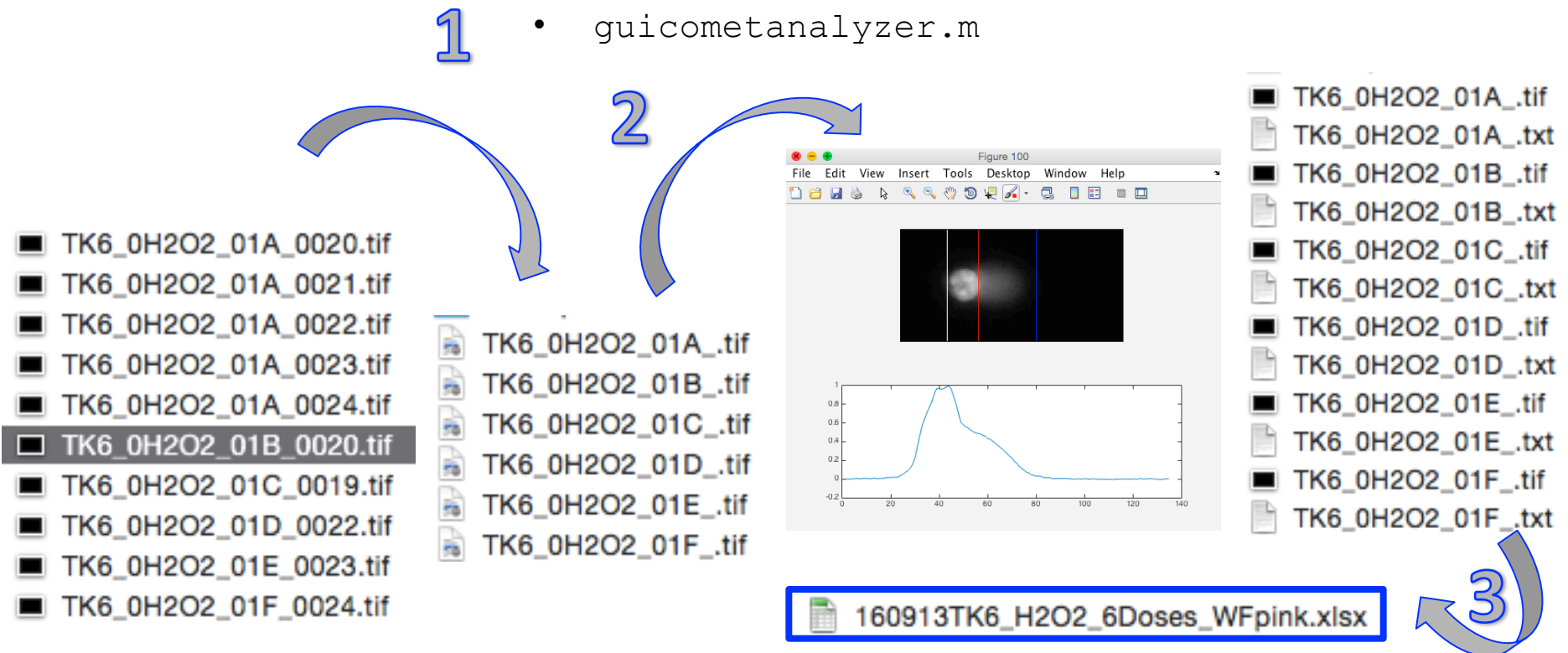
- from several images per well to one stack per well
- `GenImageStacks_singleimage.txt`

## 2. MATLAB

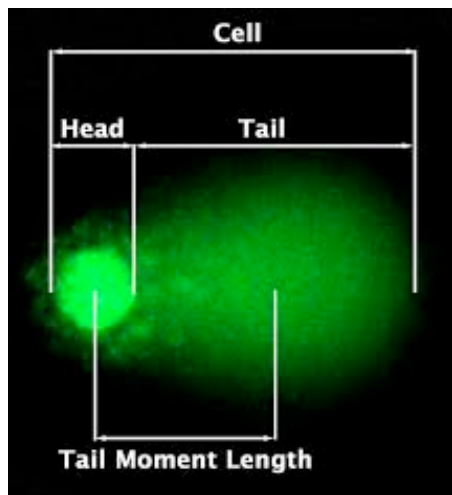
- for each comet in stack, calculates intensity of head and tail, as well as length of tail
- creates one `.txt` per comet
- `guicometalyzer.m`

## 3. Python

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



# 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 | %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 =  $100 * \text{HeadFluorescence} / (\text{HeadFluorescence} + \text{TailFluorescence})$
- %TailDNA =  $100 * \text{TailFluorescence} / (\text{HeadFluorescence} + \text{TailFluorescence})$
- Olive tail moment (OTM) =  $(\% \text{TailDNA} / 100) * (\text{TailCenterOfMass} - \text{HeadCenterOfMass})$
- 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

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

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

- 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.**

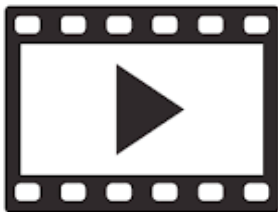
# Assignments for M1



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

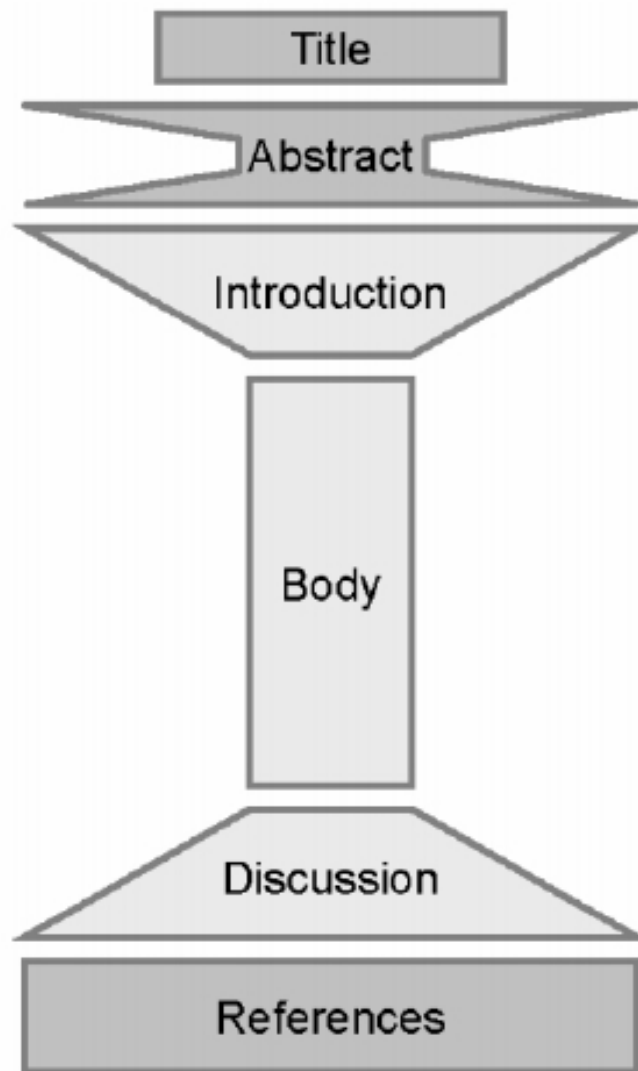
## Summary content

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)**

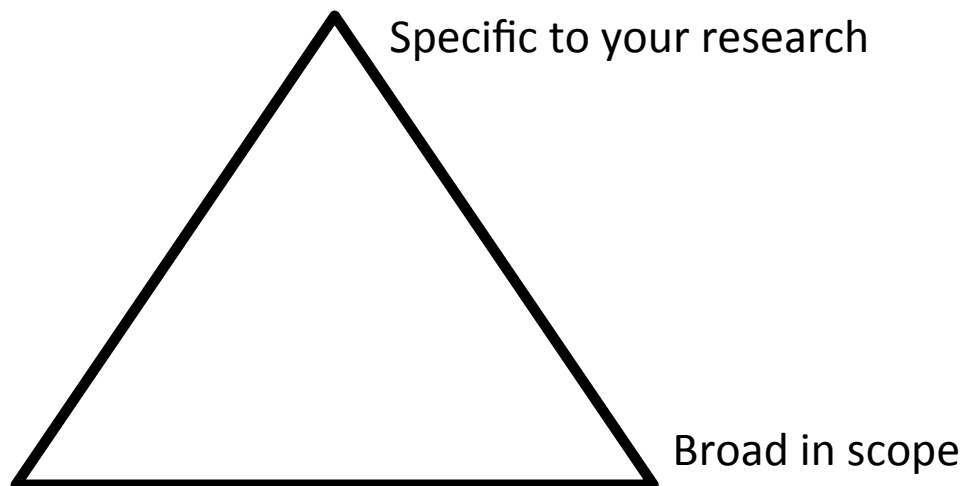
**References from Intro**

# 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%               | <ul style="list-style-type: none"><li>• Did you introduce your research?</li><li>• Did you include the key findings (and the techniques used to gather these results, if necessary)?</li><li>• Was the importance of your project clear?</li></ul>                |
| Organization | 25%               | <ul style="list-style-type: none"><li>• Is the presentation logical and easy-to-follow?</li><li>• Are the main points emphasized?</li><li>• Did you include transition statements such that the presentation 'flows' and is easily followed/understood?</li></ul> |
| Delivery     | 25%               | <ul style="list-style-type: none"><li>• Do you show confidence and enthusiasm?</li><li>• Did you use appropriate language (technical or informal, as appropriate)?</li><li>• Is your speech clear?</li></ul>  |

## 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