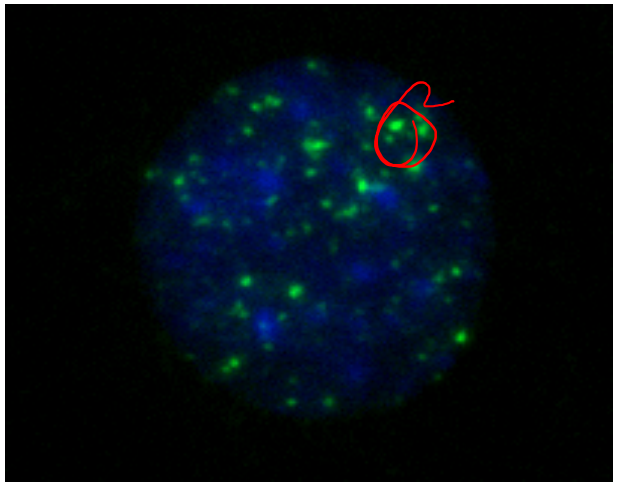
M1D2: Treat cells for gamma-H2AX and prepare CometChip

09/13/19

1. Prelab discussion
2. Treat CHO cells with MMS and As
3. Prepare CometChip during incubations

Overview of Module 1: Measuring Genomic Instability

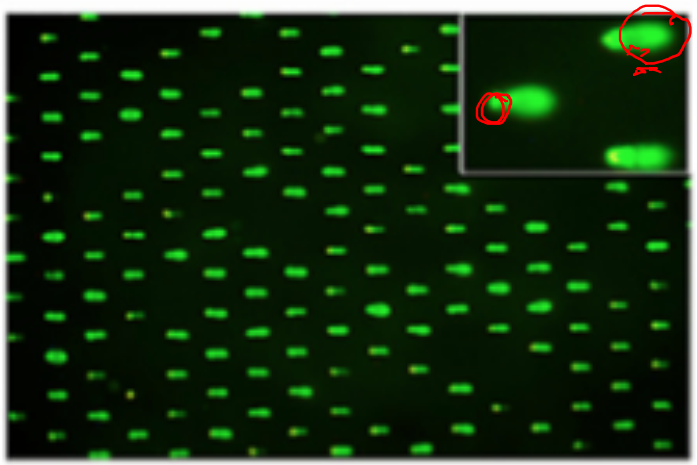
Aim: Evaluate effect of arsenic exposure on methylation-induced base excision repair (BER)



γH2AX assay:

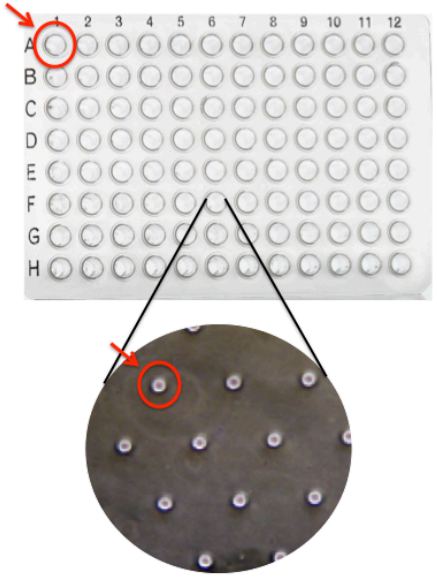
- Immunofluorescence
- Cellular response to DNA damage

DSB = foci



CometChip assay:

- single cell gel electrophoresis
- single strand breaks



SSB = tail

γH2AX experiment breakdown

<u>Last Class:</u>

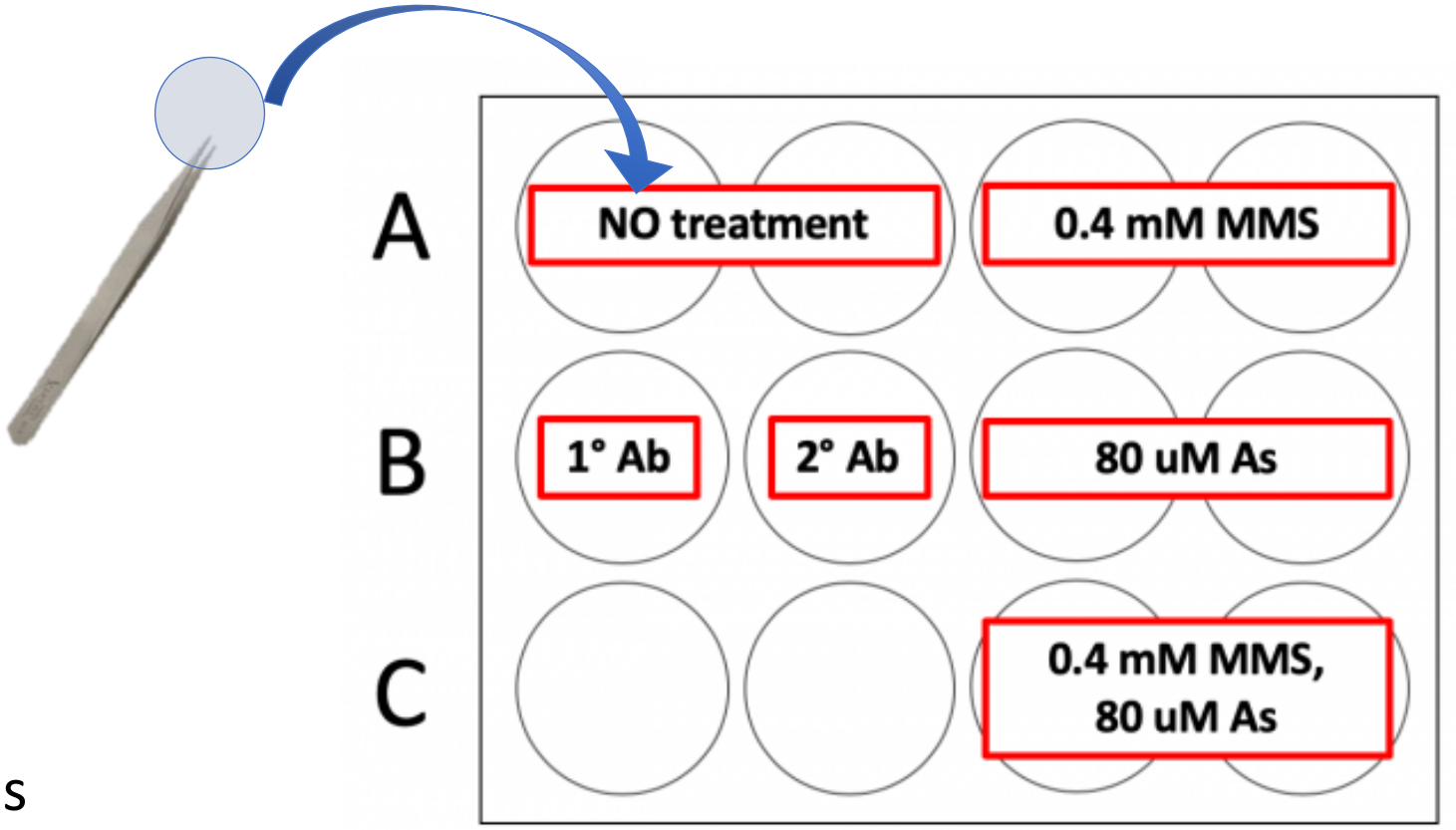
- Put coverslips in each well
- Added 25,000 CHO cells to grow on coverslips

<u>Today:</u>

- Expose cells to MMS and As

<u>Next Class:</u>

- We will remove the coverslips for immunofluorescence staining and imaging on microscope.



Research Question: Can the presence of Arsenite affect Base Excision Repair (BER) following MMS treatment?

Experimental Conditions

MMS + AS

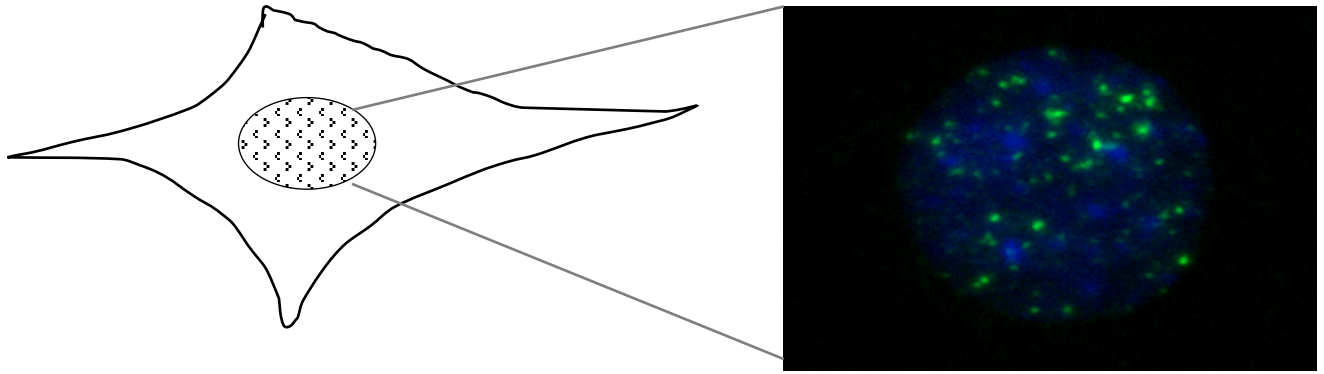
Control Conditions

No TX = Treatment

MMS - DNA damage

AS

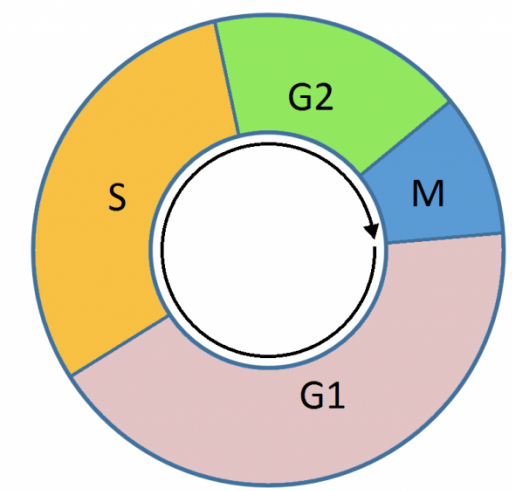
1° & 2° - reagent control



Blue: DNA (DAPI)
Green: γH2AX staining

foci

Additional Question: Which cells are in S phase at the time when Arsenite is added?

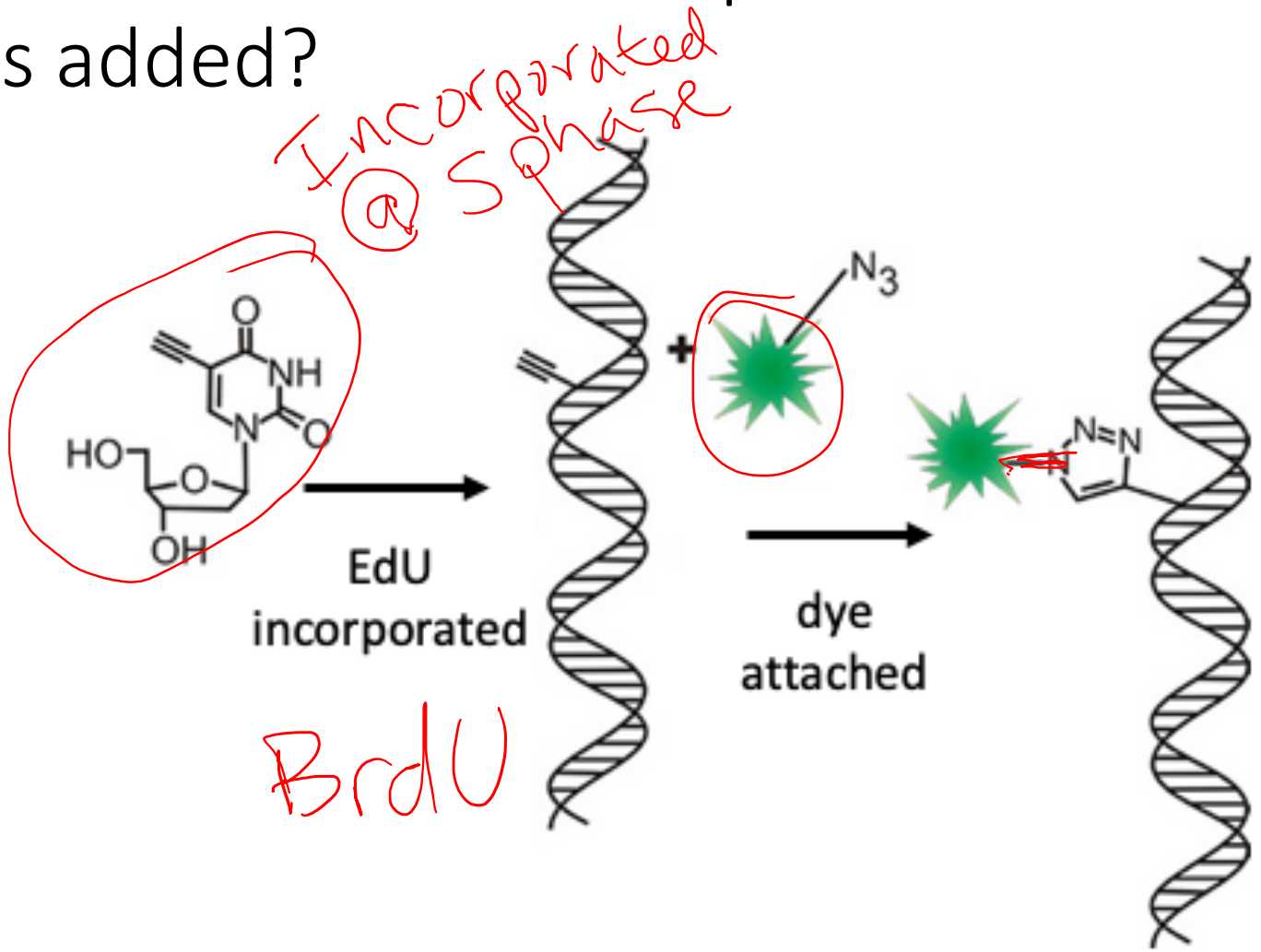


G1 - Growth

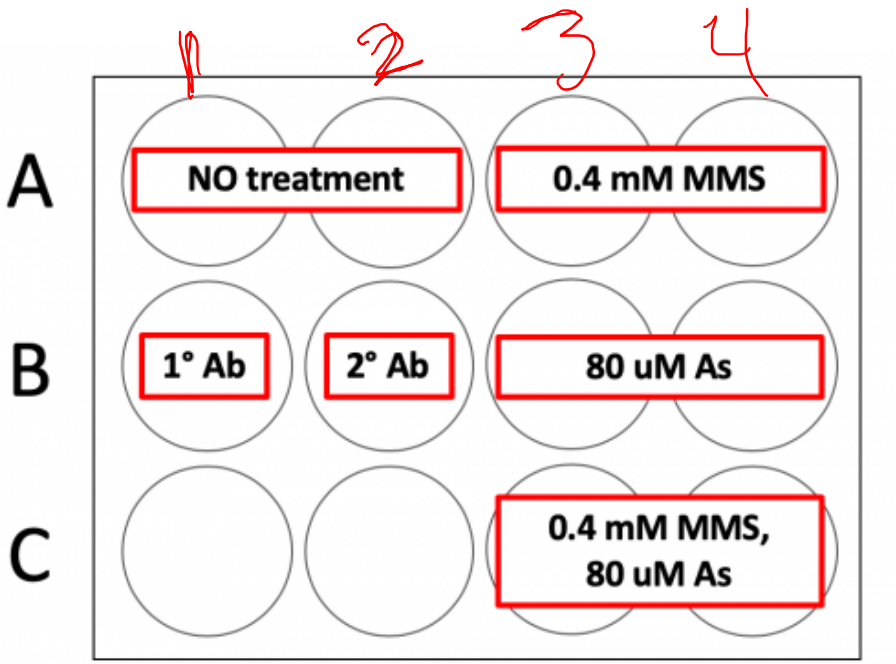
S - DNA synthesis

G2 - Growth and preparation for mitosis

M - Mitosis (cell division)



Timeline of CHO cell treatments:



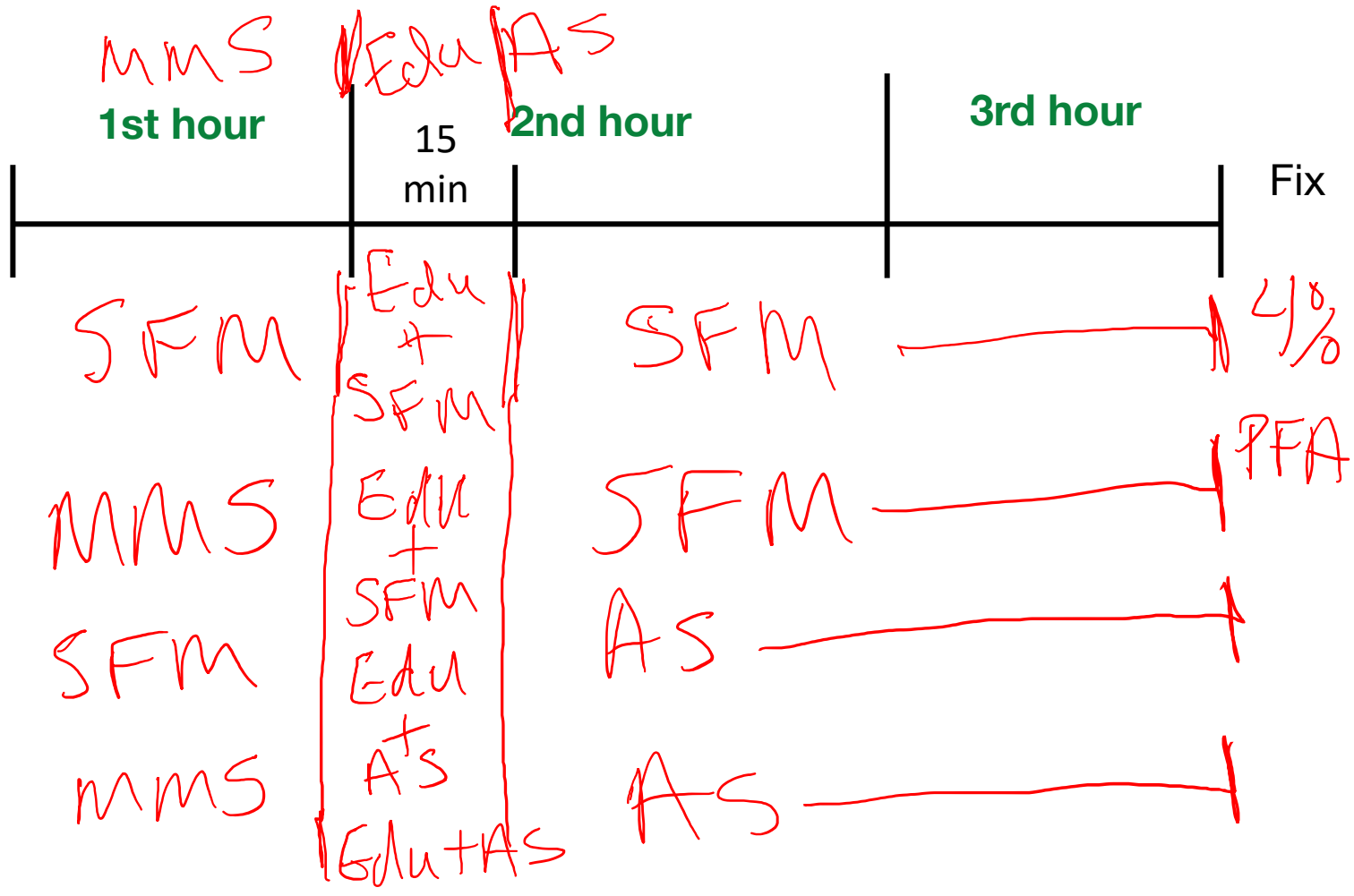
A1,2: No Treatment

A3,4: 0.4mM MMS

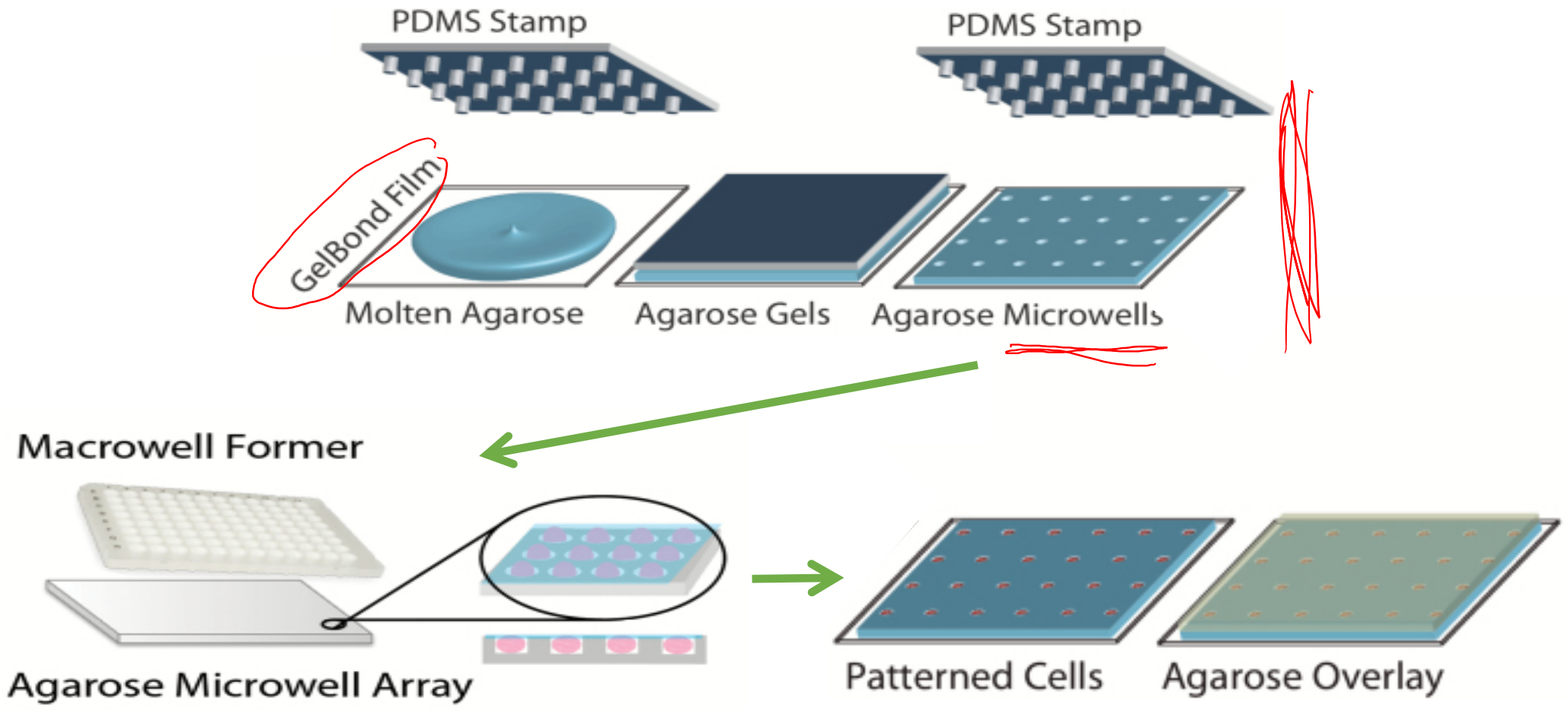
B3,4: 80uM As

C3,4: 0.4mM MMS + 80uM As

SFM = serum free media

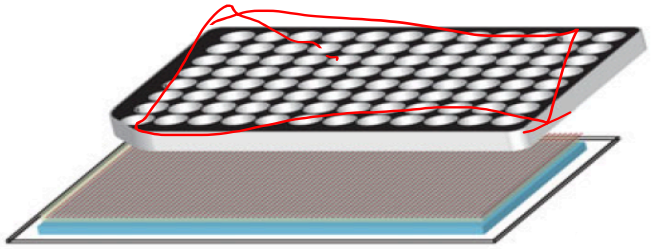


Overview of CometChip: Stamping microwells and loading cells



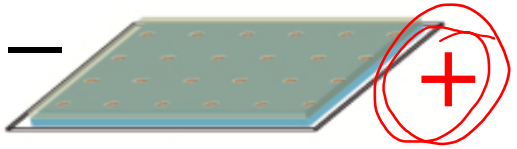
Overview of CometChip Assay: chemically treating cells and visualization

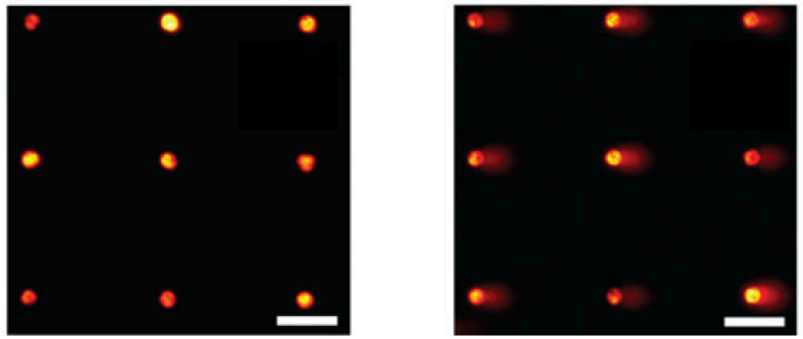
Treat captured cells in comet chip with MMS and As



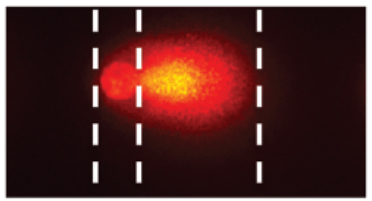
Lyse cells & unwind DNA (DNA still captured agarose in overlay)

Agarose Electrophoresis



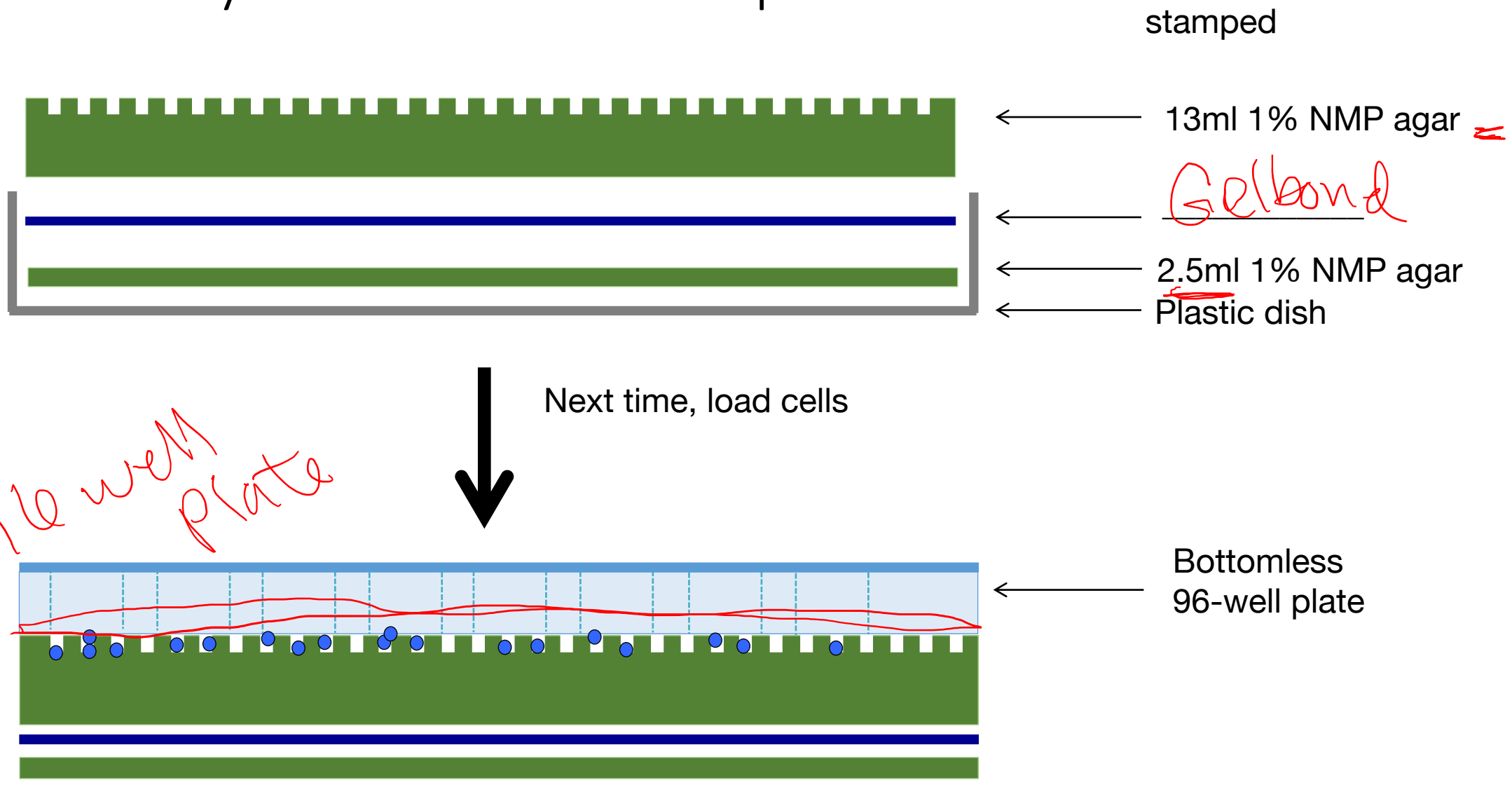


Stain DNA and image via fluorescence microscopy



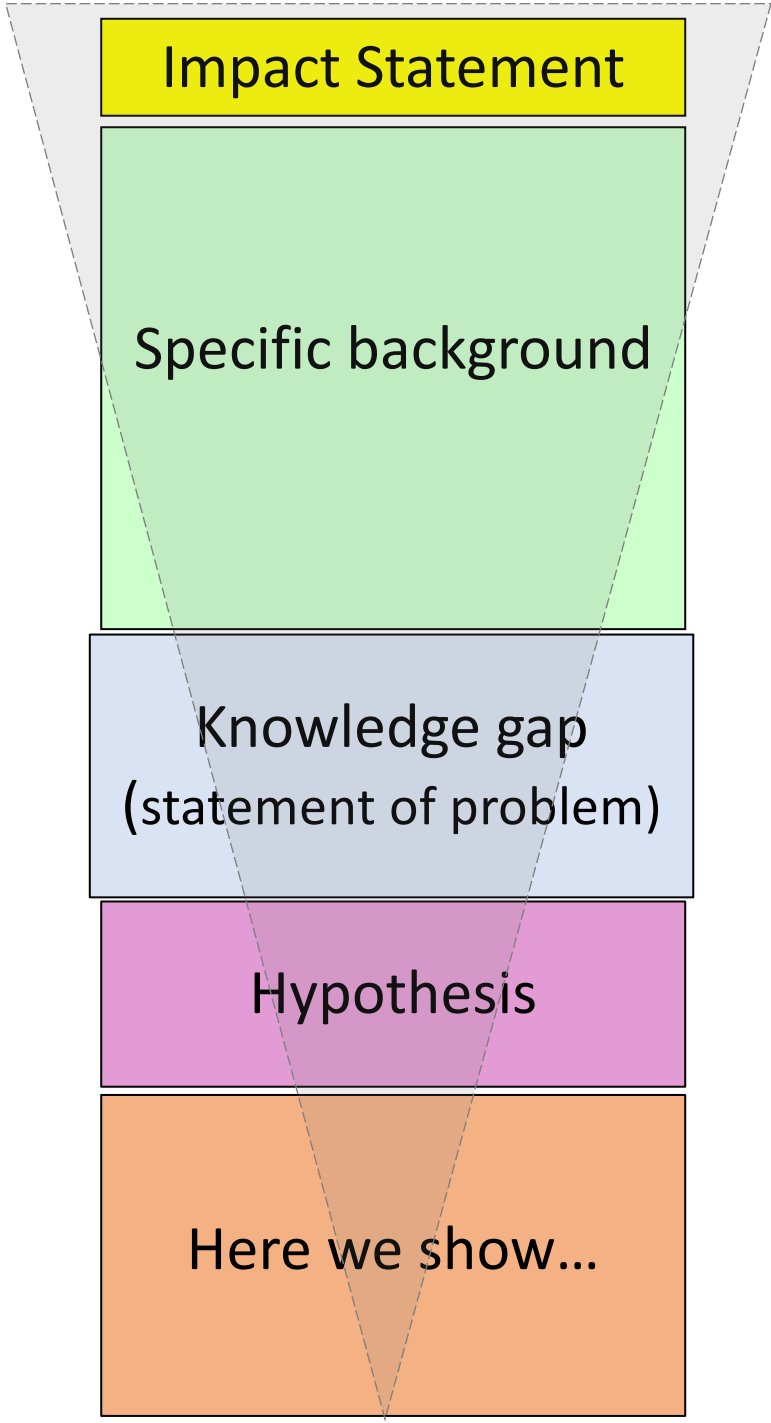
Analysis via Matlab

Today: Make a CometChip

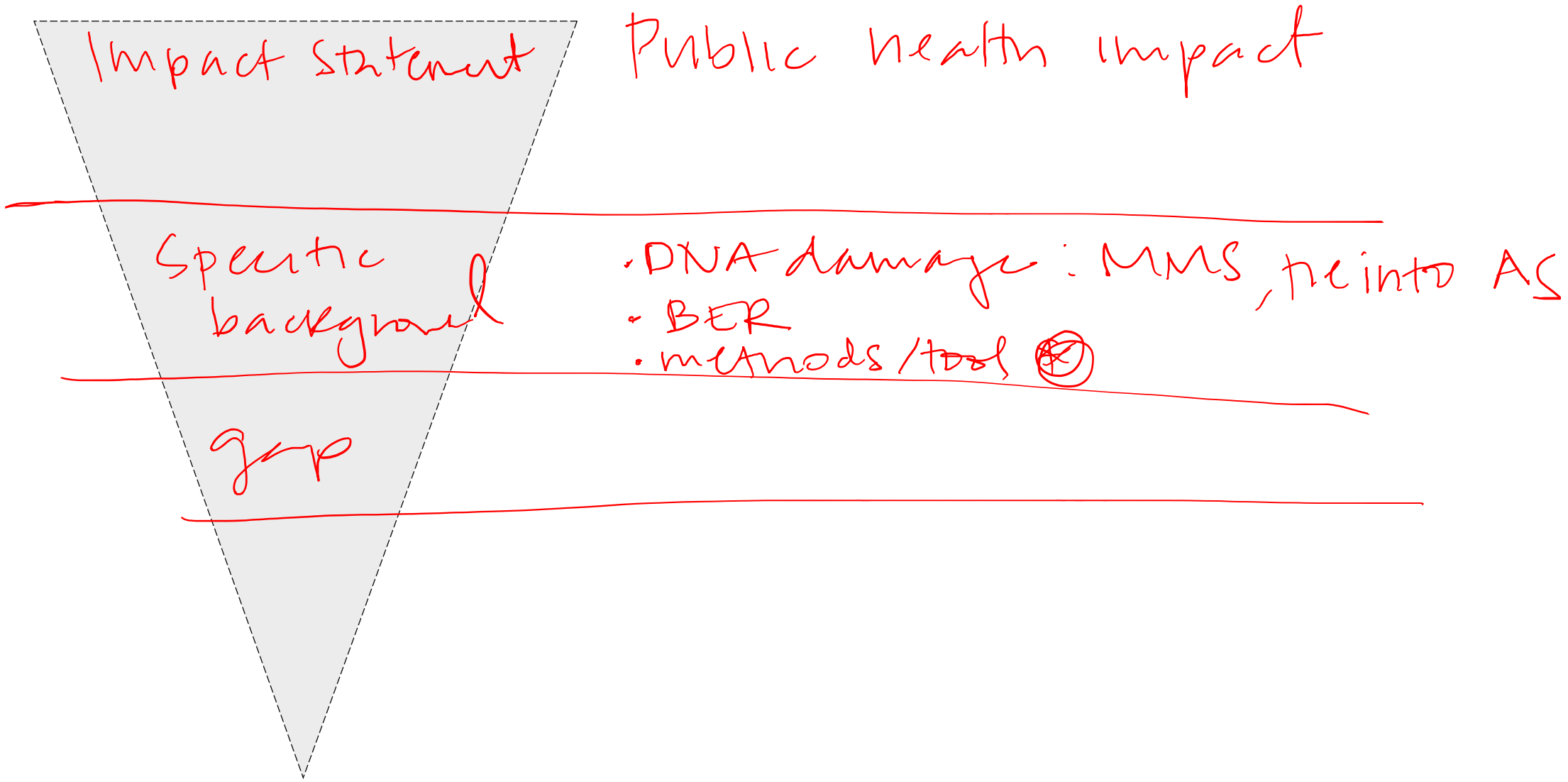


Notes on topic sentences:

- First sentence of each paragraph
- Should ‘funnel’ from big picture topic to your specific research project
  - Provide only the background needed to understand research / problem / goal
  - Clearly state what is not currently known
  - Address how you will fill knowledge gap
  - Provide preview of your results
- Include references!!



How will you introduce your story?



For next time:

M1D3HW

1. Write topic sentences (1st sentence) for each paragraph that would be in your Data Summary's Background and Motivation section
   - Remember to include references with summary & why you chose it

Reminder: Visit Comm Lab before M1D5