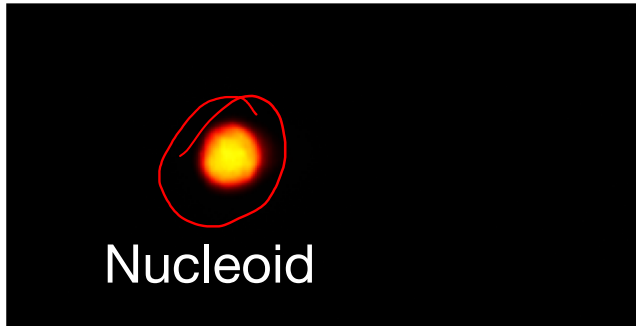


M1D6: Finish H2AX analysis and Image CometChip

10/2/19

1. ✓ Comm Lab
2. Prelab
3. Analyze CometChip with Trevigen software
4. Finish ImageJ analysis of H2AX intensity
5. Paper discussion
6. Work on Data Summary in down time– email revised version by 10pm tonight (rcmeyer@mit.edu)

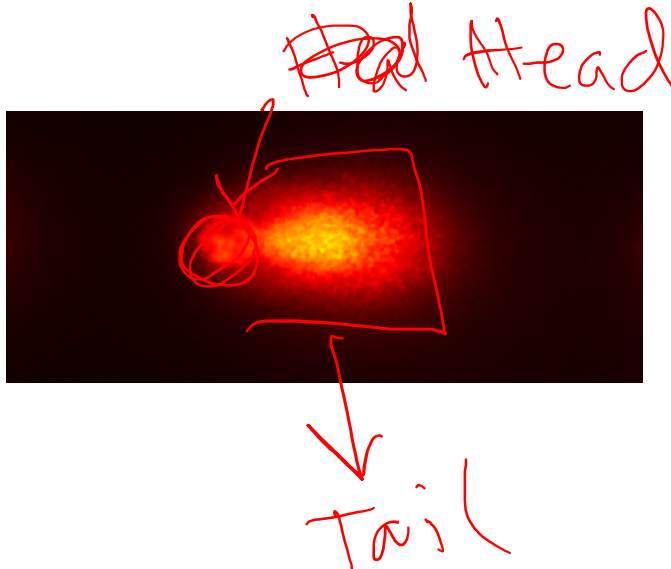
Output of Alkaline CometChip Assay



No Damage

- Supercoiled nucleoid
- Little or no migration

NT

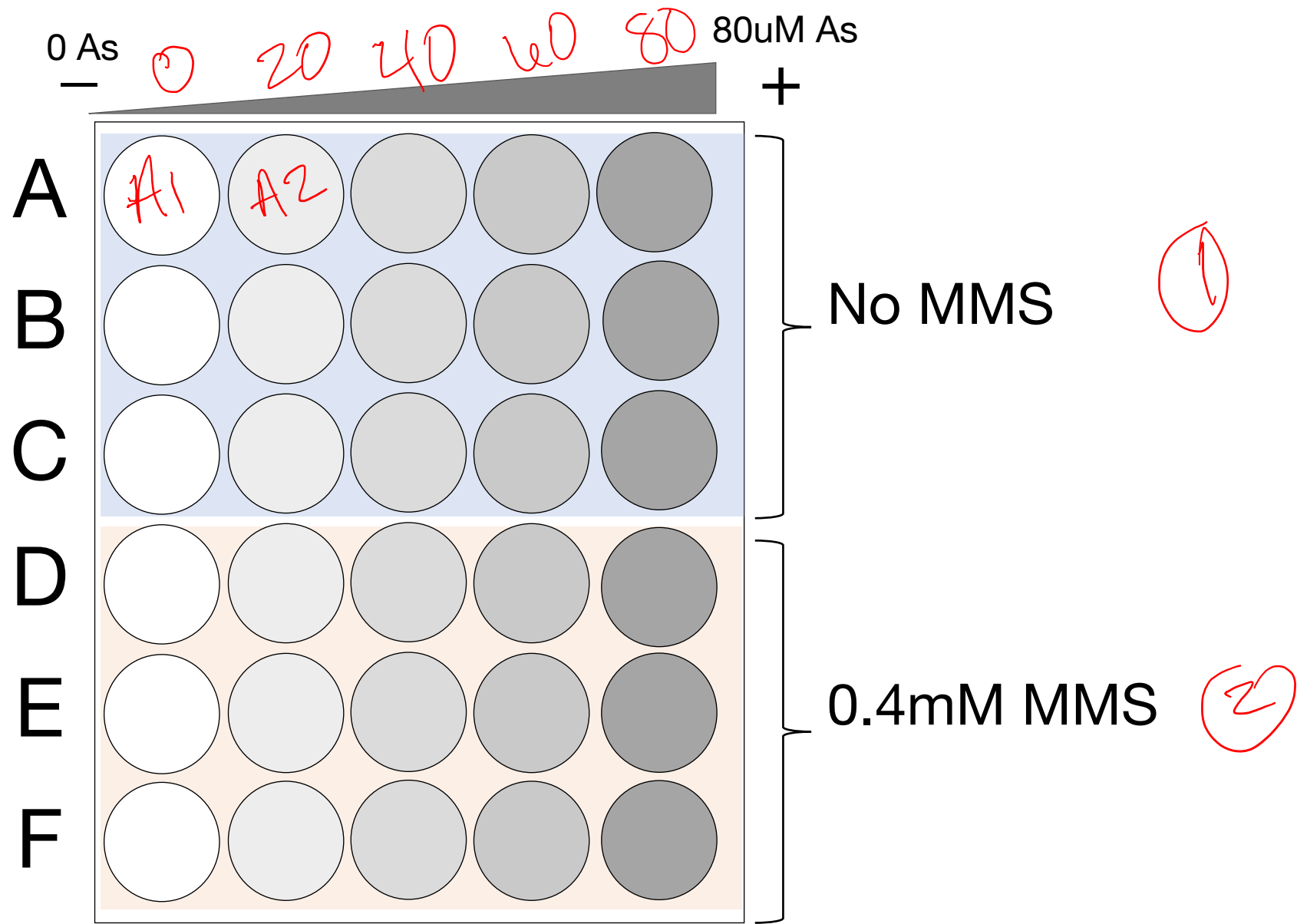


High Damage

- SSBs, DSBs, abasic sites, alkali labile sites
- forms a "Comet tail"

Genomic damage from direct strand breaks
and repair intermediates

Reminder: CometChip experimental setup



What's in the final Trevigen Excel file?

Region	#Found	#Counted	Moment_Mean	MArm_Mean	MI(inertia)_Mean	Fragment_Mean	%DNA_in_TailMean	Length_Mean
A1	107	107	0.31	1.93	2.09	1.82	11.04	5.19
A2	74	74	3.23	7.36	56.25	2.05	31.47	17.57
A3	92	92	6.36	11.68	133.94	2.8	46.53	29.73
A4	46	46	12.94	17.47	380.04	3.25	65.77	39.5
A5	93	93	10.67	16.76	284.2	3.18	58.56	38.56

Notable Columns

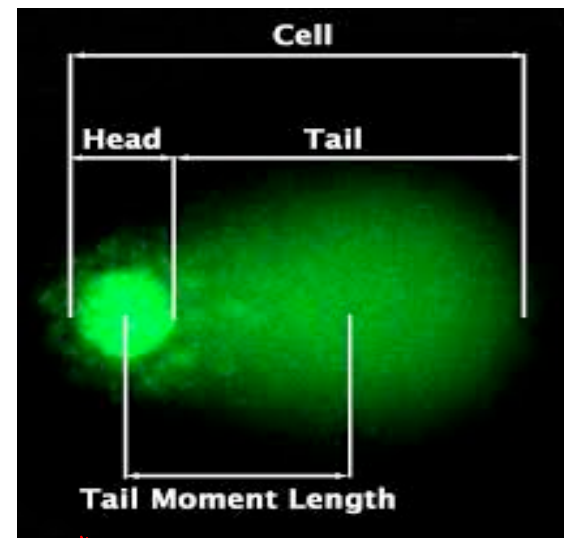
- Region: 96 well plate macrowell letter/number
- #Counted: how many comets were used for calculation in each macrowell
- %DNA in Head Mean= $\text{HeadFluorescence} / (\text{HeadFluorescence} + \text{TailFluorescence}) * 100$
- %DNA in Tail Mean= $\text{TailFluorescence} / (\text{HeadFluorescence} + \text{TailFluorescence}) * 100$
- Moment Mean= $(\% \text{TailDNA} / 100) * (\text{TailCenterOfMass} - \text{HeadCenterOfMass})$

Columns for analysis

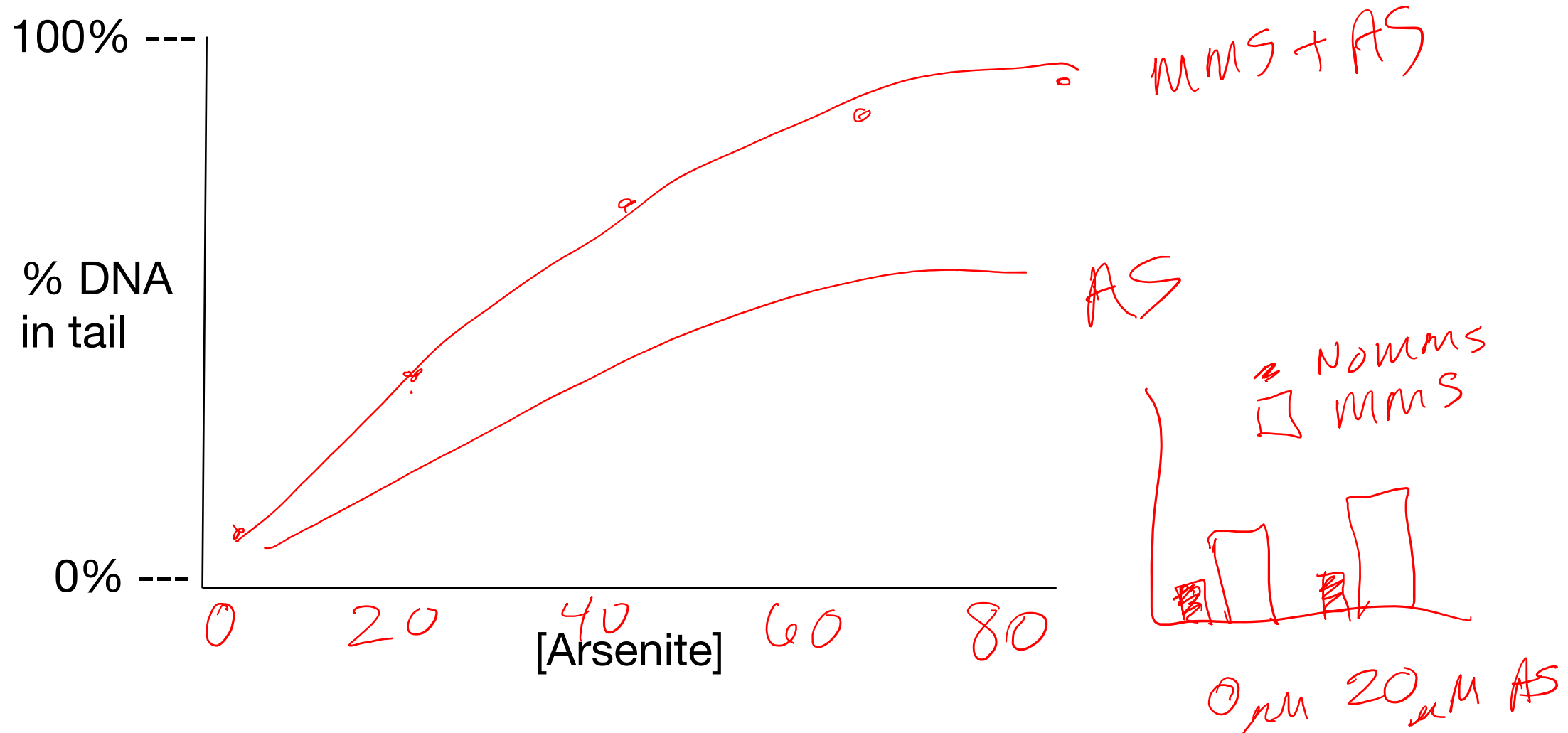
- % DNA in Tail Mean for each Region with cells

OTM

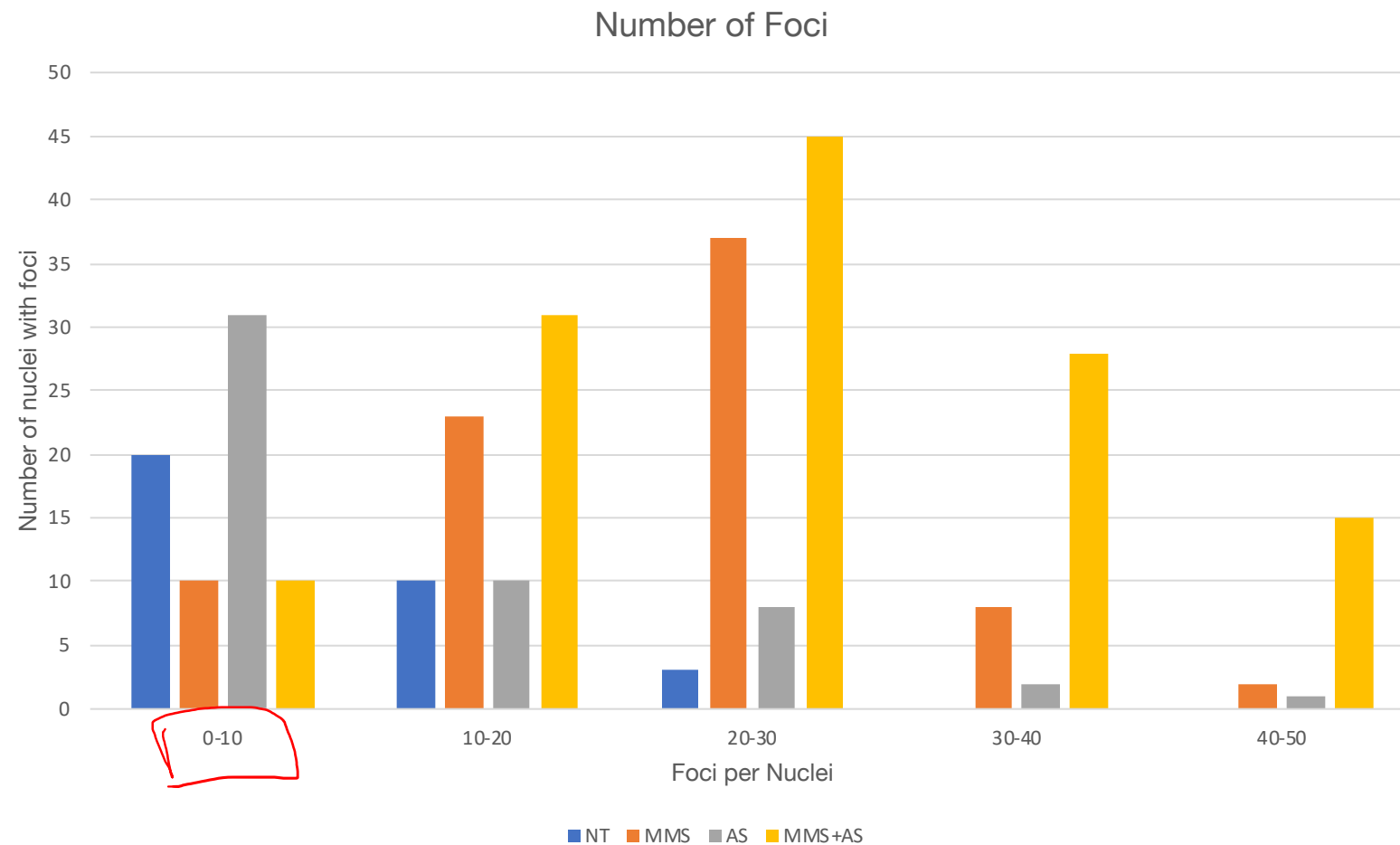
Olive Tail
Moment



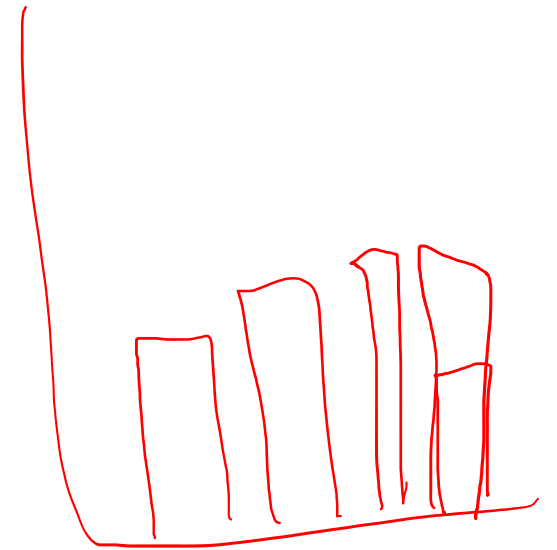
What result do we expect from the CometChip?



Binned histogram of foci counts (for H2AX portion of data summary)



Is the title acceptable?
Are the axis labels helpful?



Major assignments for Mod1

- Data summary draft
 - due by 10pm on Mon., October 14
 - revision due by 10pm on Sat., October 26

Summary content

1. Title
2. Abstract
3. Background & Motivation
4. Figures, Results & Interpretation
5. Implications & Future Work

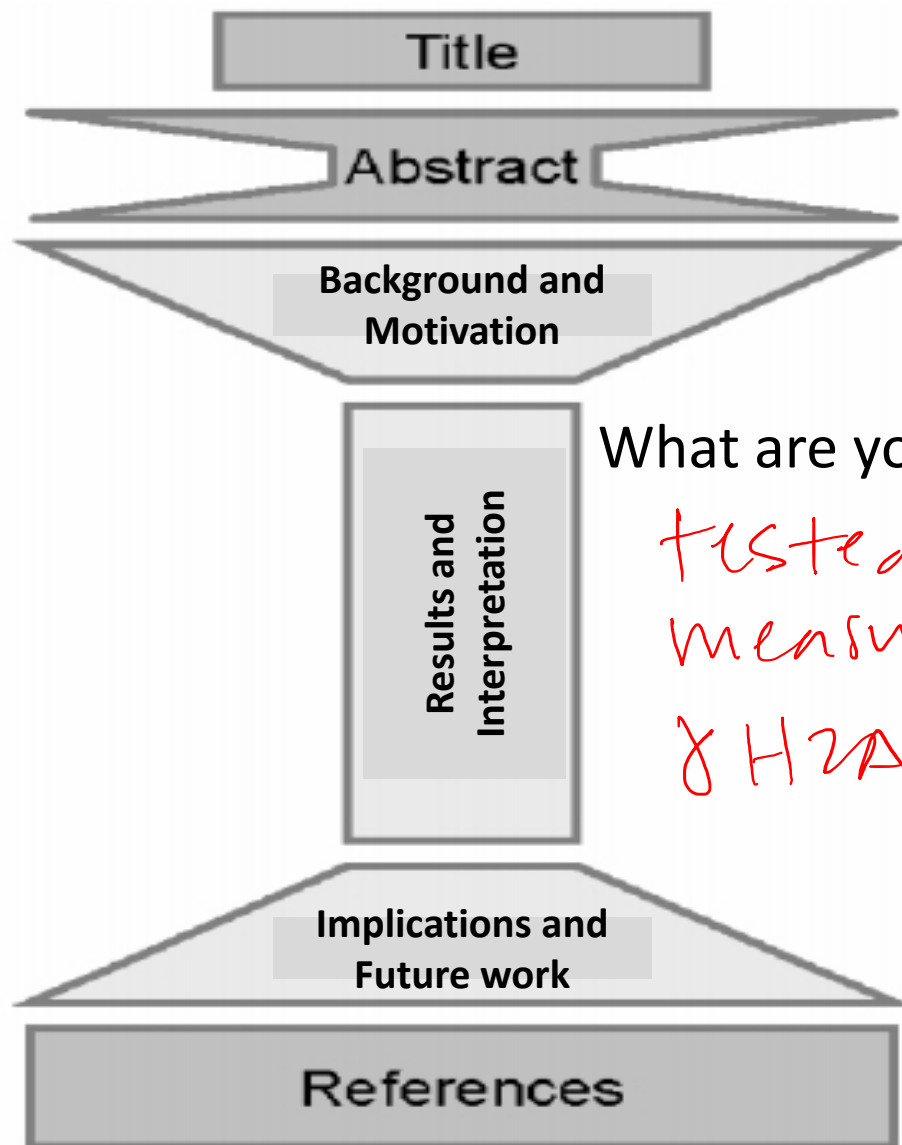
bullets!

> 1 page

up to 2 pgs.

up to 2 pgs

M1 Data summary architecture



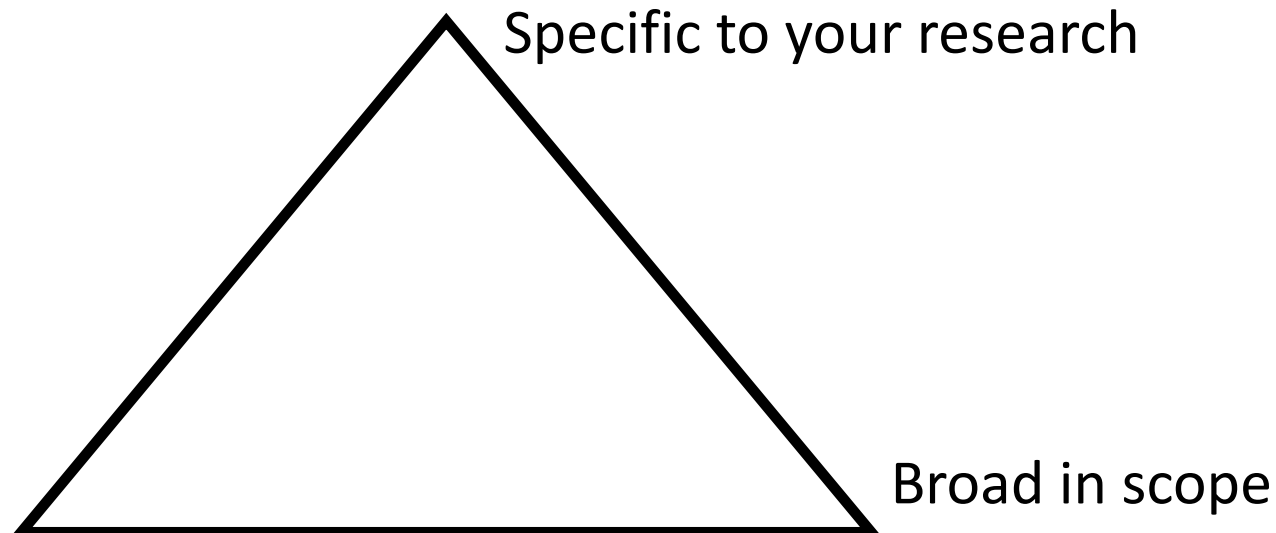
What are your data?

tested cell loading
measured DNA damage } comet
chip
γH2AX < # of foci (MATLAB)
Amount of fluor (Images)

HW M1D7: Implications & Future Works

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?



Your Implications & Future Works should:

1. tie back to your Background & Motivation!
2. synthesize the results such that the hypothesis / research question is answered!!

Notes on Implications & Future Works...

- Start with a very similar paragraph to the last paragraph in your Background/Motivation (restate major results and broad implications)
- Follow same order as in Figures/Results
 - Tie together the conclusions from your data
 - If necessary describe caveats of experiment and suggest improvements
 - Identify unknowns and speculate (within reason)
 - Don't make huge generalizations or overreach the results shown
- Propose future experiments, identify new questions that arise
 - Incremental next steps that can be tested / measured
- Come back to the big picture / impact statement topic introduced in background