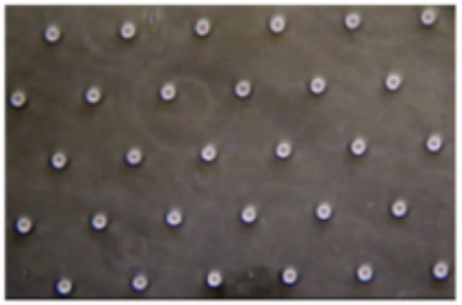
M1D3: Evaluate cell loading results

09/19/16

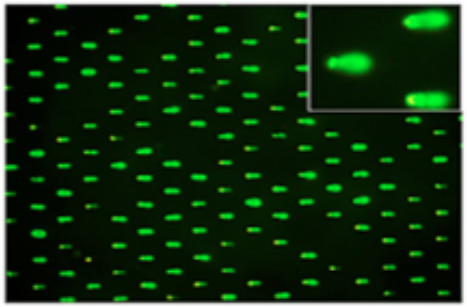
1. Communication workshop 56-614
2. Image CometChip on fluorescent scope
3. Prepare CometChip for next experiment
4. “Post” lab Discussion
   - Determine optimal cell loading for next experiment

Overview of
Module 1: Measuring Genomic Instability



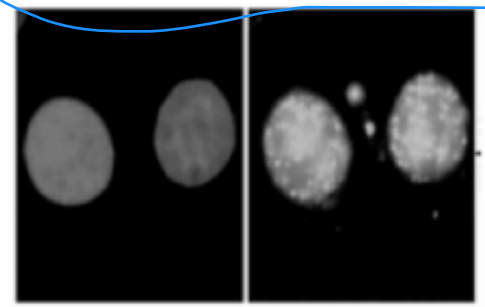
1. Optimize comet chip assay

- Test loading variables



2. Use comet chip assay to measure DNA repair

- Measure effects of MMS and H_2O_2 on BER

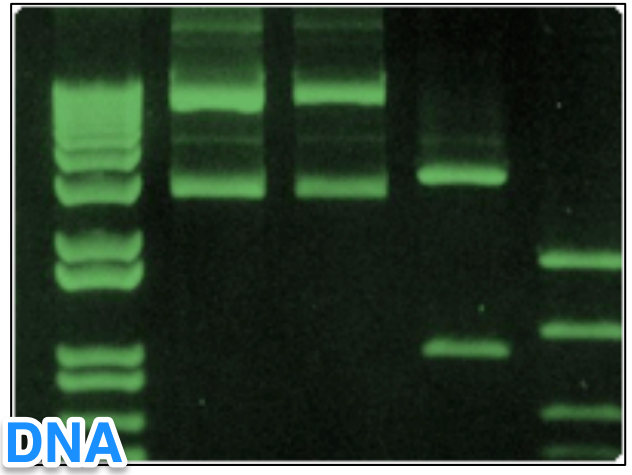


3. Use immuno-fluorescence assay to measure DNA repair

- Examine effect of MMS and H_2O_2 on DSB abundance

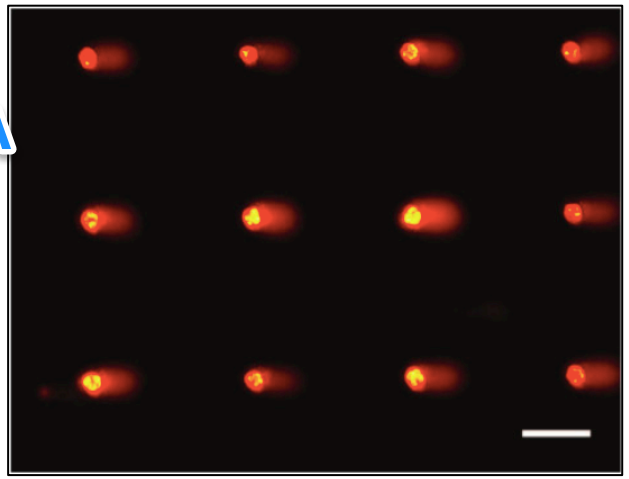
Lysis & staining CometChips

- Alkaline lysis solution **promotes breakdown of membrane/denatures proteins**
  - 2.5 M NaCl, 100 mM Na_2EDTA, 10 mM Tris
  - pH 10 **high pH (alkaline)**
  - Triton X-100 **detergent**



- Neutralize & dye
  - 0.4M Tris **optimize SYBRgold binding to DNA**
  - pH 7.5
  - SYBR Gold DNA stain **DNA intercalator**

increases 1000X signal after binds DNA



- What are impt. considerations for visualizing DNA?

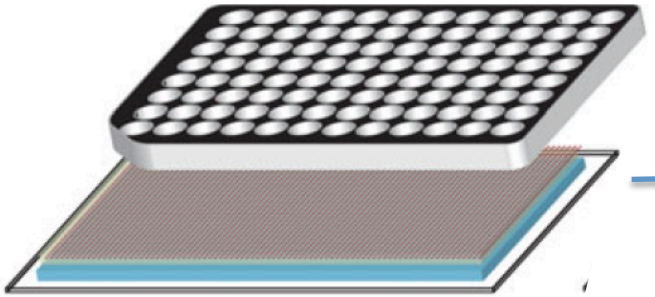
ssDNA
dsDNA
visualize (UV)
sensitivity

Team	Row B cell # loaded (% filled)	Row C cell # loaded (% filled)
Red	40,000 (88%) *	
Orange	75,000(57%)	
Yellow	60,000 (30%)	
Green	18,000 (83%)	37,200 (95%) *
Blue	25,000 (85%)	50,000 (85%)
Pink	36,000 (60%)	67,500 (77%)
Purple	60,000 (100%) *	
White	36,000 (63%)	

Load 45,000 cells/macrowell

Next time: test role of biochemical factors (mutagens) in genomic stability (DNA damage)

Load and capture cells in 2 CometChips, then treat with mutagens



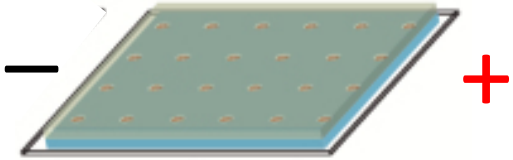
Lyse cells

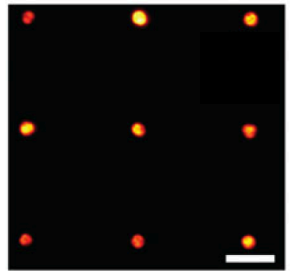
Chip 1: No enzyme treatment

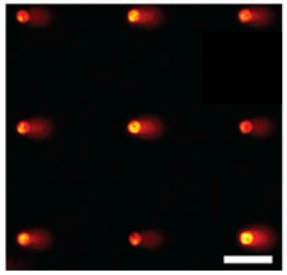
Chip 2: Treat with purified enzymes that cleave DNA at damaged bases

Alkaline unwinding

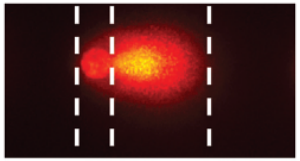
Electrophoresis







stain DNA and image via fluorescence microscopy



analysis via Matlab

Major assignments for Mod1

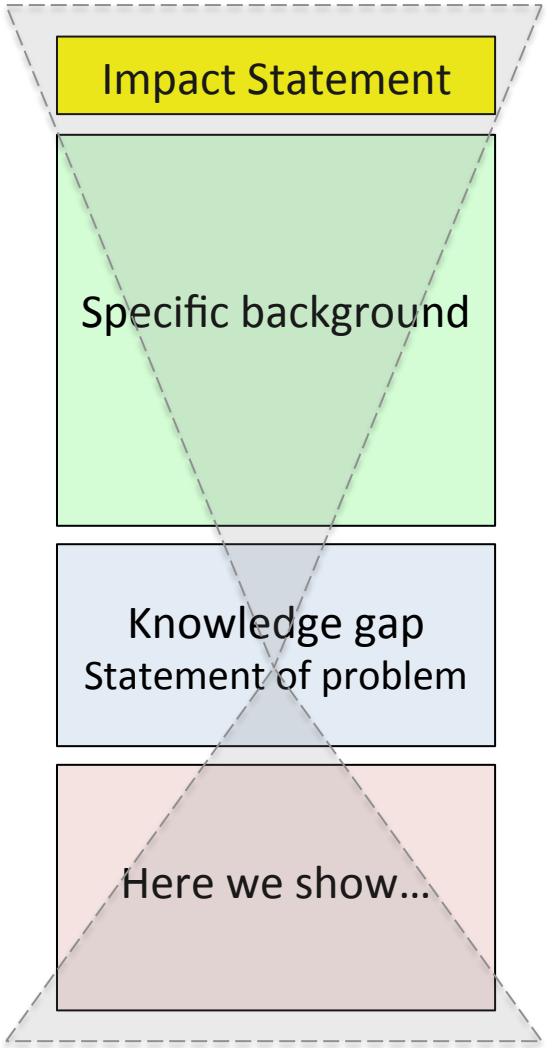
- Data summary draft
  - due by 10pm on Mon., October 9
  - revision due by 10pm on Sun., October 22

Summary content

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

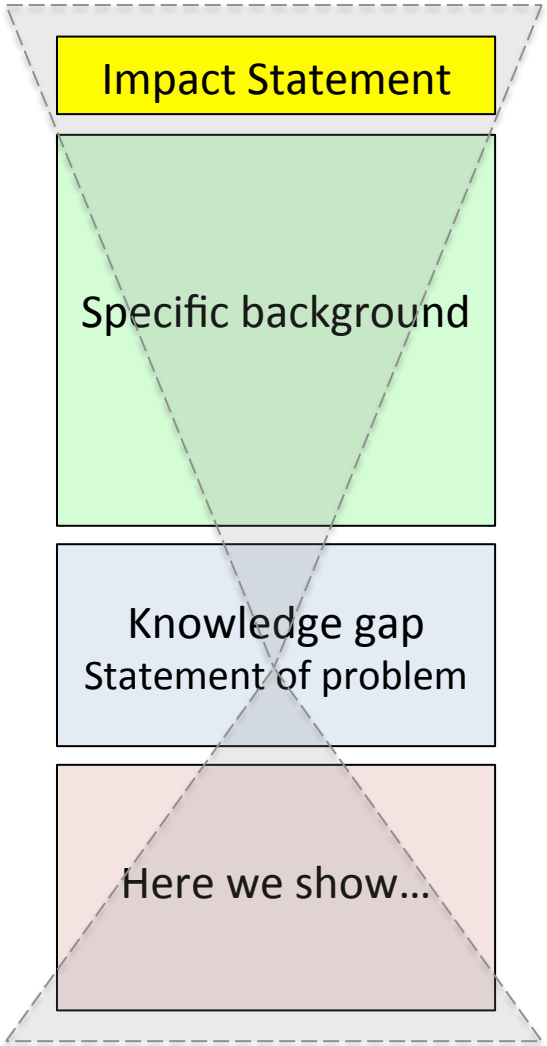
- Mini presentation due by 10pm on Sat., October 14
- Blog post for M1 due by 10pm on Mon., October 23

What goes into a background/motivation section?



- Your research is anchored in a general topic that your audience cares about or could be interested in.
  - focus on describing previous work in the field
- Specific background connects your project with the general background.
  - minimum essential information
  - references current work in the field
  - introduce specific technologies necessary for understanding the project
- The question you address is clearly articulated, connected to the background, and has appropriate scope for the project
  - give evidence of incompleteness of current understanding therefore motivating the investigation
  - state your hypothesis
- A preview of your findings and their implications
  - light on Methods

What goes into your introduction?



Choose one narrative