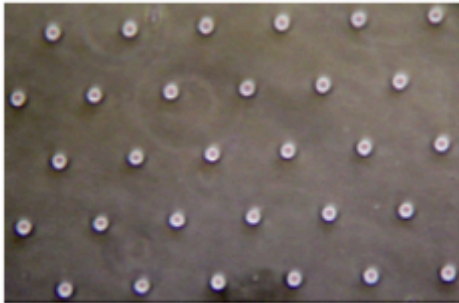


M1D5:
Seed cells for
immunofluorescence assay
CometChip data analysis

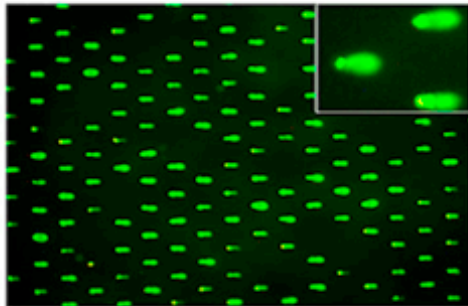
09/30/2016

Overview of “M1: Measuring Genomic Instability”



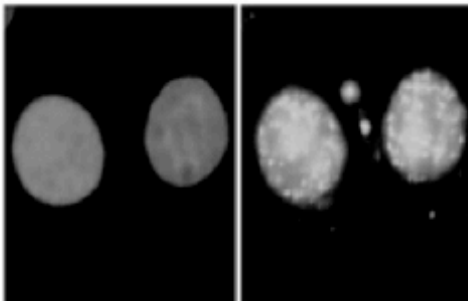
1. Optimize comet chip assay

- Test loading variables



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

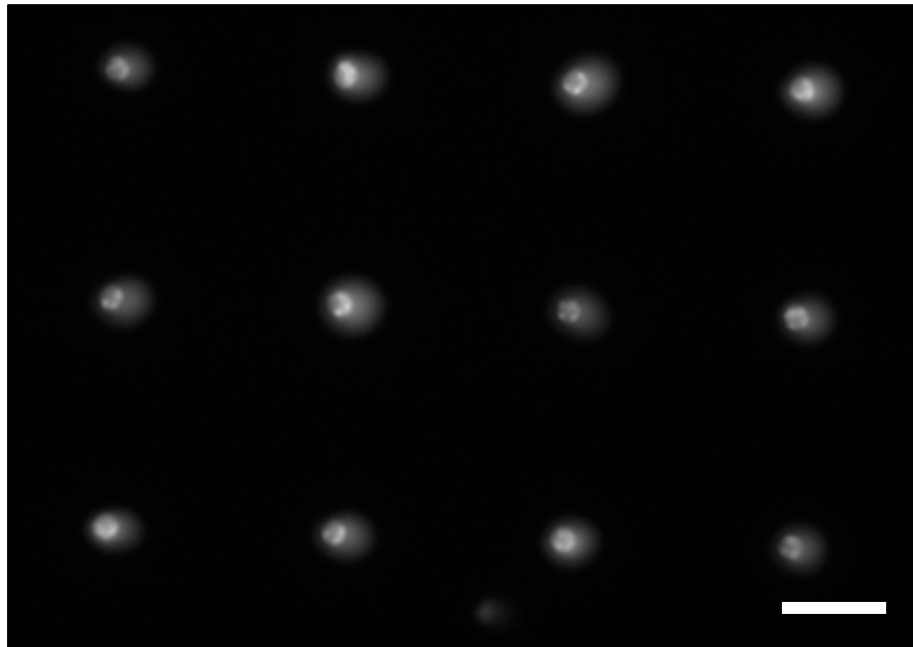
- Measure effects of MMS and H_2O_2 on BER
- Assess repair variability in healthy individuals



3. Use immuno-fluorescence assay to visualize DNA repair

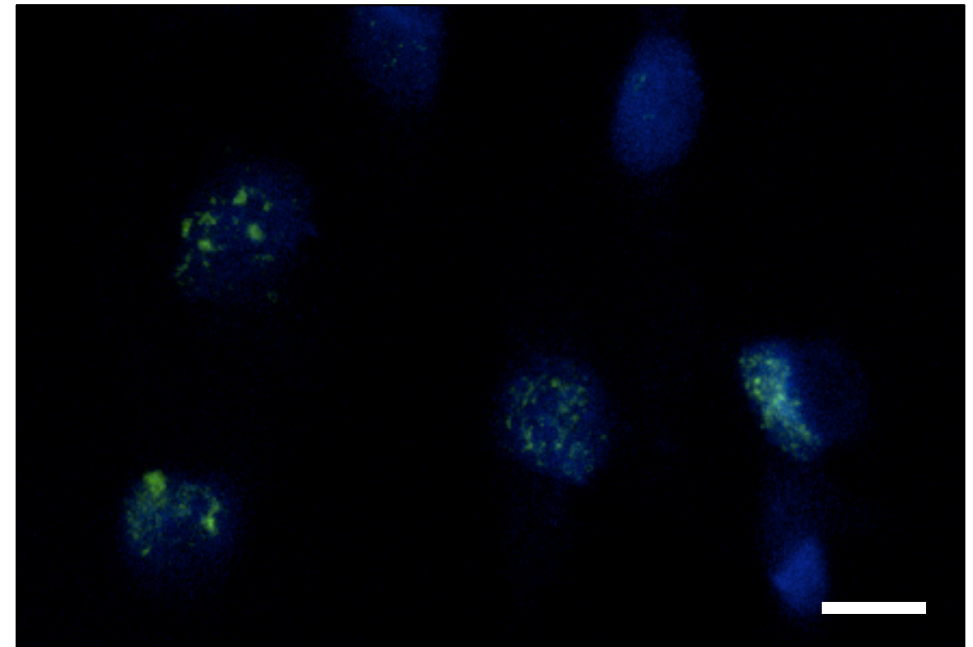
- Examine effect of H_2O_2 on DSB abundance

CometChip & γ H2AX assays both rely on fluorescence to detect DNA breaks



DNA

- TK6 cells in suspension
- lysed
- ss or ds DNA break

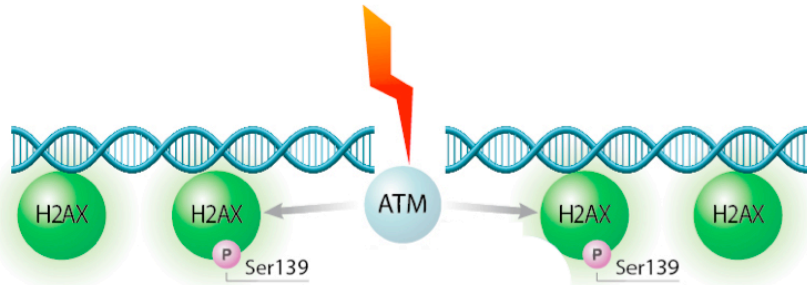


nucleus
(DAPI)

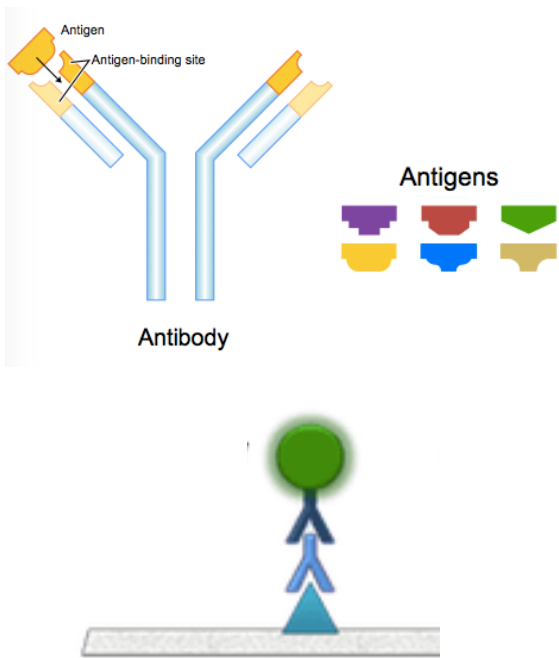
double strand break
(AlexaFluor 488)





- adherent M059J/K cells
- fixed but structurally intact
- ds only DNA break

Using immunofluorescence: γ H2AX assay to detect double-strand DNA breaks



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

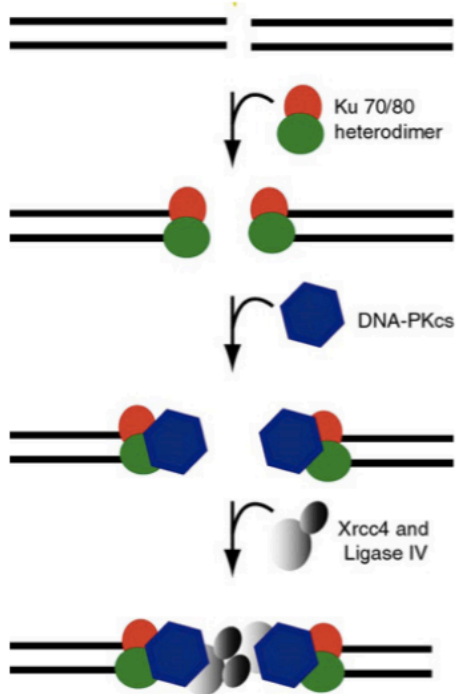


protein of interest	 γ H2AX
primary antibody	 mouse anti-human anti- γ H2AX
secondary antibody	 goat anti-mouse
green fluorescent dye exc./ em. wavelengths	 488 / 520 nm

M059K and M059J cell lines

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

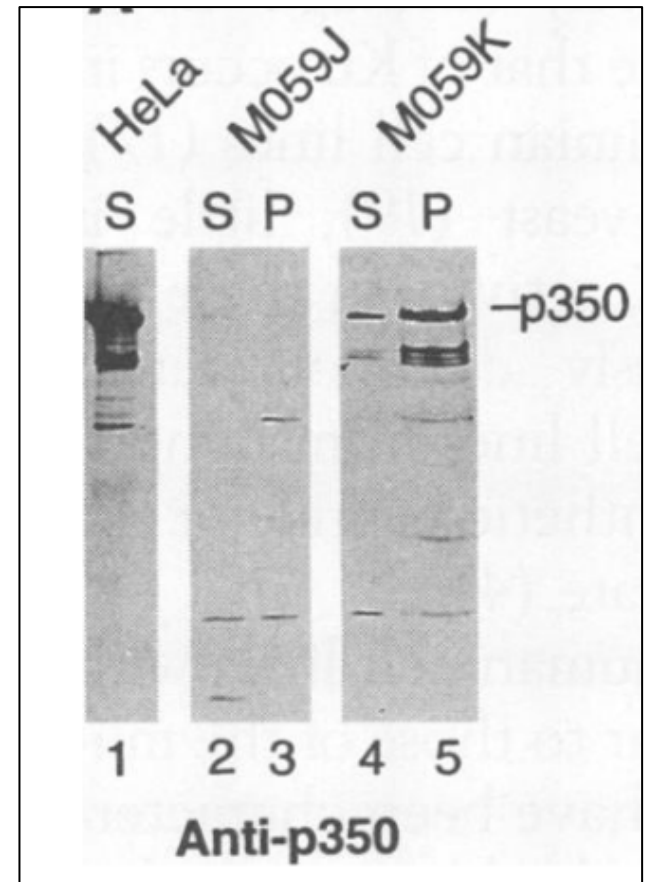
Non-Homologous End Joining (NHEJ)



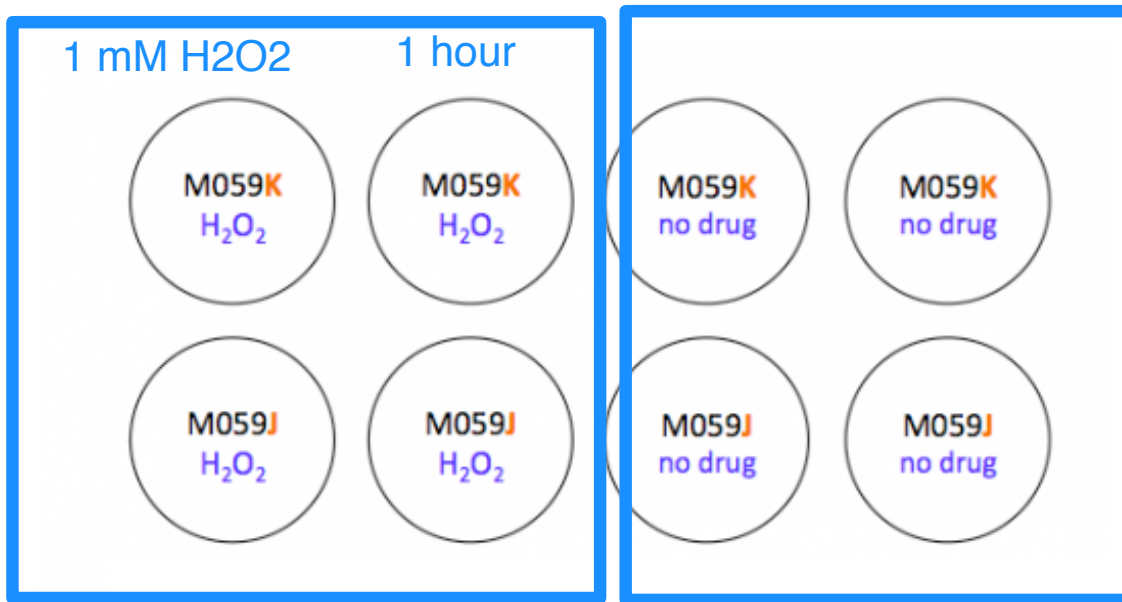
Ku70
Ku80

DNA-PKcs
(incl. p350)

Xrcc4
Ligase IV

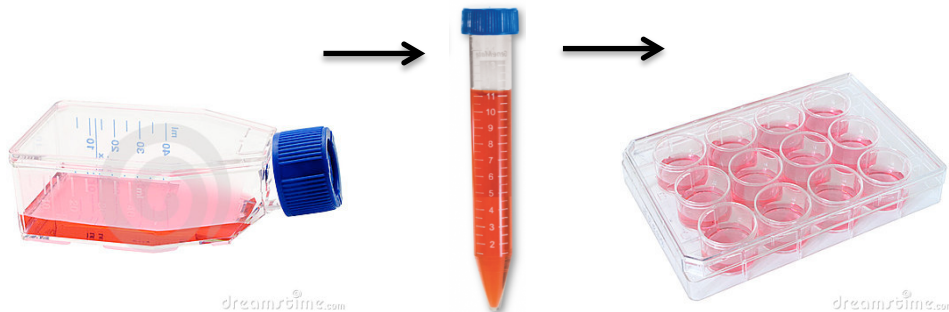


Cell plating in the tissue culture hood



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

so we can remove cells from plate later to image



- trypsinize adherent cells before counting

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

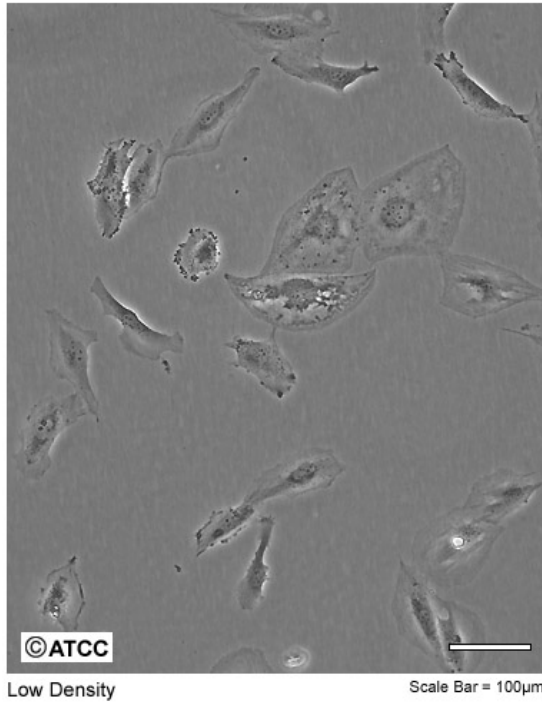
glucose primarily used for growth

Non-food:

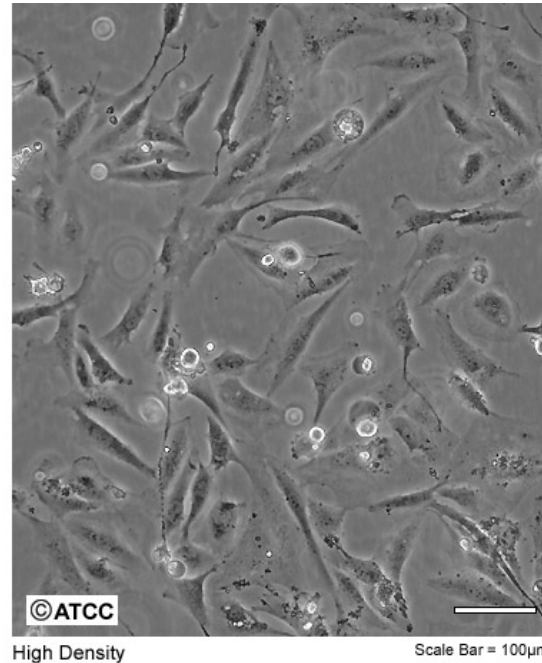
- antibiotics:
 - penicillin
 - streptomycin



low (~ 20%) confluency



high (~ 90%) confluency



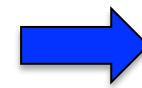
Seeding your M059J/K cells

why?

1. Rinse with PBS
cleans from dead cells, debris
wash away anti-trypsin agents
2. Detach with trypsin
serine protease
3. Calculate number of cells
4. Seed new culture dish



flask



12-well plate

Today, we split into two halves



- in tissue culture
 - red & blue teams



- data analysis
 - green & purple teams
 - using new MacBooks

Data analysis in ImageJ, MATLAB, and Python

1. ImageJ

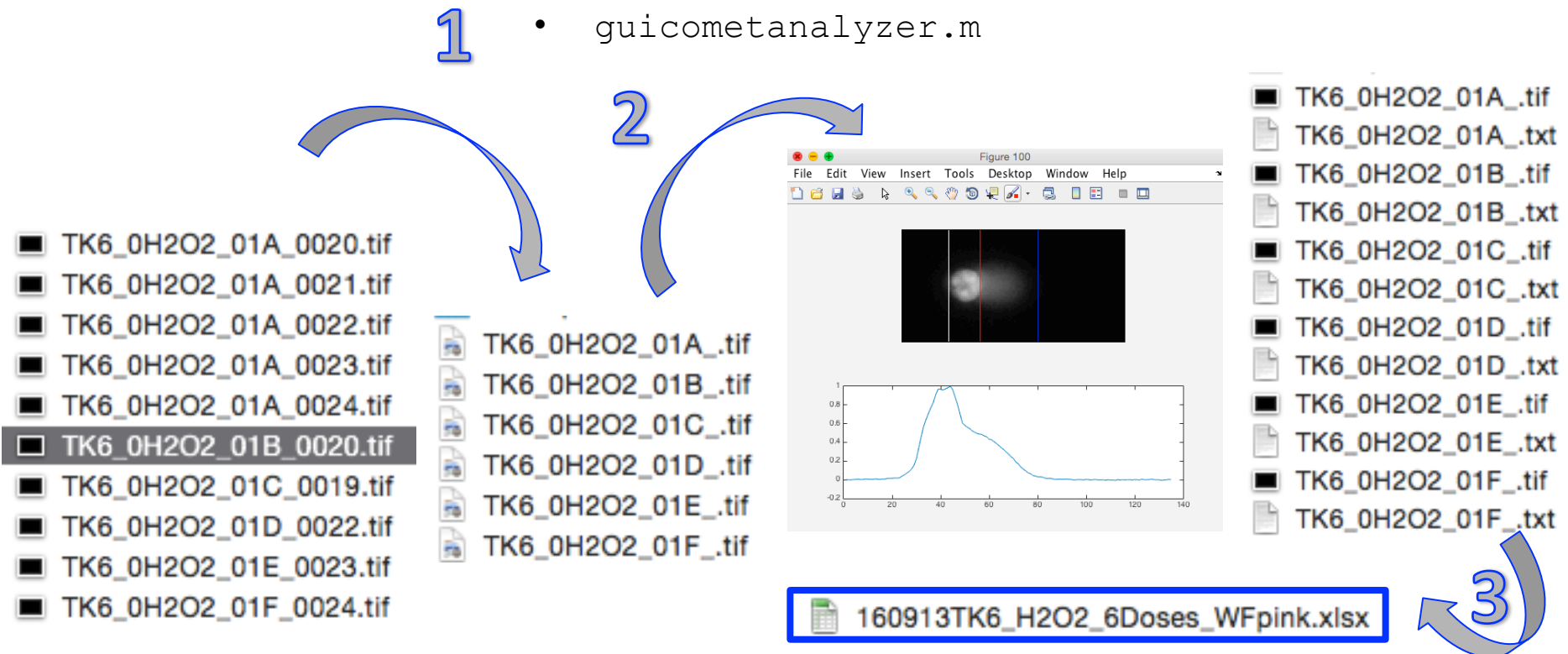
- from several images per well to one stack per well
- GenImageStacks_singleimage.txt

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

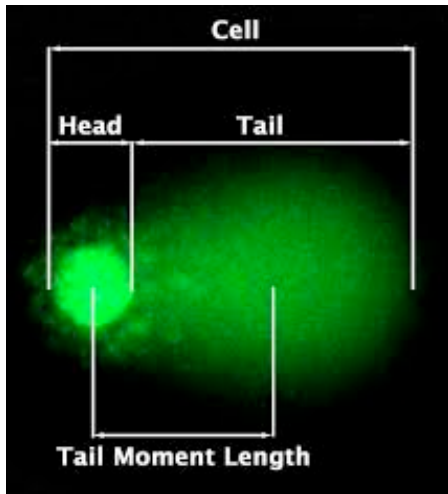
3. Python

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



for Coriell cells data: A-B: Coriell # 10
 C-D: # 20
 E-F: # 24

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]
 live
 dead

triplicates

recovery: no 0 20 40 60 min
 drug

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

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

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

$$t_{table} = TINV(0.05, n - 1) \quad \text{in Excel}$$

- 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

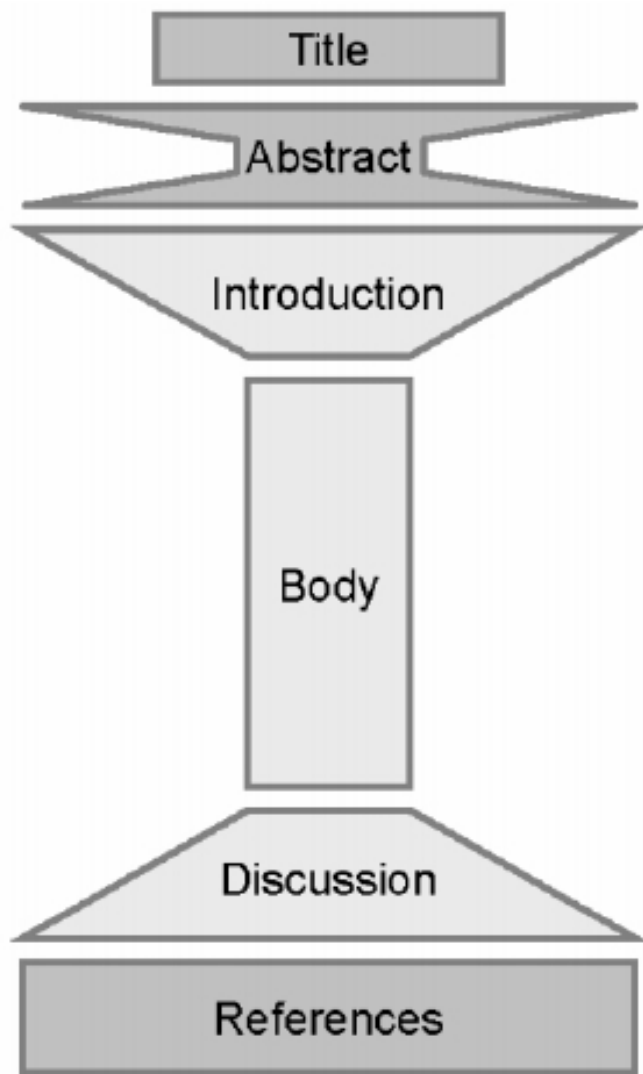
Summary content

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 vs. M1 Data Summary



All (except abstract) in BULLET POINTS

For M1D6: Implications and future work

Specific to your research

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?

Broad in scope

➤ In your Data Summary, tie together your intro and implications and future work

For M1D6: outline of mini presentation

- Follow **time** and content guidelines
- Introduce yourself and your research
- Clearly state your **hypothesis** to identify main question
- Be **quantitative** when stating your findings

Category	Approximate worth	Elements of a strong presentation
Content	50%	<ul style="list-style-type: none">• 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%	<ul style="list-style-type: none">• 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%	<ul style="list-style-type: none">• Do you show confidence and enthusiasm?• Did you use appropriate language (technical or informal, as appropriate)?• Is your speech clear?