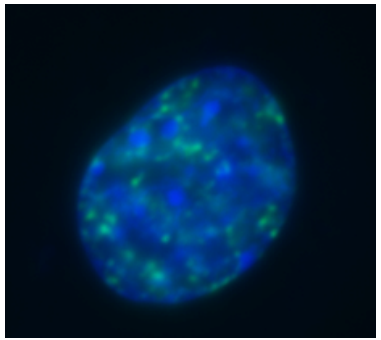
M1D3: Gamma-H2AX assay antibody staining and optimize CometChip loading

09/17/19

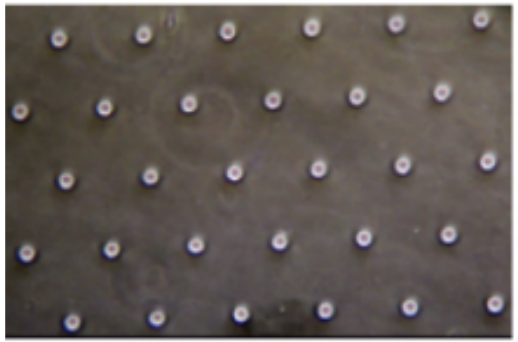
1. Pre-lab Discussion
2. Start H2AX assay EdU and antibody staining
3. Load CometChip with CHO cells
4. Image CHO cell loading

Overview of Module 1: Measuring Genomic Instability

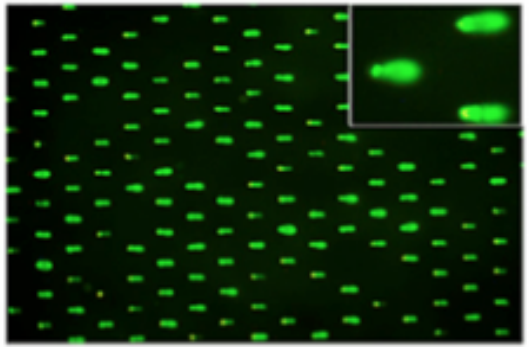
Aim: Evaluate effect of Arsenic exposure on methylation induced base excision repair (BER)



γH2AX assay

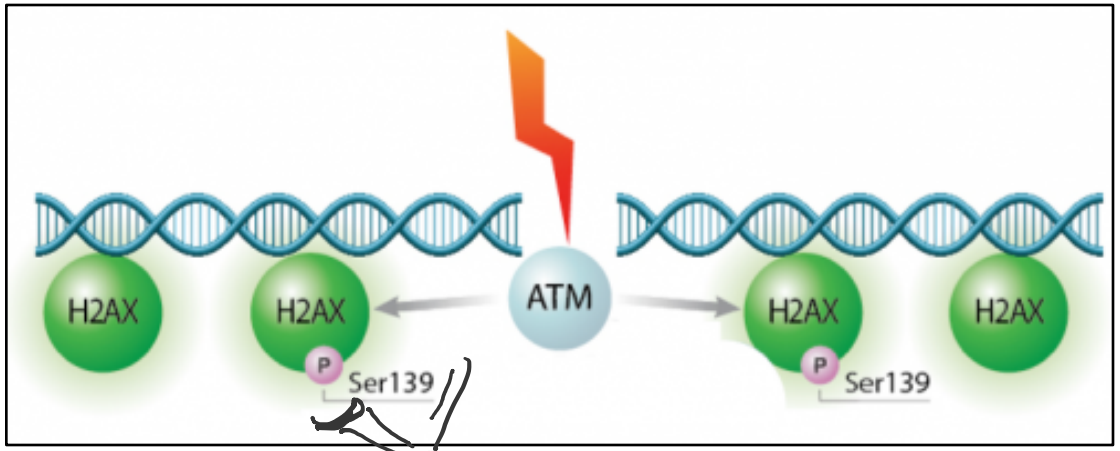


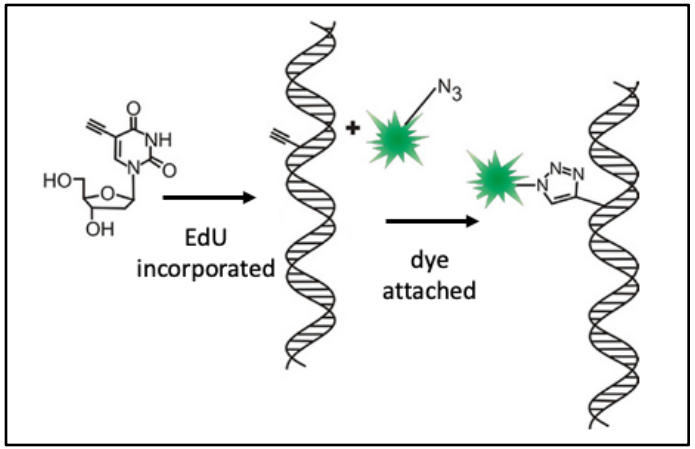
Optimize CometChip loading

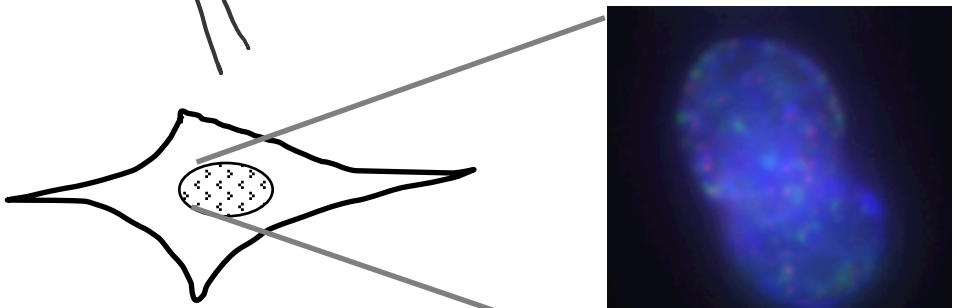


CometChip assay

Measuring dsDNA damage and determining if cells are in S phase







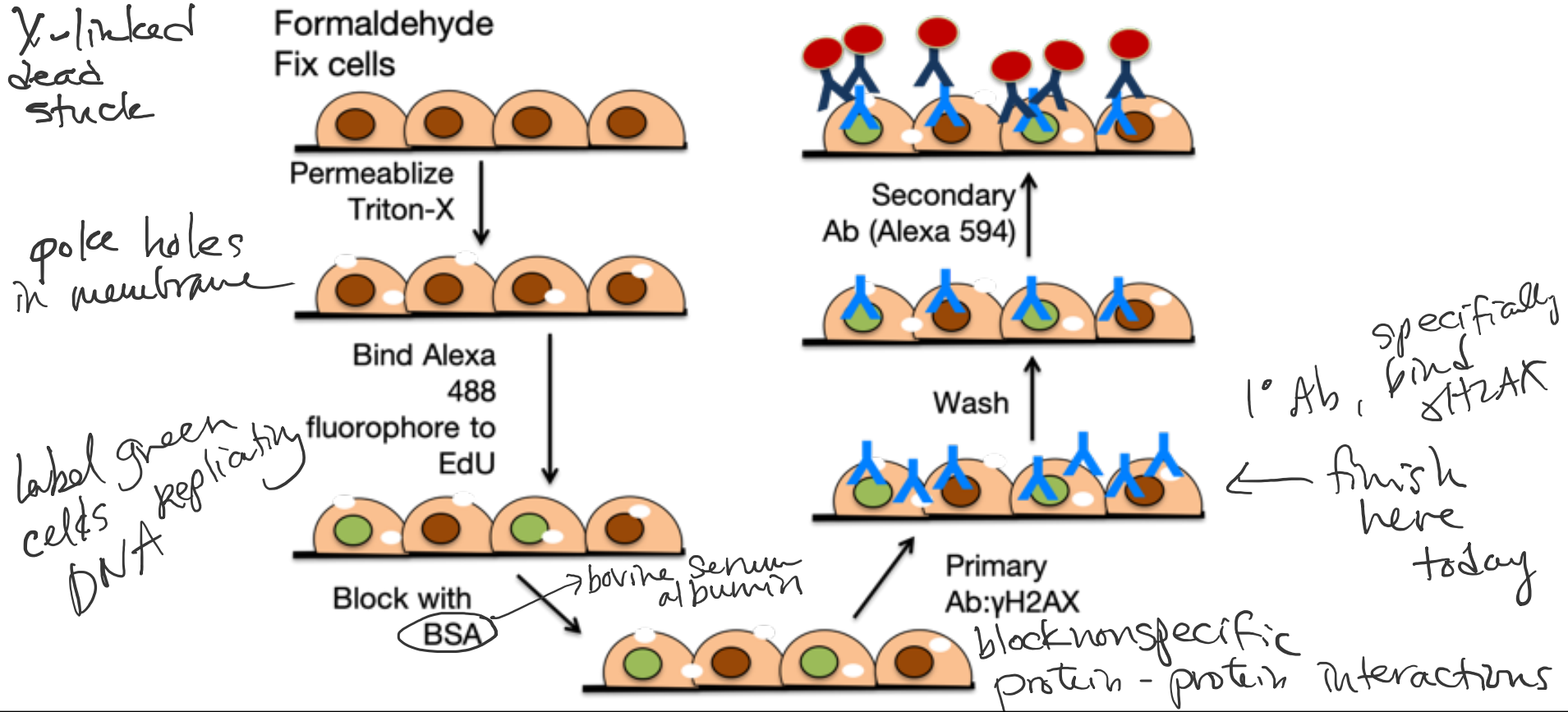
Blue: DNA
Green: EdU incorporation
Red: γH2AX staining

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

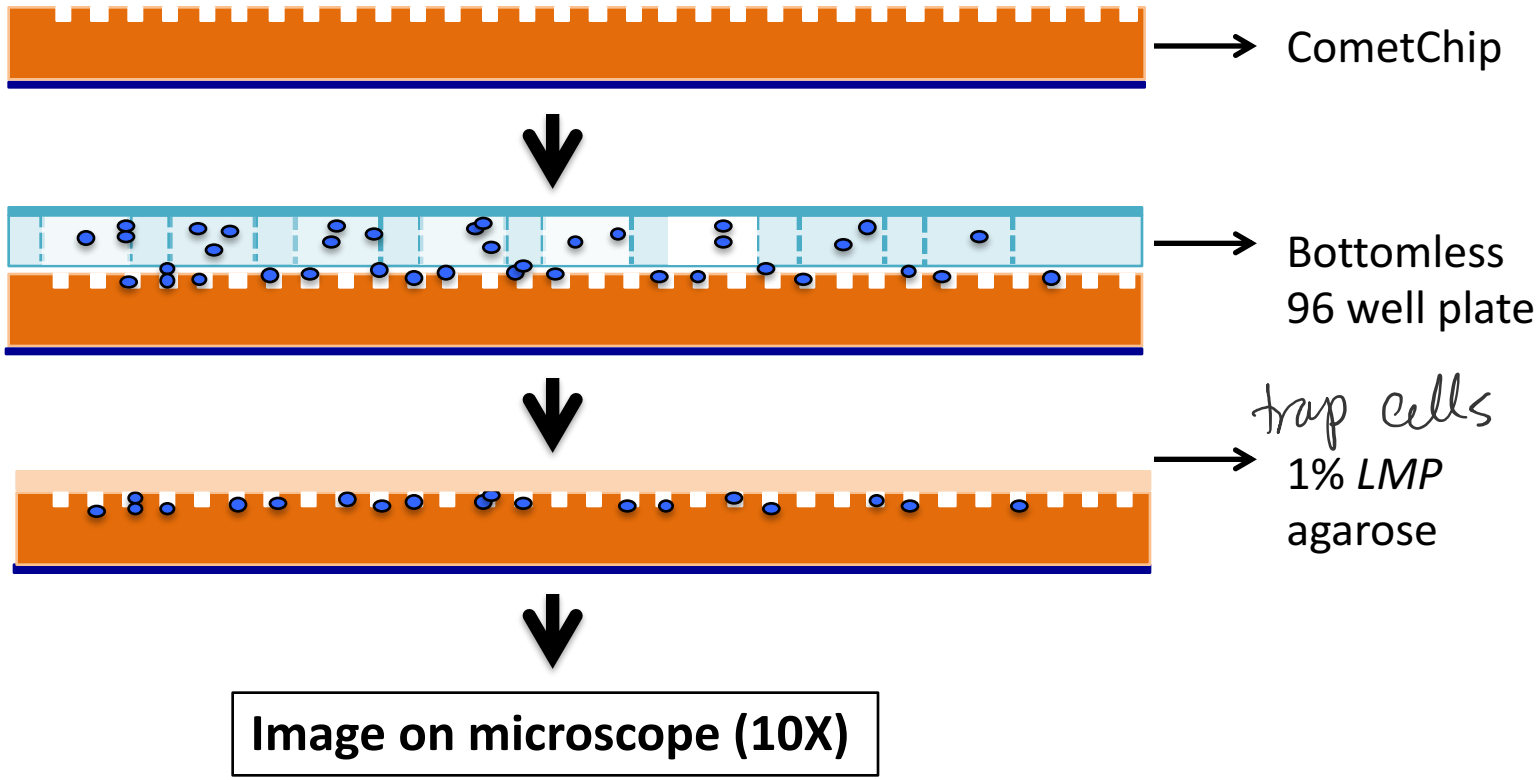
- Histone H2AX phosphorylated at Ser139 if DSB
- Antibodies against γH2AX (phosphorylated form)

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

Using immunofluorescence (IF) and EdU reaction:



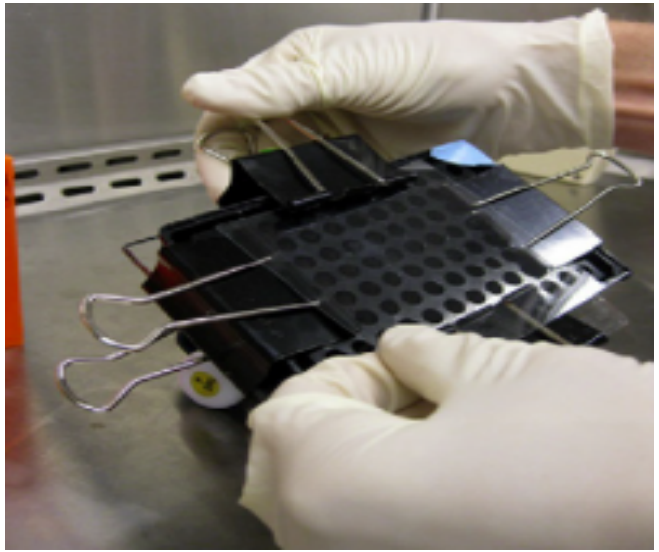
Today: Load cells onto the CometChip

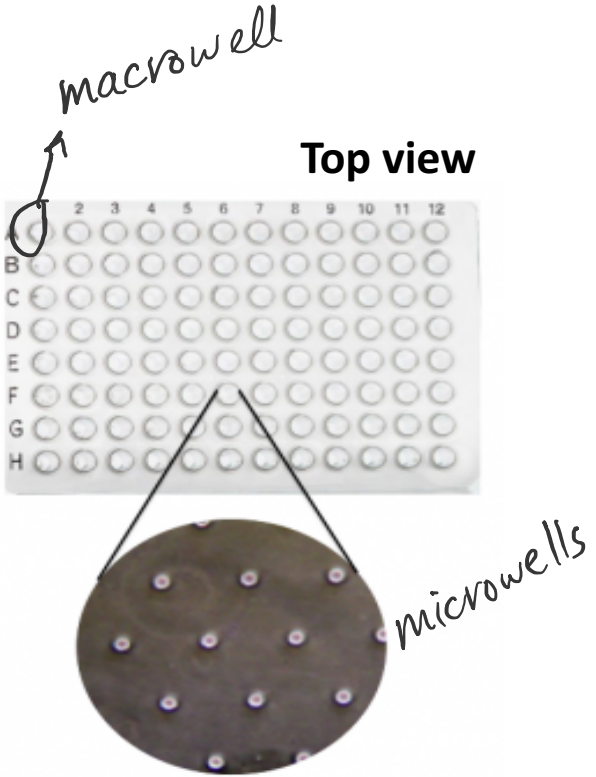


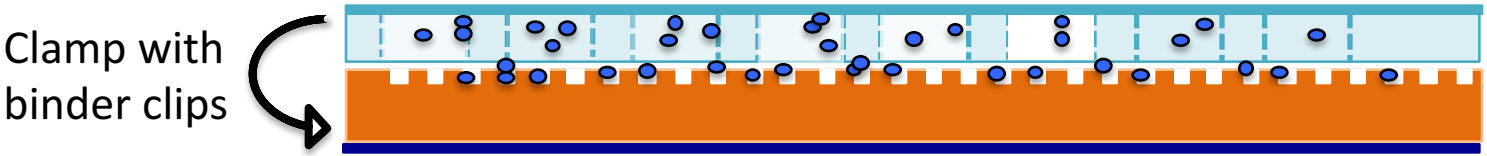
side view

What this looks like in real life

- Glass plate
- Bottomless 96-well plate
- 4 binder clips
- 37°C incubator



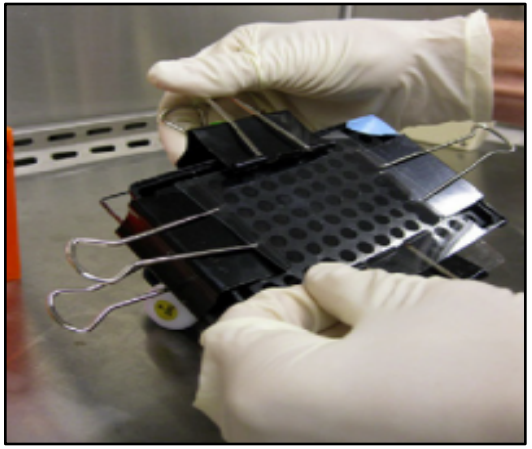


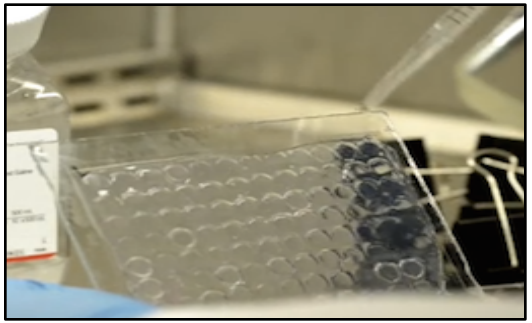


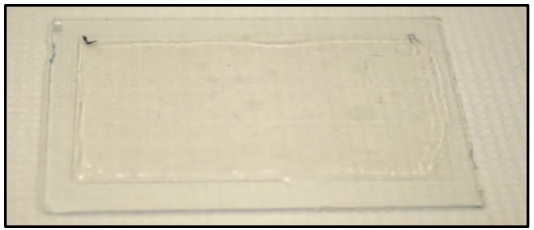
Critical steps:

- Cell loading
  - Line up macrowells carefully within the WASH pattern drawn on gel bond
- Washing
  - Not too much!
  - Across the top of the glass plate
  - Wash from low to high concentration
- 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 5 min

A no cell
B low
C high







Designing the cell loading experiment

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

Considerations:
- size of cell / shape of cell
- microwell size (40μm)
- # of microwells per macrowell
- area of macrowell

Variable:
concentration of cell loaded [low # / high #]

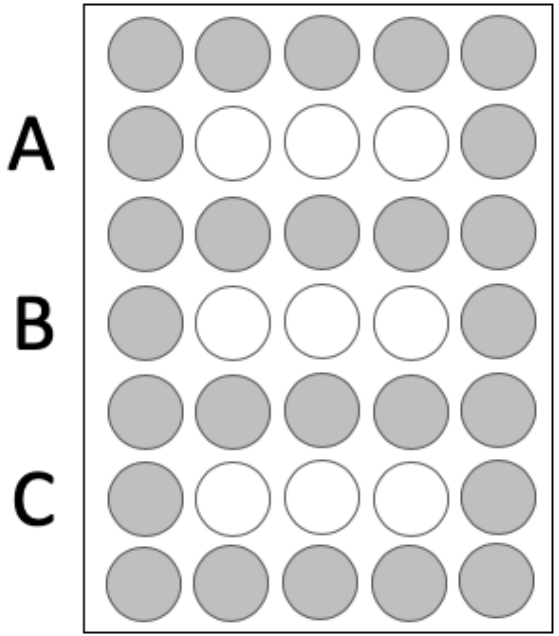
Control:
no cells (negative control)

Repeatability:
triplicate 3 macrowells / condition

Designing the 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

Shaded = no cells



Incubate cells at 37°C for **15min**

Major assignments for Mod1

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

Summary content

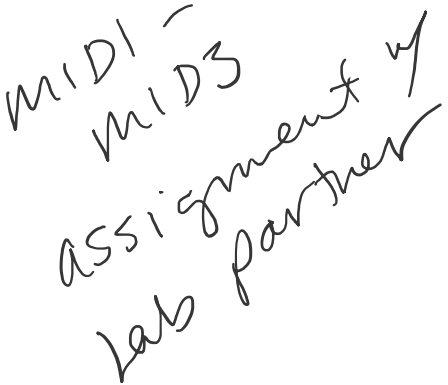
1. Title
2. Abstract
3. Background & Motivation
4. Figures, Results & Interpretation
5. Implications & Future Work

- Mini presentation due by 10pm on Sat., October 19
- Blog post for M1 due by 10pm on Tues., October 15

How do we communicate our protocol?

Tips to write Methods (due M1D4)

- Include enough information to replicate the experiment
  - list manufacturers name
- Use subsections with descriptive titles
  - Put in logical order
  - Begin each subsection with sentence to introduce purpose
- Use **clear and concise** full sentences
  - **NO** tables and lists
  - Passive voice expected
- 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



Example methods section~

Tissue Culture:

human lym. (gift from Engelward lab)

TK6 cells were grown in a flask with ~~12ml~~ RPMI

10% (concentration of P/S (Invitrogen))

supplemented with FBS. Cells were split using

0.25%

trypsin. The cells were kept in an incubator at 37°C.

Improving a Methods paragraph

Maintaining lymphoblastoid cell line(s):

TK6 human lymphoblastoids (gift of the Engelward Lab, MIT) were cultured at 1 x 10^4 cells/cm^2, cell number calculated via hemocytometer and trypan blue stain. Cells were grown in RPMI medium 1640 (Invitrogen) supplemented with 10% fetal bovine serum (Atlanta Biologicals) and 100 units/mL penicillin-streptomycin (Invitrogen). Culture conditions were maintained at 37°C, 5% CO2 and 95% relative humidity.

In lab today

1. Transfer coverslips to staining chambers and star 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 (with lab partner)

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