M1D1:
Prepare microwell array and practice tissue culture

1. Laboratory orientation quiz
2. Pre-lab discussion
3. Benchwork
   - We will complete Part 3 first

We are here to help!

Noreen

- Monday 2pm-5pm
- in 16-317

Leslie

- Friday 9am-10am
- Friday 3pm-4pm
- in 56-341c

Josephine

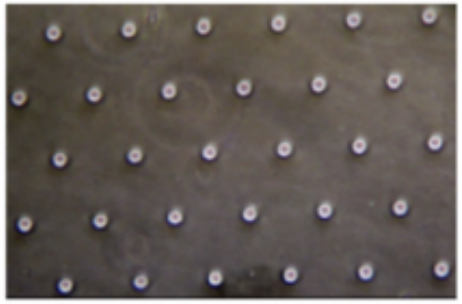
- Thursday 2pm-3pm
- in 56-341c

by appointment: nllyell@, lesliemm@, joshaw@

Mark your calendars!

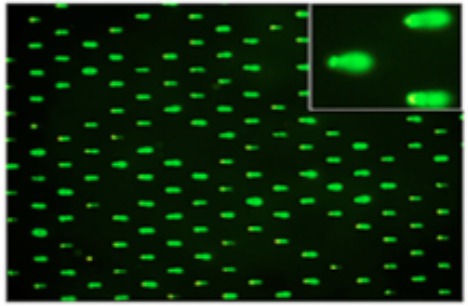
- Data summary (15%)
  - completed in teams and submitted via Stellar
  - draft due 10/9, final revision due 10/22
  - format in bullet points, .PPTX
- Mini-presentation (5%)
  - completed individually and submitted via Gmail
  - due 10/14
- Laboratory quizzes
  - scheduled for M1D5 and M1D7
- Notebook (part of 10% Homework and Notebook)
  - one entry will be graded by Eric on M1D7
- Blog (part of 5% Participation)
  - due by 10/23 via Blogspot

Overview of Mod1 experiments



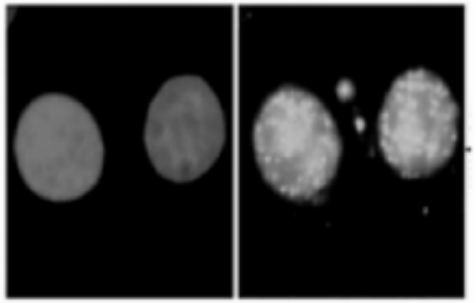
1. Optimize comet chip assay

- Test loading variables



2. Use comet chip assay to measure DNA repair

- Measure effects of MMS and H_2O_2 on BER

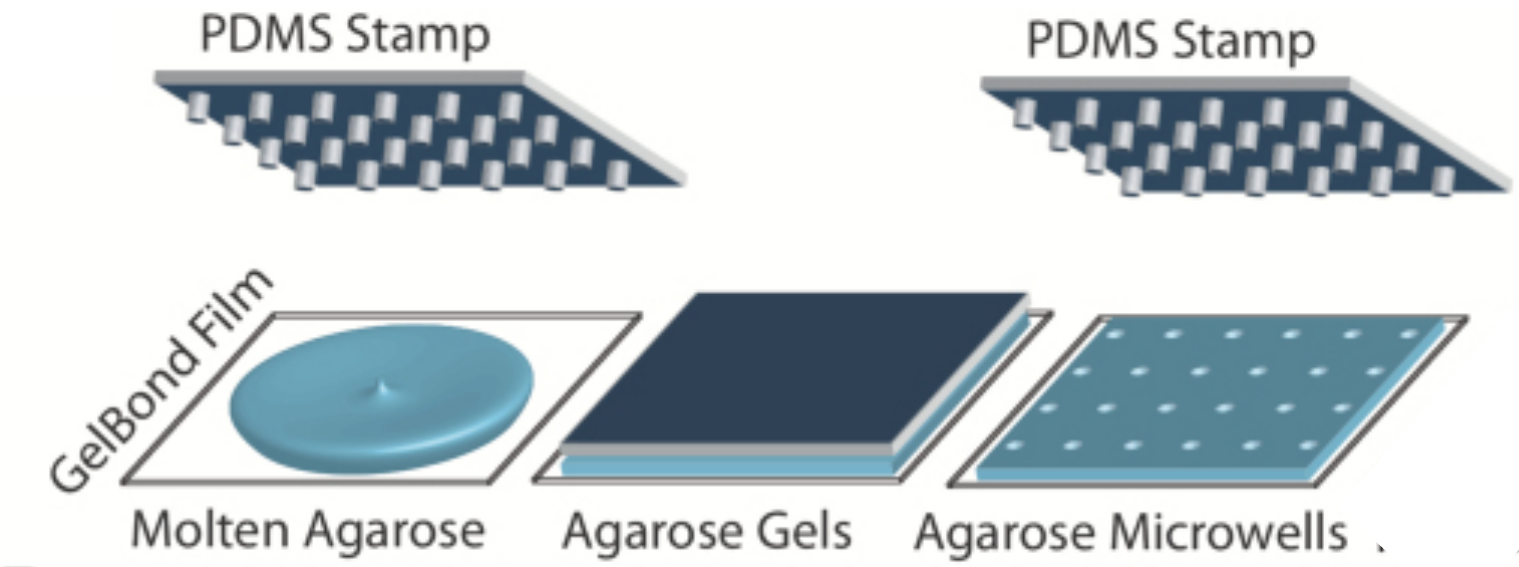


3. Use immuno-fluorescence assay to measure DNA repair

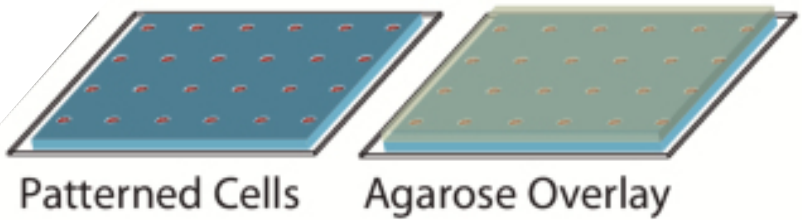
- Examine effect of MMS and H_2O_2 on DSB abundance

Exp1: Optimize CometChip loading

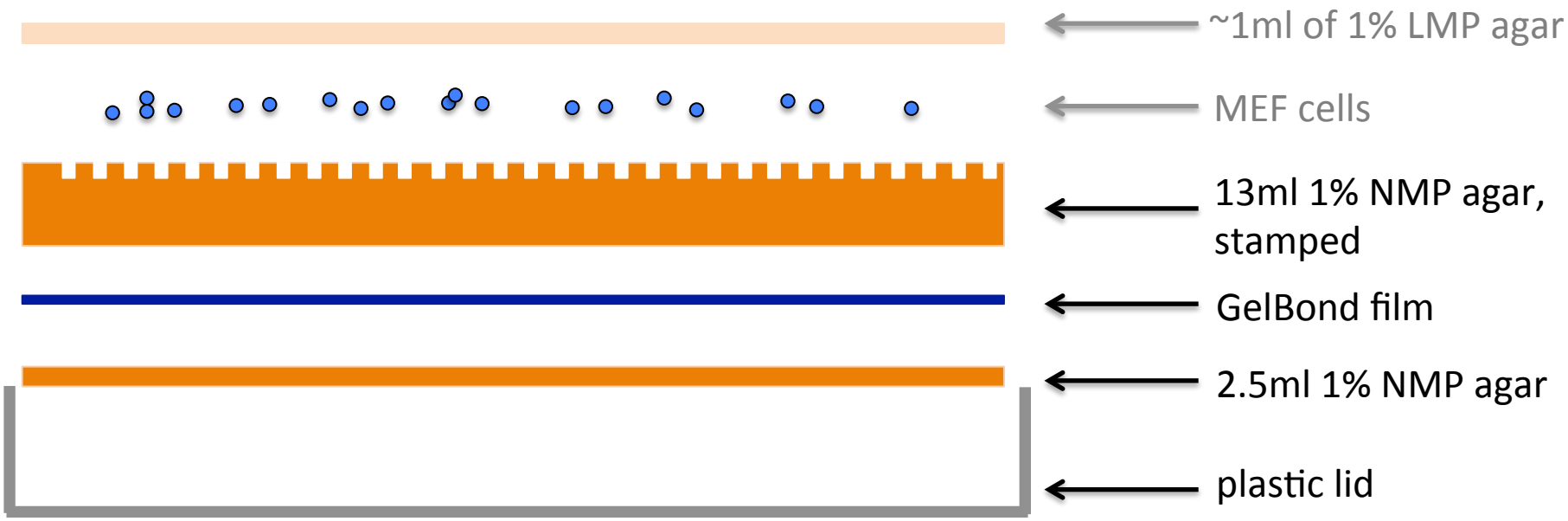
Preparing the CometChip (M1D1):



Loading the CometChip (M1D2):



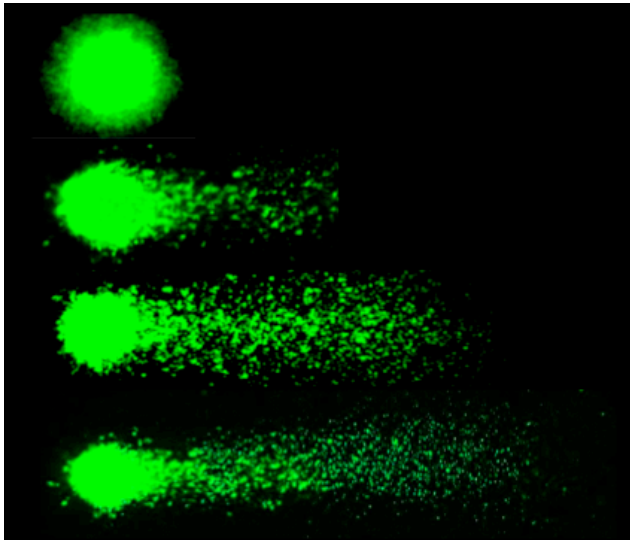
Let’s take a closer look



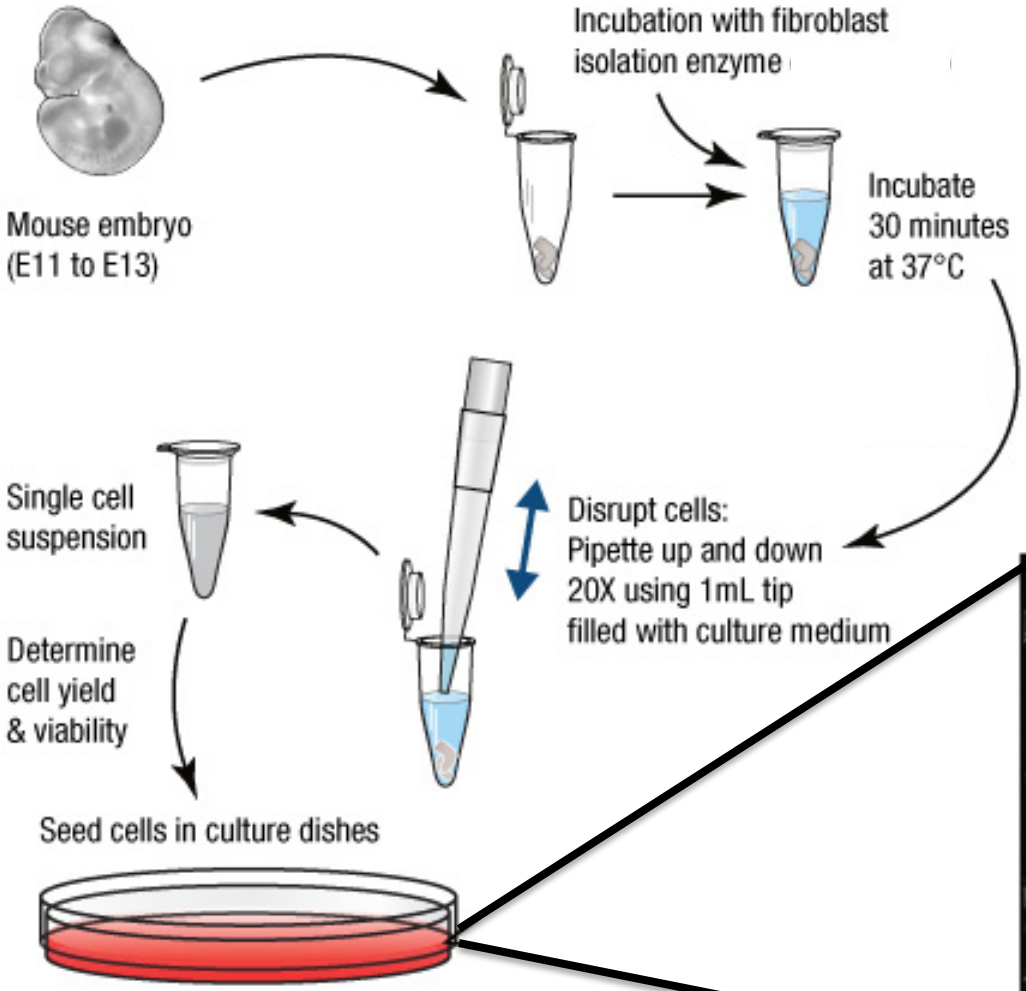
Exp2: Biochemical testing using CometChip

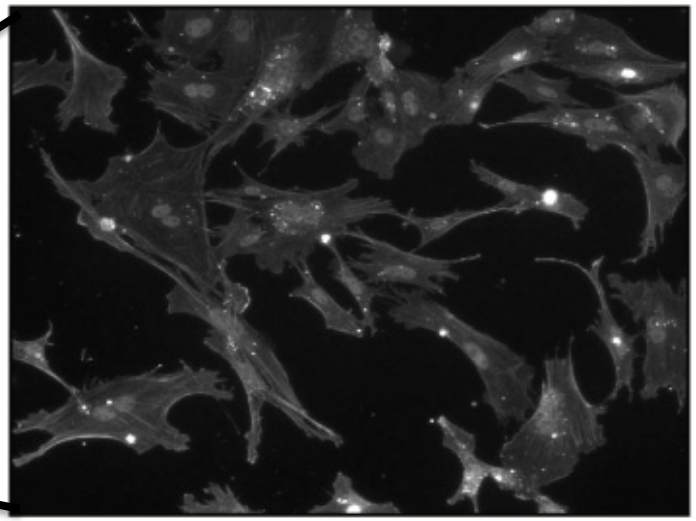
What are the effects of H_2O_2 and MMS on genomic stability?

1. Comparison between treatments
2. Comparison between doses
3. Comparison with added enzymes (cleave at damaged bases)



But first, we need to prepare our cells



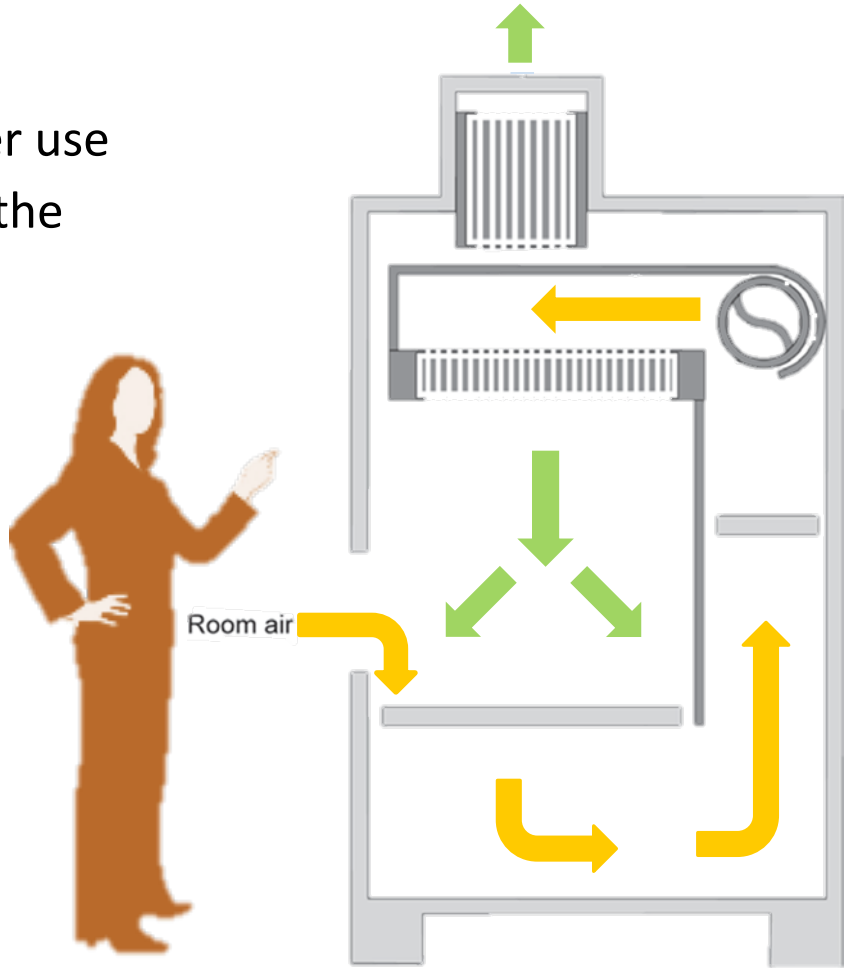


Day 7 (β-actin, 40X)

ThermoFisher: Isolation of Primary Mouse Embryonic Fibroblasts

Maintaining sterility is important!

- **70% ethanol everything:**
  - wipe cabinet before and after use
  - wipe everything that enters the cabinet
  - do not spray cells with EtOH
- **Do not disturb air flow:**
  - do no block grille or slots
  - minimize side-to-side arm movements
  - work > 6” away from sash
  - leave blower *on always*
- Do not talk into incubator
- Only open sterile media in hood



Keeping your cells happy

Food:

- DMEM (Dulbecco's Modified Eagle's medium) defined
  (phenol red = indicator dye)
  glucose
  amino acids
  vitamins
  buffers
  salts
- FBS (fetal bovine serum) undefined
  growth factors
  cholesterol
  lipids
  cytokines

Non-food:

- antibiotics:
  - penicillin
  - streptomycin

control bacterial contamination

Learning the jargon

- Confluence

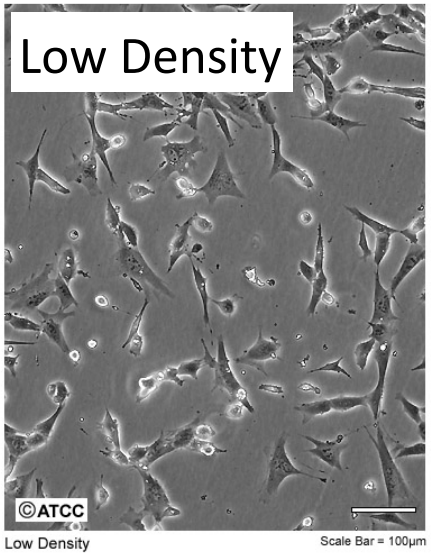
  eye ball estimation of culture density

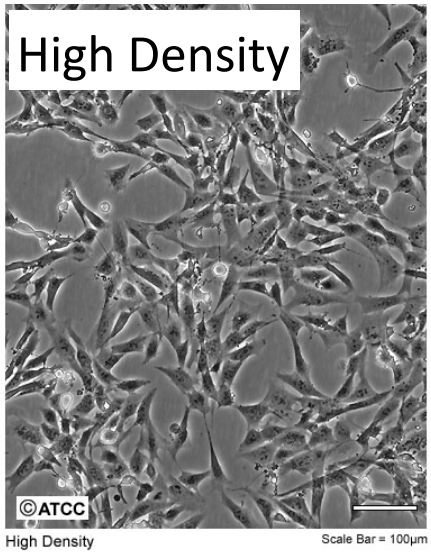
- Splitting

  'sub-culturing'
  reducing density of population to maintain growth conditions

- Seeding

  putting cells into a fresh dish for growth





Splitting cells

1. Look at cells with microscope and estimate confluence

   make sure alive and present, check for contamination

2. Rinse with PBS

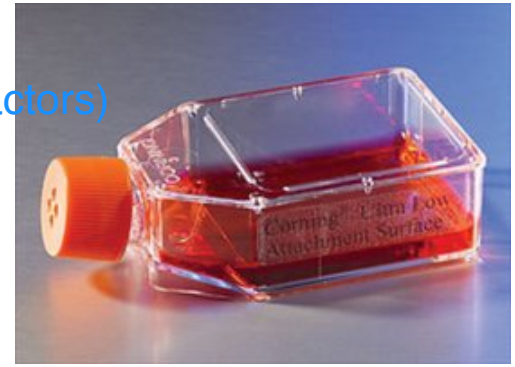
   wash away excess media, cellular proteins (anti trypsin factors)

3. Detach with trypsin

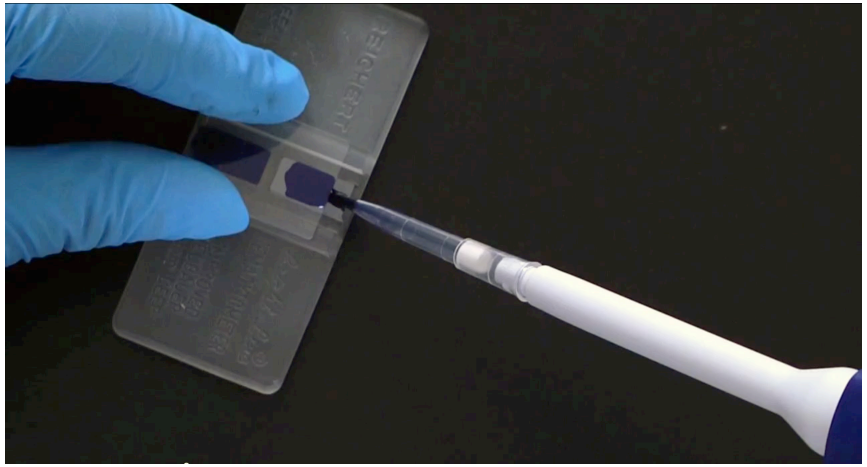
   cleave attachment proteins

4. Count using hemacytometer*

5. ‘Seed’ into fresh culture flask



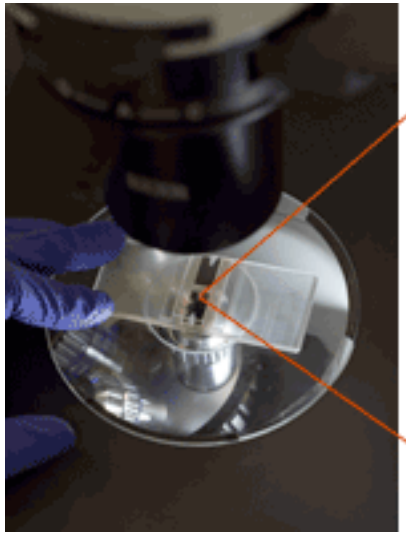
Counting cells

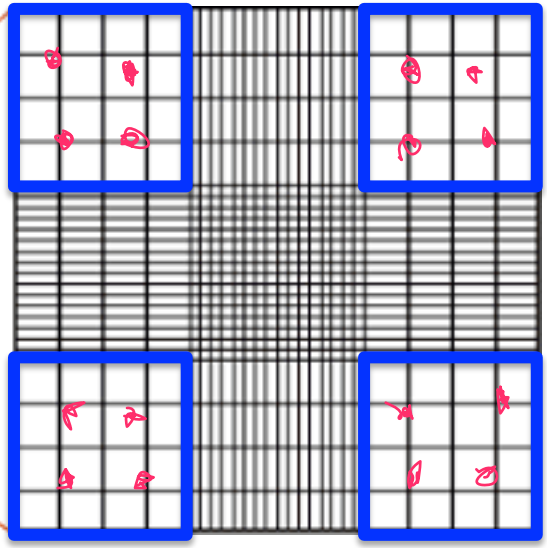


- Hemocytometer

  glass slide with etched pattern and weighted coverslip (holds 10 uL)
- Trypan blue

  stains dead cells blue





cells / mL = 10,000 x average of 4 corners

16/4 = 4

= 40 000 cells/mL

Today in lab…

- All teams will start in TC (Part 3)
  - Pay close attention to demonstration
- Then each team will prepare one CometChip
  - Read through protocol before you begin
  - Be careful with CometChip stamps
  - Watch JOVE video during downtime
- Record your science in Benchling!
  - Template example on due M2D2 HW