M1D3: Continue gamma-H2AX assay and optimize Cometchip

9/18/19

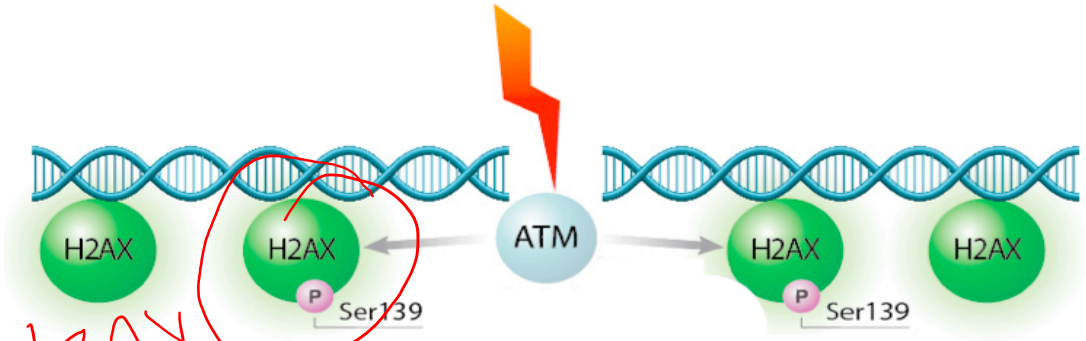
1. Prelab discussion
   A. Discuss methods homework
2. Begin immunofluorescence staining of coverslips
3. Optimize cell loading for cometchip

Turn in Homework at front bench…

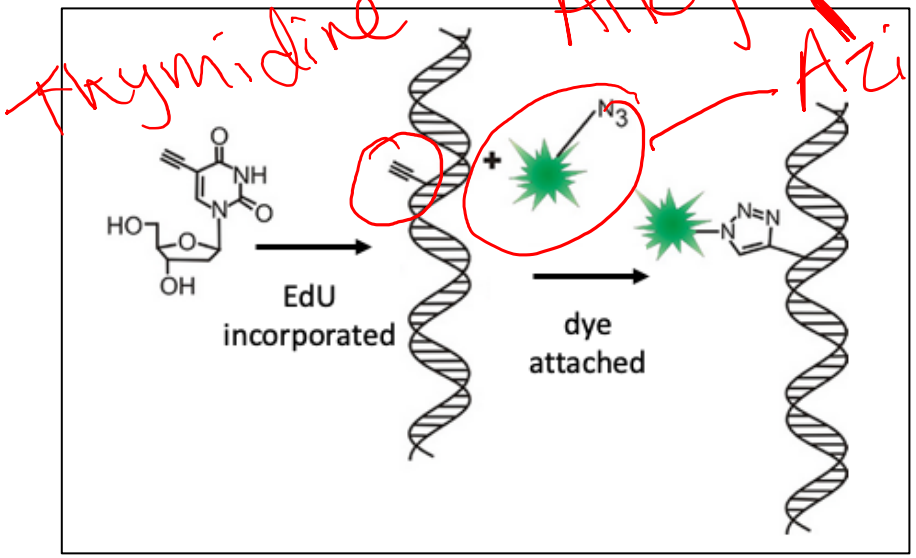
Last time…

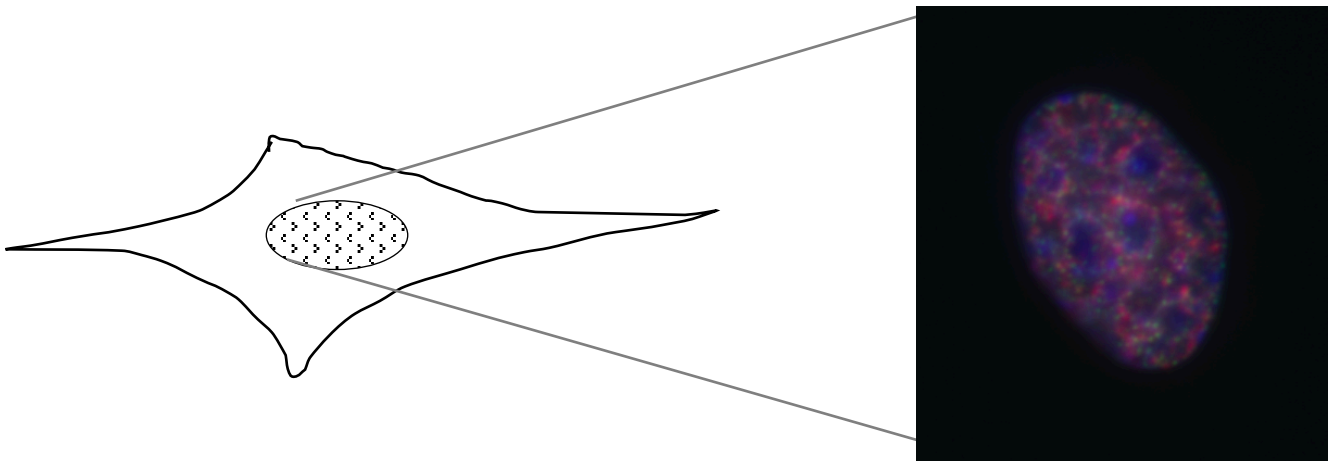
Chemical Added	Effect of Chemical
MMS	methylate DNA (initiate BER)
AS	inhibit Repair
Edu	S phase

Aims: Evaluate effect of arsenic exposure on methylation-induced base excision repair (BER) in S phase

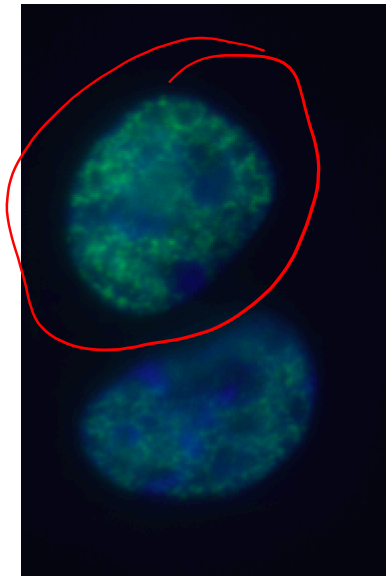


γ H2AX

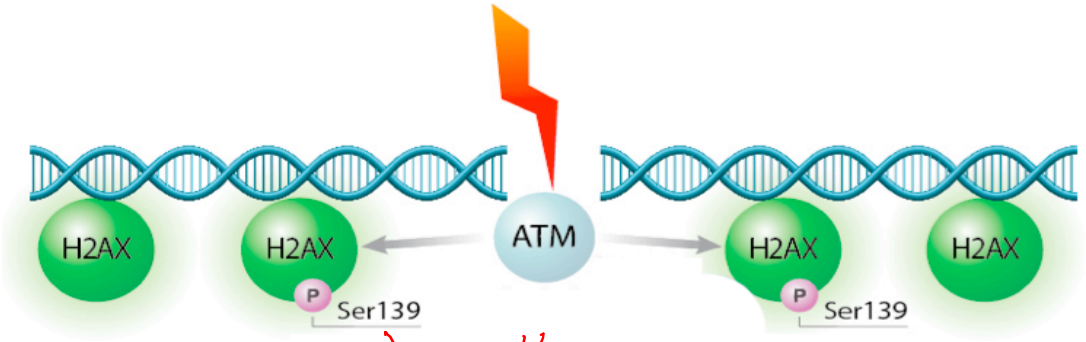




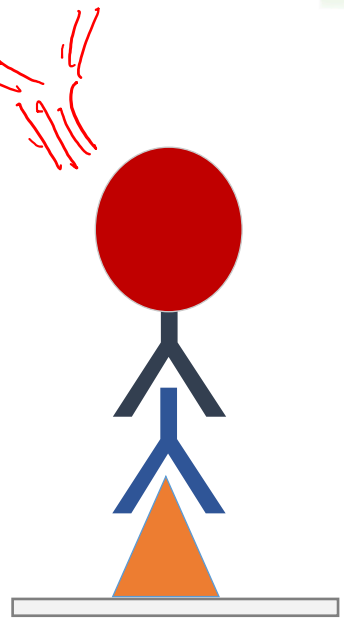
Blue: DNA DAPI
Green: EdU incorporation
Red: γH2AX staining



Using immunofluorescence (IF): γH2AX assay to detect double-strand DNA breaks



- Histone H2AX phosphorylated at Ser139 if DSB
- Antibodies against γH2AX (phosphorylated form)

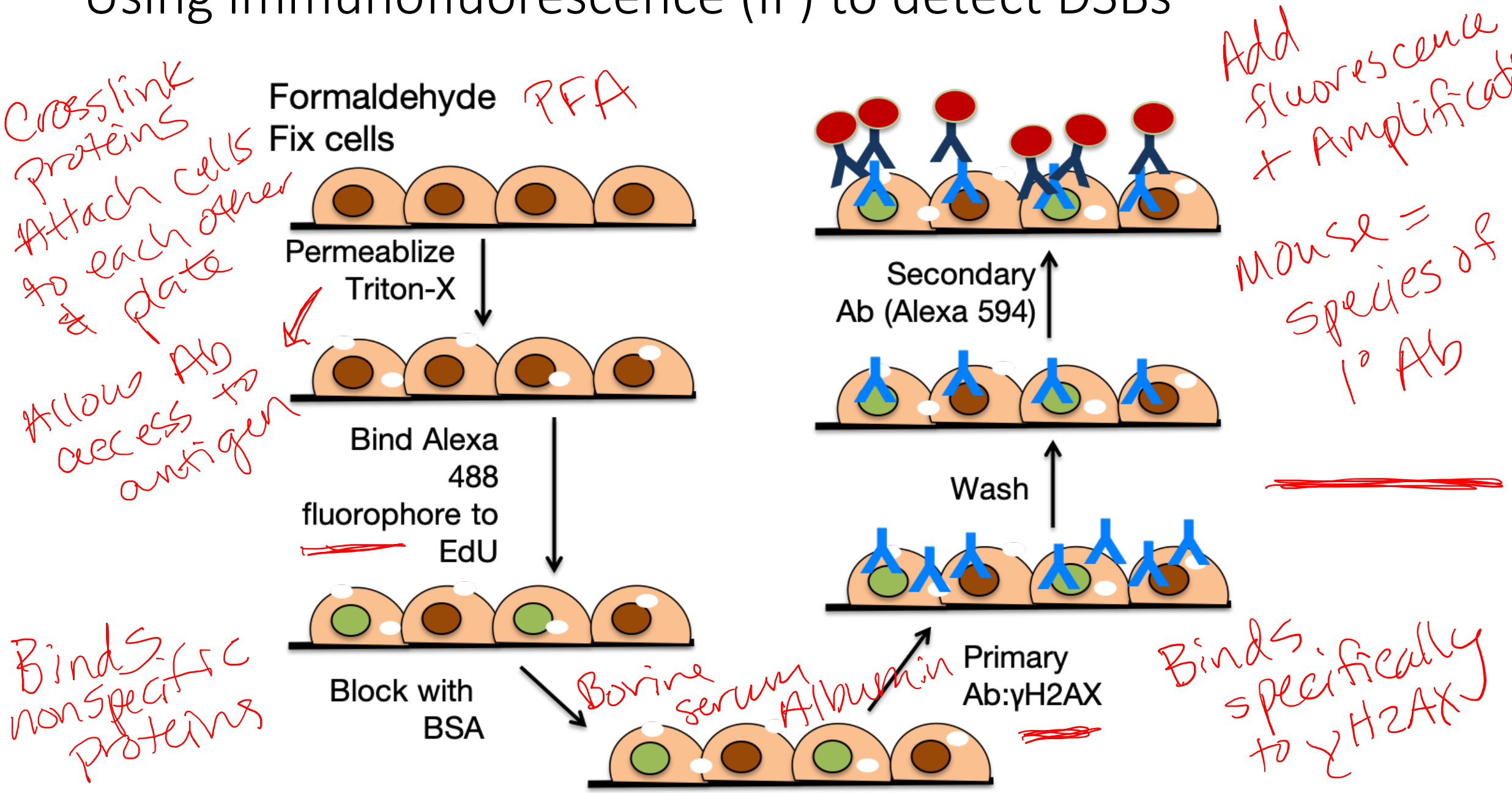


protein of interest	γH2AX
primary antibody	mouse anti-human anti-γH2AX
secondary antibody	goat anti-mouse
Fluorophore (conjugated to secondary antibody) exc./ em. wavelengths	594 / 617 nm

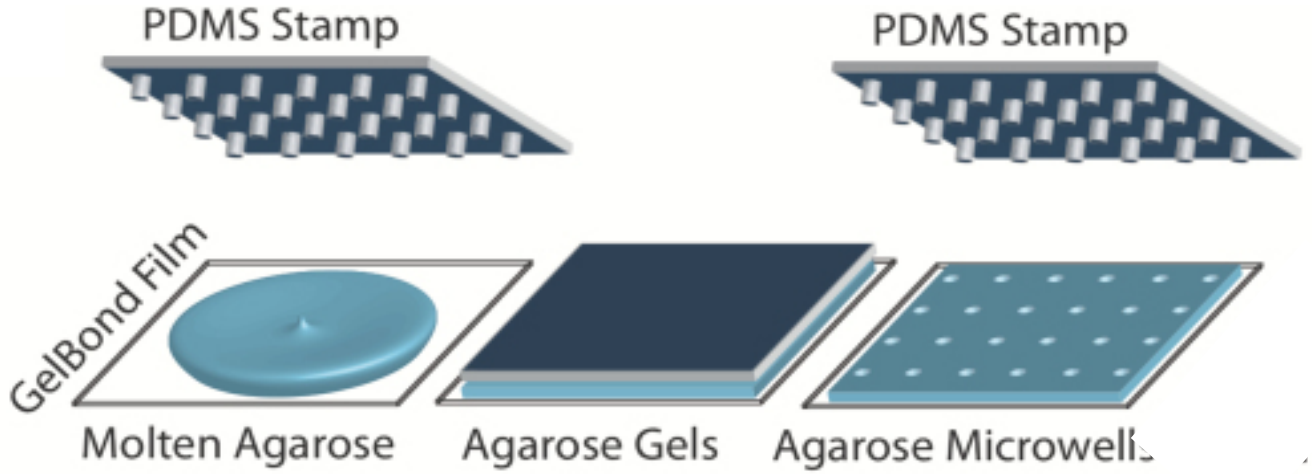
1°

2°

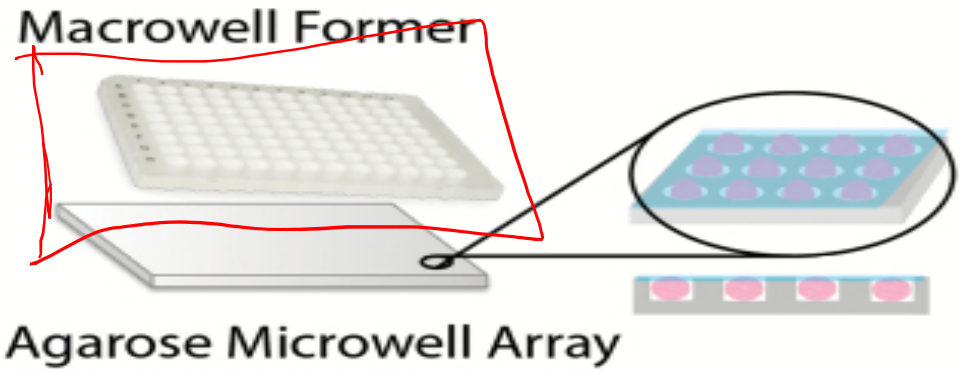
Using immunofluorescence (IF) to detect DSBs

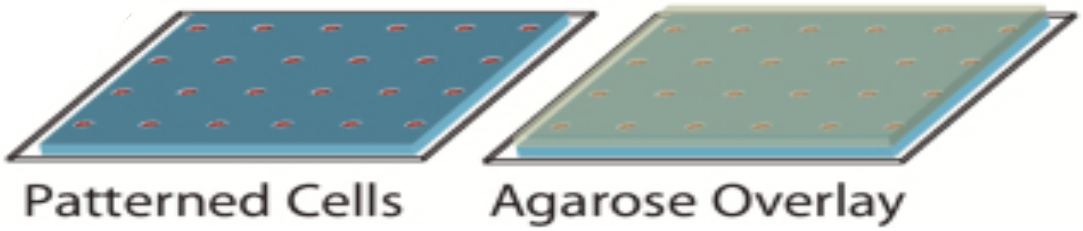


Optimize cometchip cell loading:

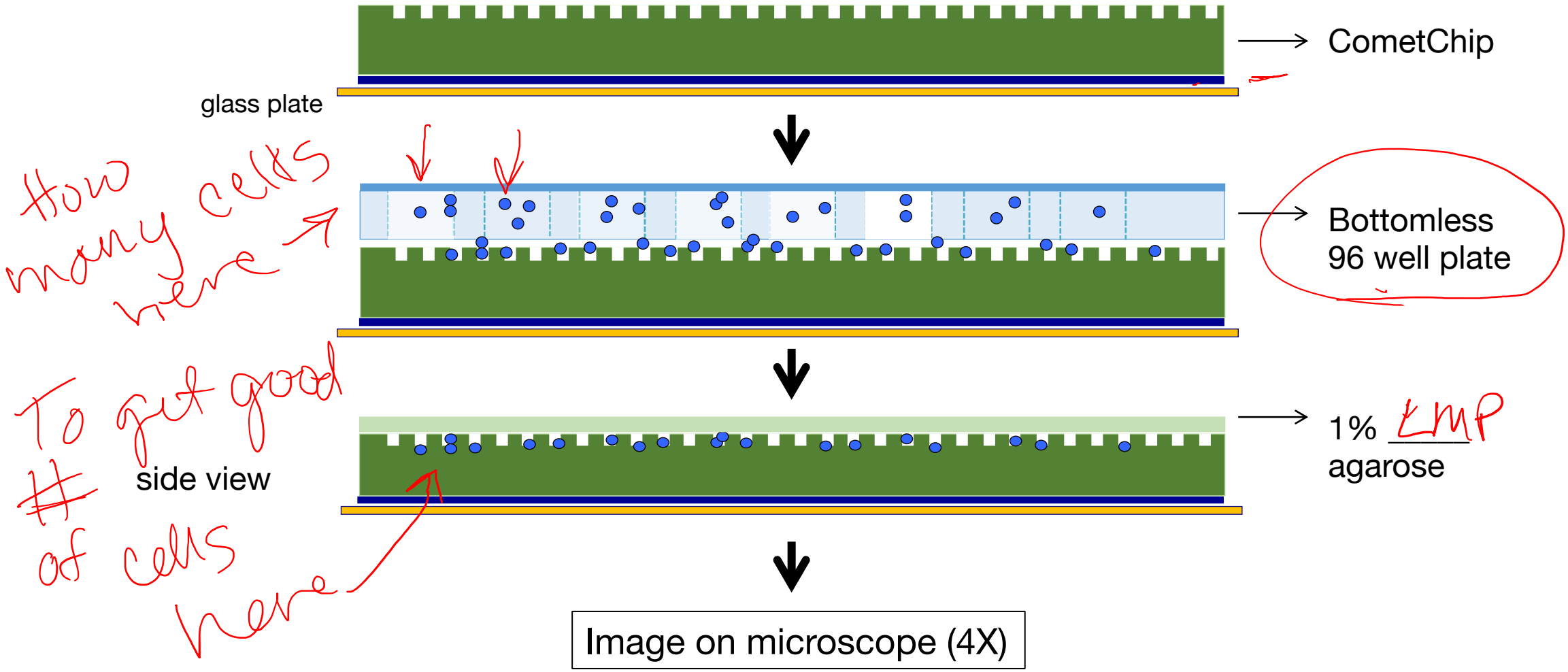


Last Time



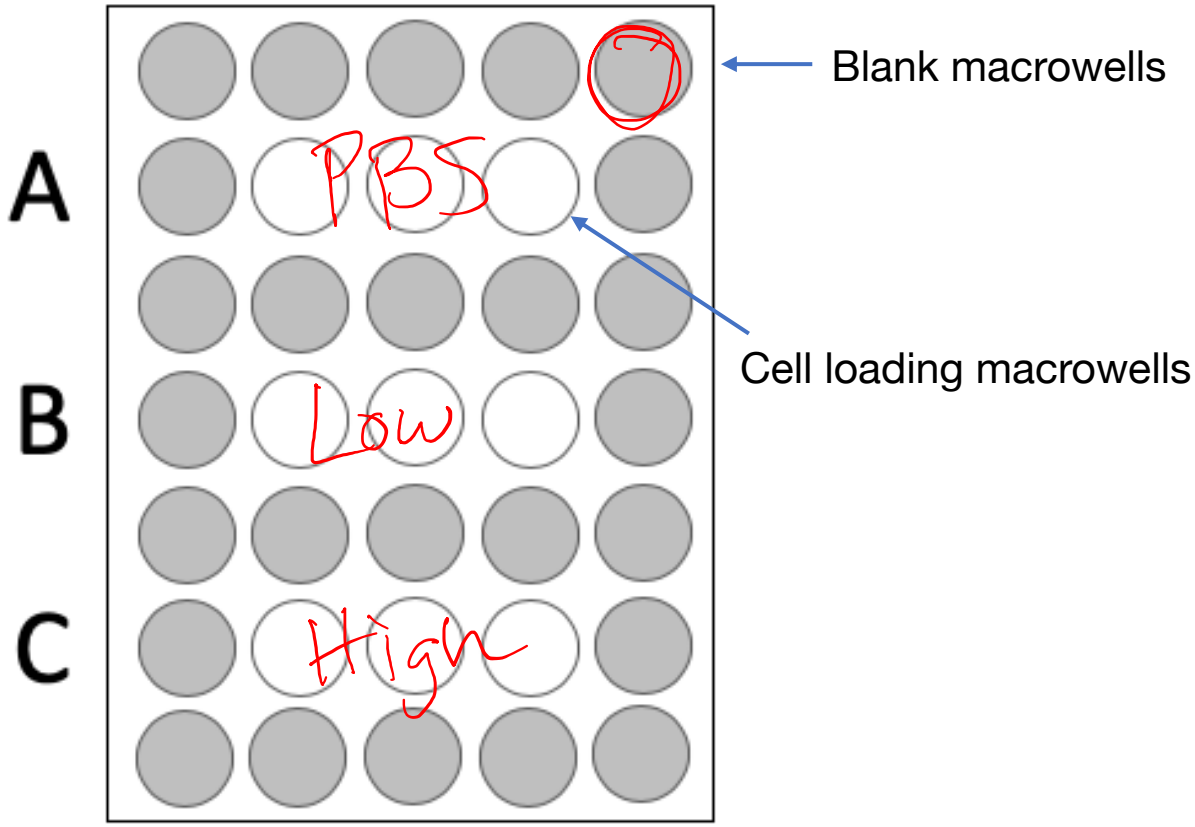


Today: Load cells onto the CometChip



Designing your cell loading experiment

A	Condition A will be 'no cells loaded' control
B	Condition B will be the lower cell number decided by your team
C	Condition C will be the higher cell number decided by your team



Incubate cells at 37°C for 15min

Designing the cell loading experiment

Experimental question: What is the minimum number of cells needed in each macrowell to obtain efficient loading?

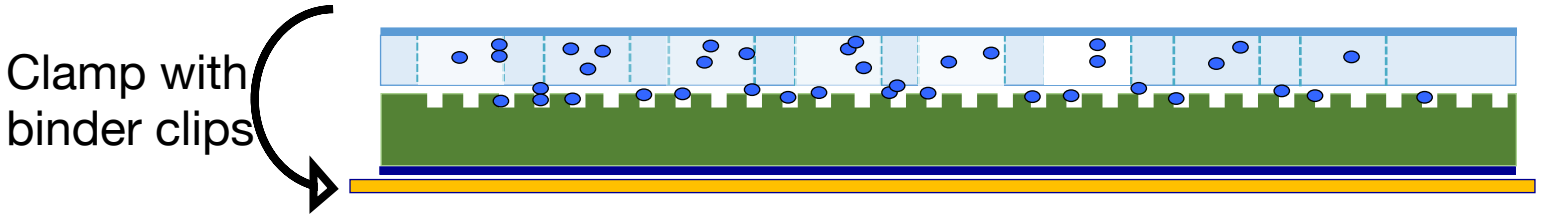
Considerations:
- How big microwell is # how many in a macrowell
- size of cells

Variable: Concentration of cells

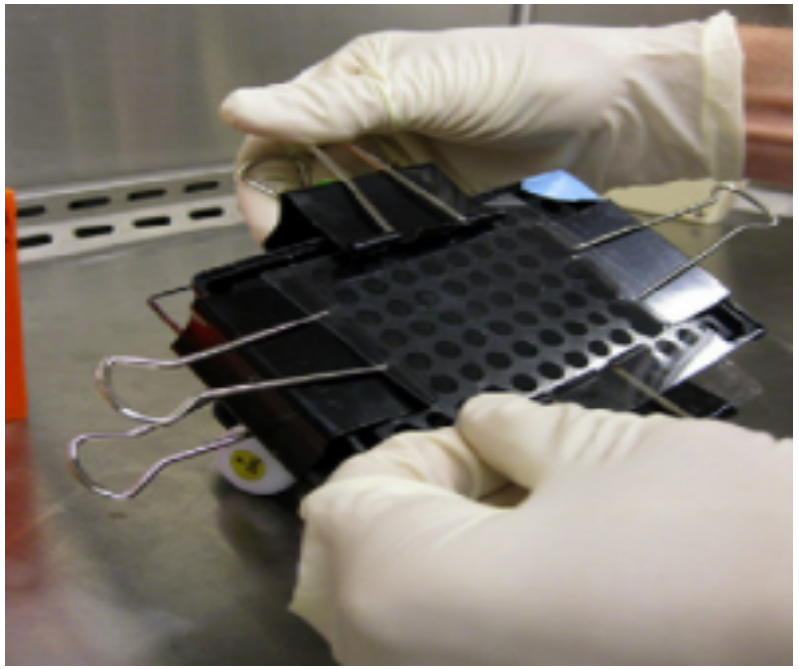
Control: PBS (negative control)

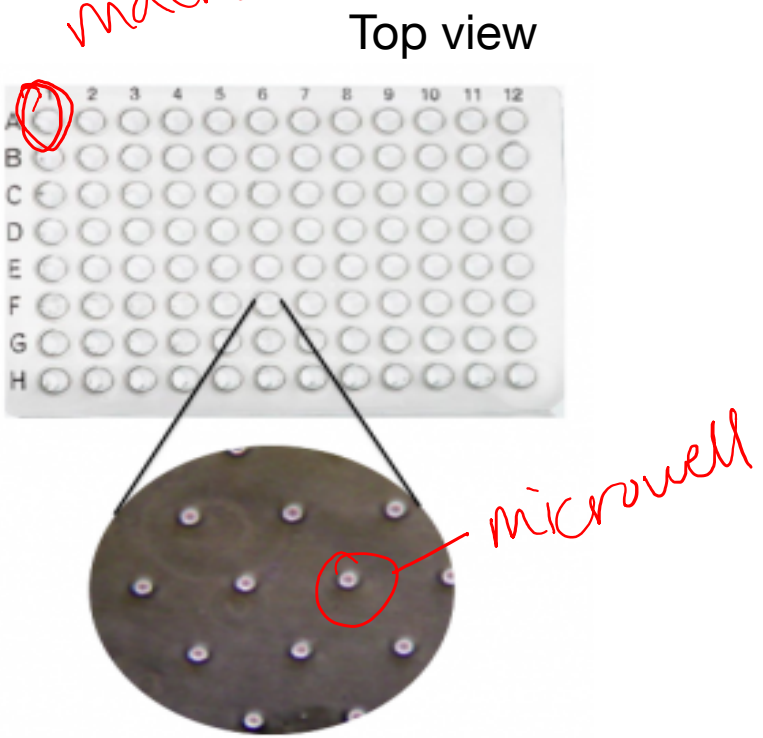
Repeatability: 3 wells – triplicate

What this looks like IRL



- Glass plate
- Bottomless 96- well plate
- 4 binder clips
- 37°C incubator (in main lab)

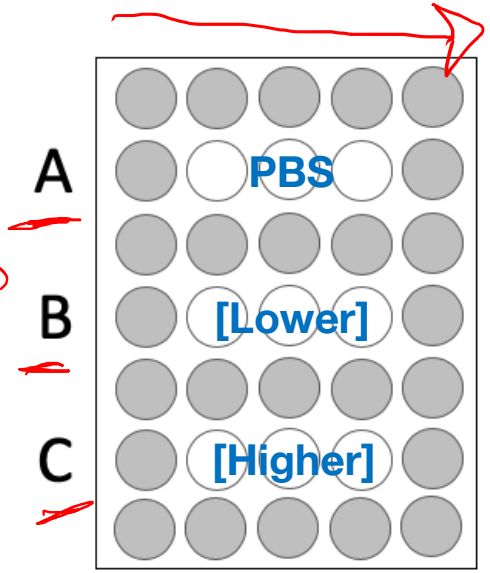




Major critical steps

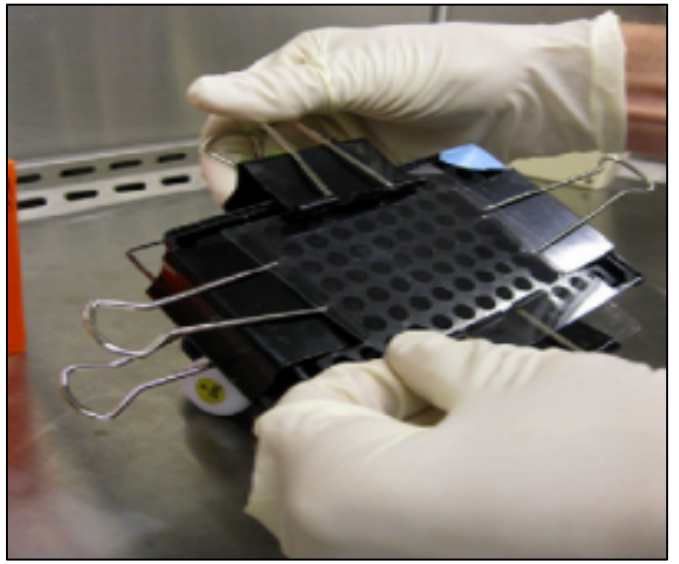
- Cell loading
  - Line up macrowells carefully so they are straight across gel in line with letters
  - Load cells and place in 37deg incubator
  - Leave for 15 minutes (why?)

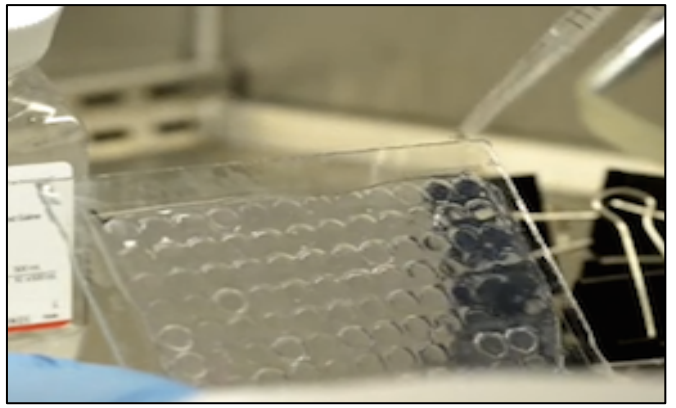
cells sink into micro wells

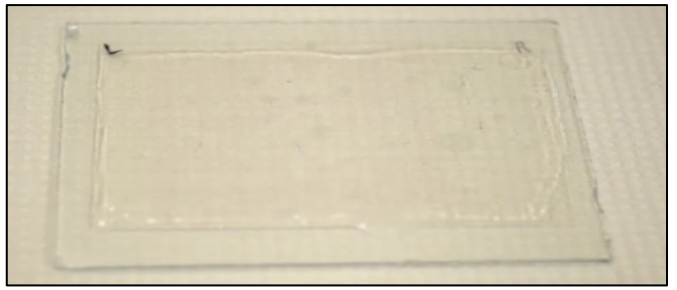


- Washing
  - Not too much!
  - Across the top of the glass plate
  - Wash from low to high concentration

- Cover the cells
  - 1ml 1% LMP agarose
  - 1% LMP agarose gels *quickly*
  - Leave glass plate under comet chip
  - Dispense it drop-by-drop with P1000
  - Leave it undisturbed for 3 min then move to 4°C for 3 min







Pro tips for writing a methods section

Include enough information to replicate the experiment

- List manufacturer's name and location (Company)
- Be **concise and clear** in your description

Use subsections with descriptive titles

- Put in logical order, rather than chronological order
- Begin with topic sentence to introduce purpose / goal of each experimental procedure

Use clear and concise full sentences

- NO tables or lists, all information should be provided in full sentences and paragraphs
- Write in passive voice and use past tense

Use the most flexible units

- Write concentrations (when known) rather than volumes

Eliminate 20.109 specific details

- Example "labeled Row A, Row B…"
- Do not include details about tubes and water!
- Assume reader has some biology experience
- Include parts of the protocol that the teaching faculty completed, but do not say "completed by teaching faculty."

How can you improve this example?

"Cells were grown in 12 mL of DMEM supplemented with FBS. We split cells with trypsin and counted them with a hemacytometer. Flasks were incubated in 37 C incubator."

How can you improve this example?

What cells? From where were the cells attained?

How much? What else was added to the media?

"Cells were grown in 12 mL of DMEM supplemented with FBS. We split

Volume here does not have context as based on the flask used. When might flask / plate size be helpful??

Define all abbreviations and include supplier / manufacturer.

Use passive voice and avoid jargon!

cells with trypsin and counted them with a hemocytometer. Flasks

Be specific about the purpose of each of the steps used…cells were harvested with (be sure to included concentration used) trypsin then counted using a hemocytometer. And what else was used? At what final concentration / percent?

Be specific about the subject of each action / step.

were incubated in 37 C incubator."

Specific location / equipment used is not important, just the temperature conditions. What other growth conditions were maintained?

Revised example…

Maintaining CHO cell line

Chinese hamster ovary (CHO) cells (gift of Engelward Laboratory, MIT) were grown in Dulbecco's… (DMEM) (Manufacturer) supplemented with 10% fetal bovine serum (FBS) (Manufacturer) and 100 U / mL of penicillin and streptomycin (Manufacturer). To harvest, cells were washed with phosphate buffered saline (PBS) (Manufacturer) then 0.25% trypsin (Manufacturer) was used. Cells were counted using 10% (v/v) trypan blue and a hemocytometer. Cultures were maintained at 37 C, 5% CO_2, and 95% relative humidity.

In lab today:

1. Transfer coverslips to staining chambers and start EdU and antibody staining
2. Determine loading conditions and load CHO cells onto CometChip
3. Take images of CHO cells loading before you leave

HW due M1D4 (group)

1. Write methods section for protocols on M1D1 through M1D3
   - Consider how to divide the work amongst all of you
   - Follow guidelines discussed today
2. (Individual) Visit Comm Lab before M1D5.