M1D1: Prepare microwell array and practice tissue culture

09/12/17

1. Lab Orientation Quiz
2. Pre-lab Discussion
3. ½ class goes to the Tissue Culture Room
4. ½ class prepares a CometChip

20.109 Office hours

Noreen

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

Leslie

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

Josephine

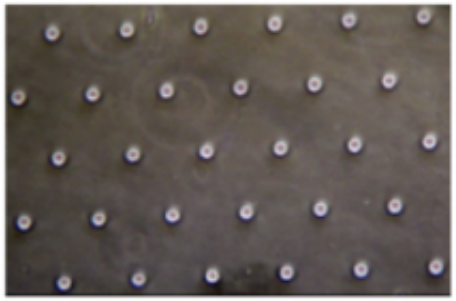
- Thursday 2pm-3pm
- in 56-341c

by appointment: nllyell@, lesliemm@, joshaw@

M1 major assignments

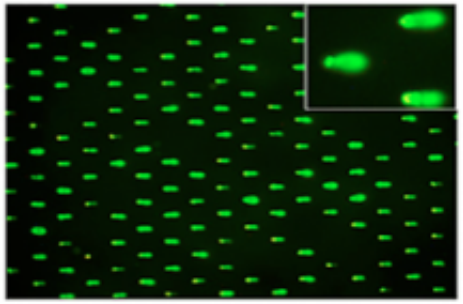
- Data summary (15%)
  - in teams, on Stellar
  - draft due 10/9, final revision due 10/22
  - bullet points, .PPTX
- Mini-presentation (5%)
  - individual, video via Gmail
  - due 10/14
- Lab quizzes
  - M1D5 and M1D7
- Notebook (part of 10% Homework and Notebook)
  - one day will be graded by Eric on M1D7
- Blog: http://be20109f17.blogspot.com/ (part of 5% Participation)
  - by 10/23

Overview of
Module 1: Measuring Genomic Instability



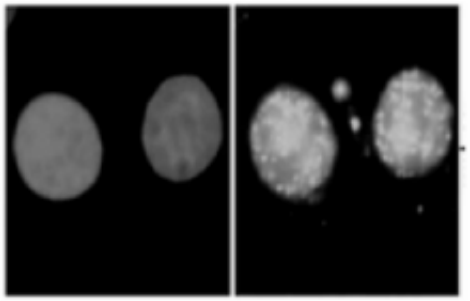
1. Optimize comet chip assay

- Test loading variables



2. Use comet chip assay to measure DNA repair

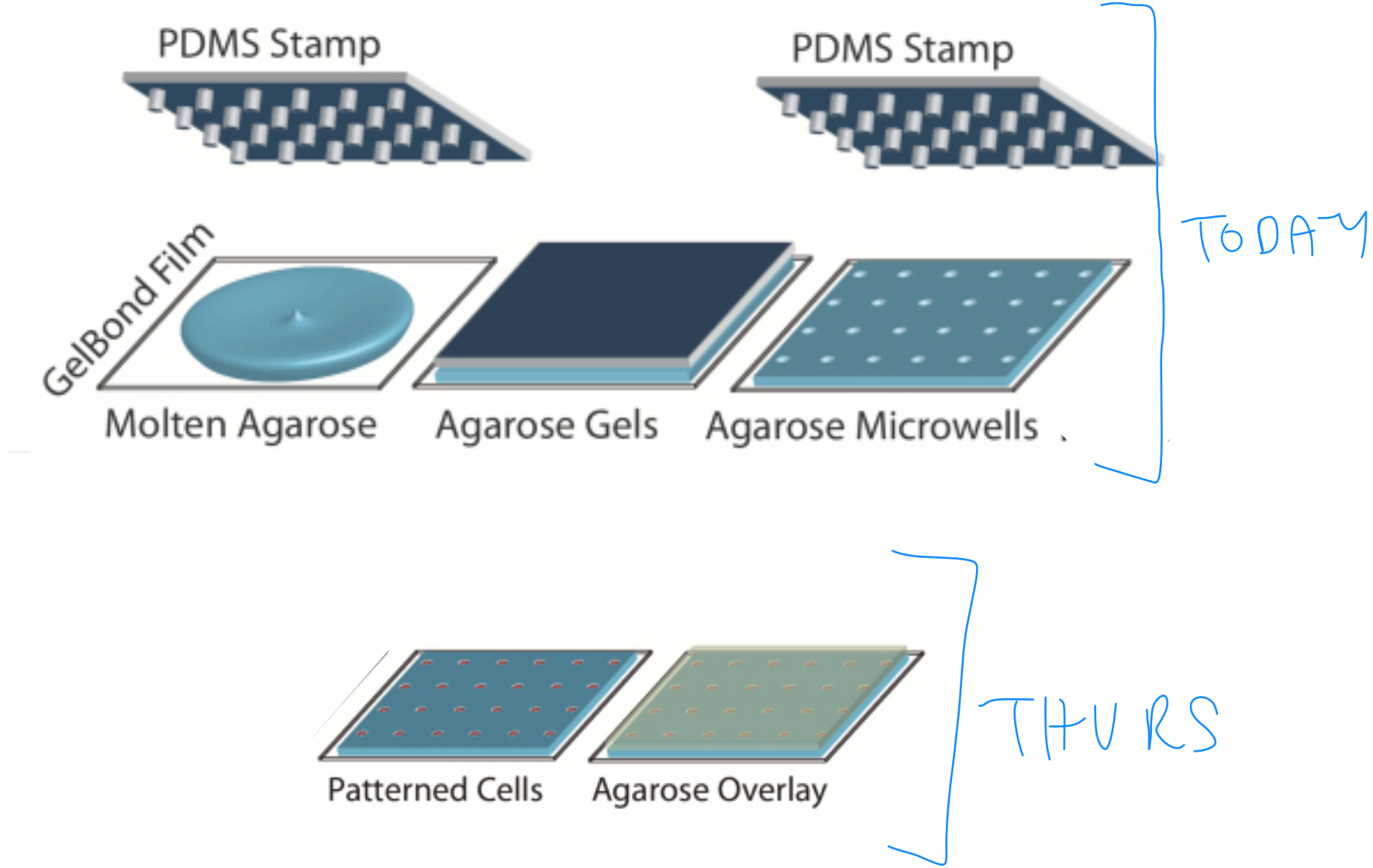
- Measure effects of MMS and H_2O_2 on BER



3. Use immuno-fluorescence assay to measure DNA repair

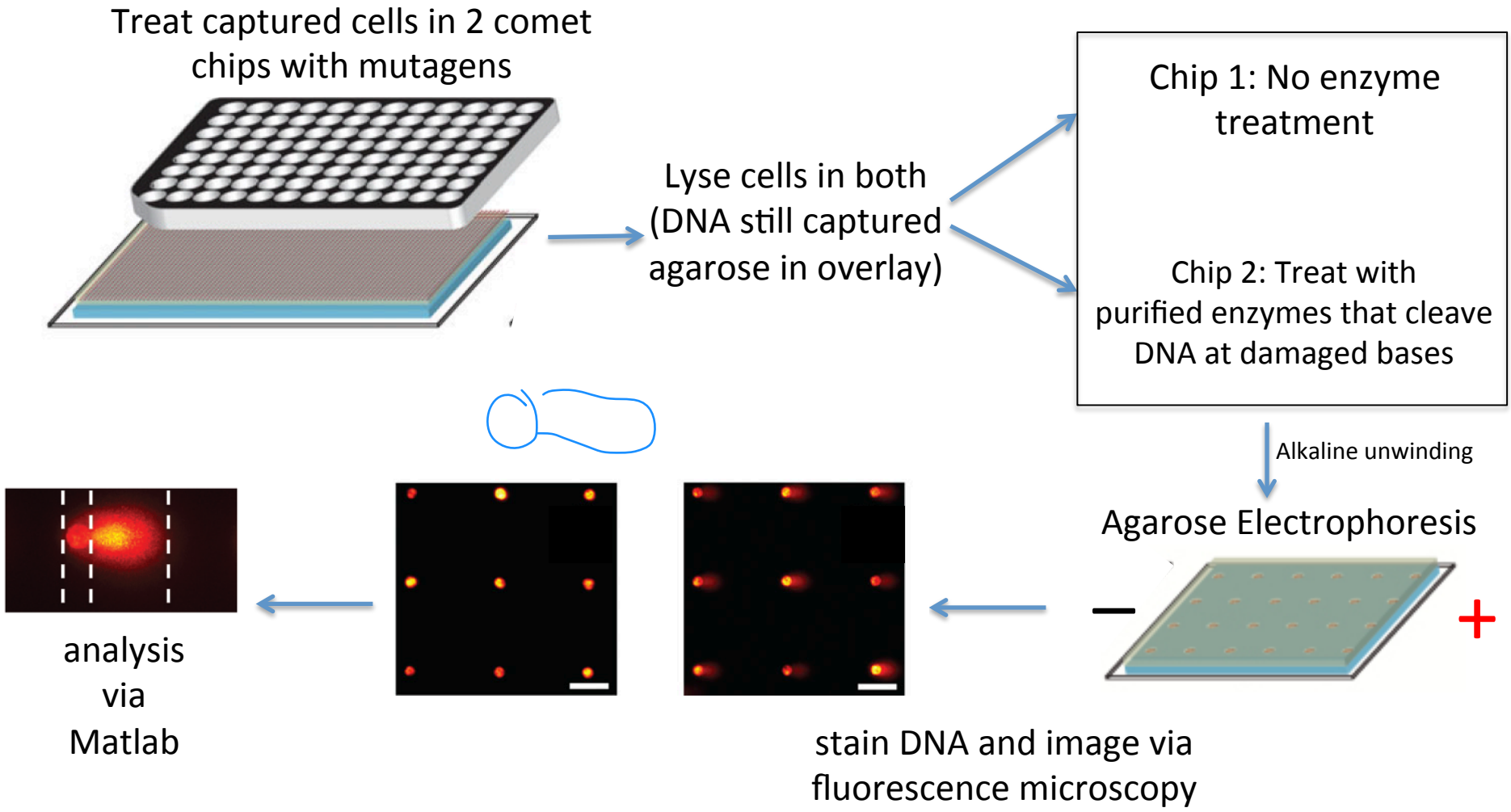
- Examine effect of MMS and H_2O_2 on DSB abundance

This week: Creating a CometChip and optimize loading cells



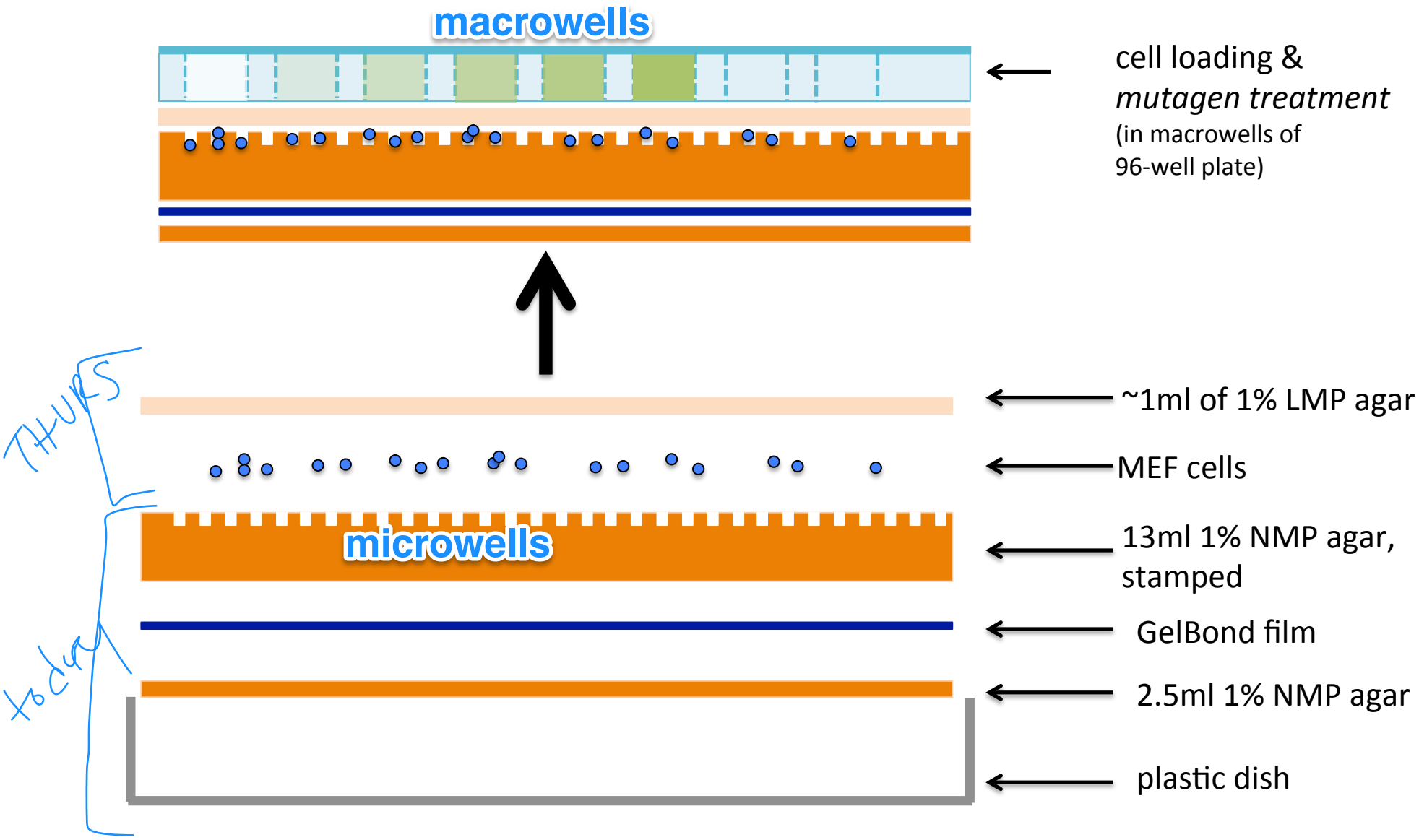
few

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

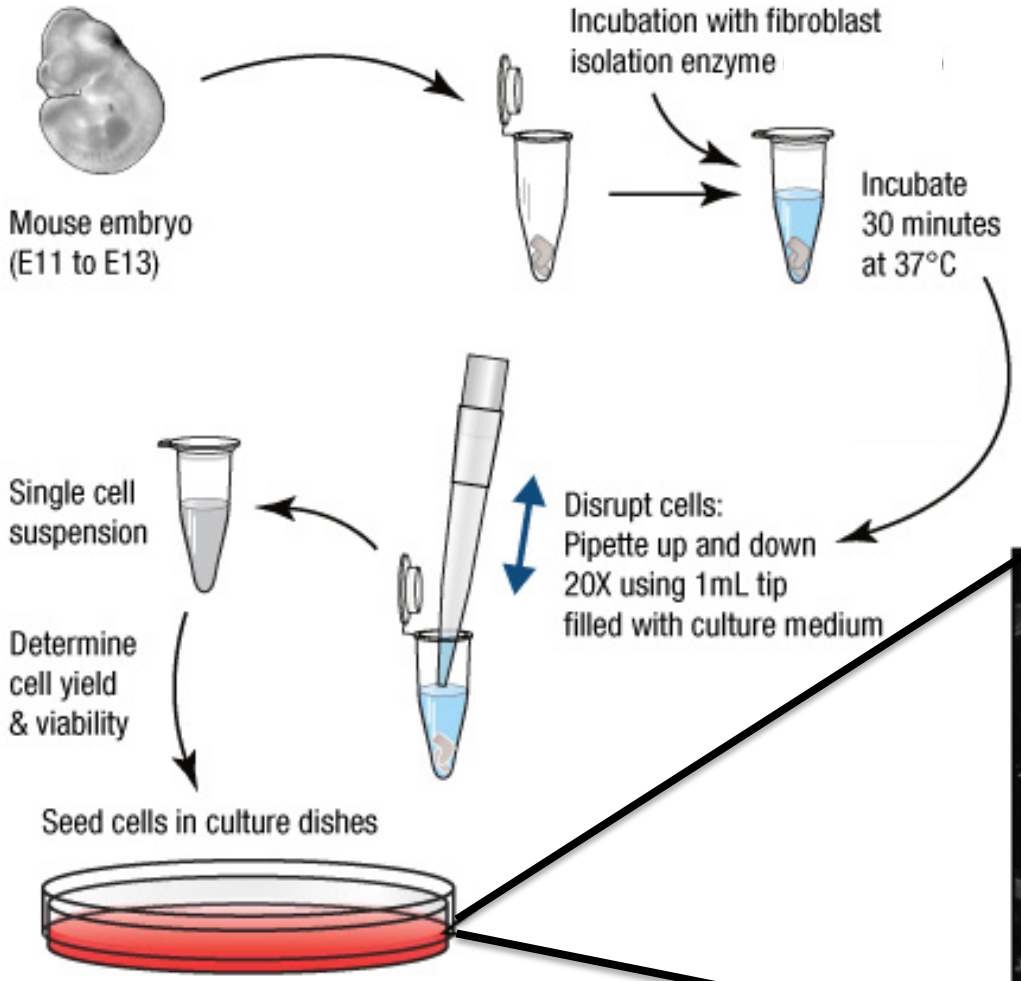


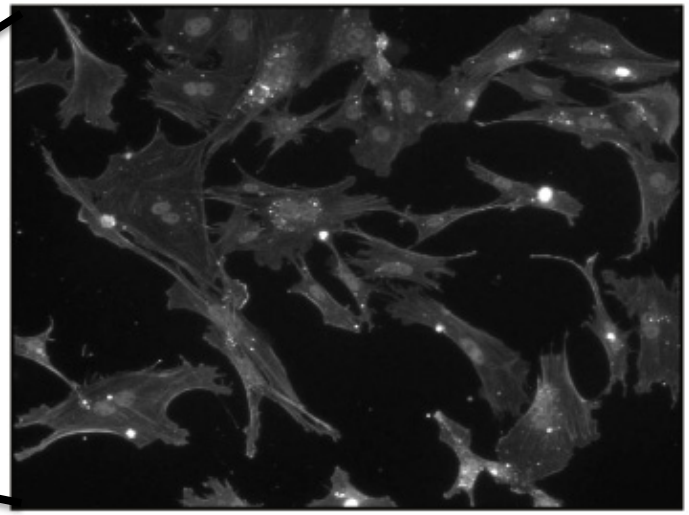
The CometChip layers

Side view



Mouse Embryonic Fibroblast Isolation



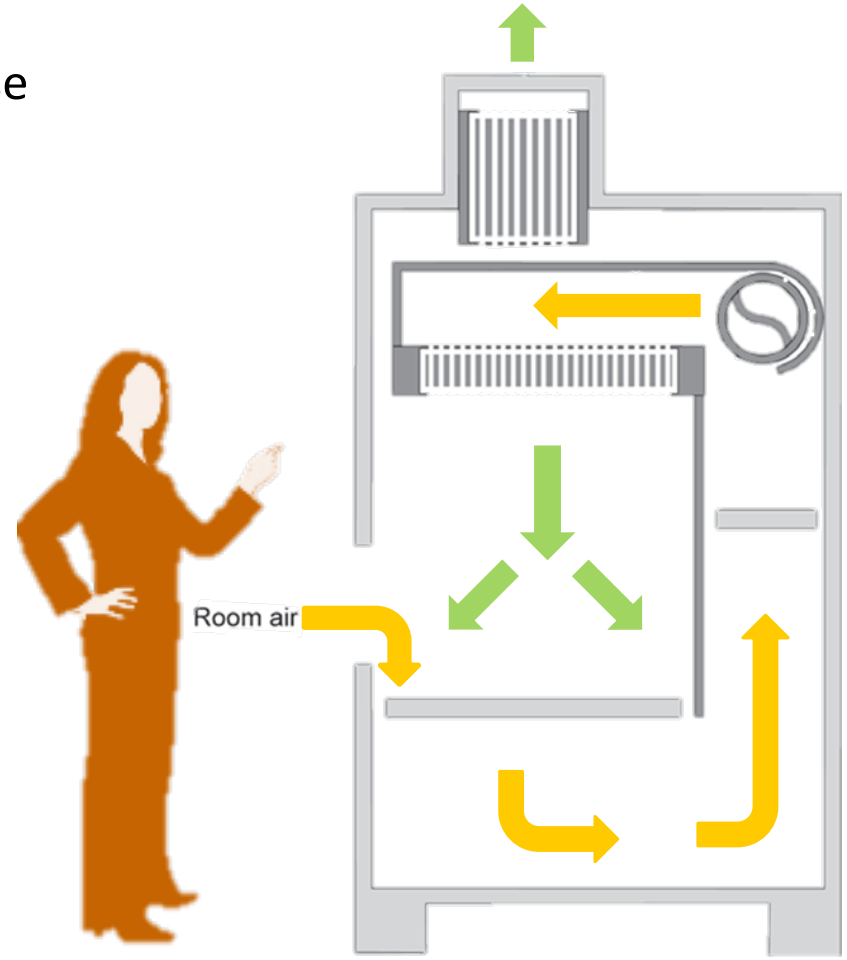


Day 7 (β-actin, 40X)

ThermoFisher: Isolation of Primary Mouse Embryonic Fibroblasts

Tissue culture sterile technique

- **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



Mammalian cell culture medium

What do cells need to survive? grow, divide and viable

Food:

- DMEM (Dulbecco's Modified Eagle's medium) defined

  glucose
  amino acids
  pH buffer
  salts
  vitamins

- FBS (fetal bovine serum) undefined

  growth factors
  lipids
  cholesterol
  cytokines

Non-food:

- antibiotics:
  - penicillin
  - streptomycin

  prevent bacterial growth

Mammalian cell culture terminology

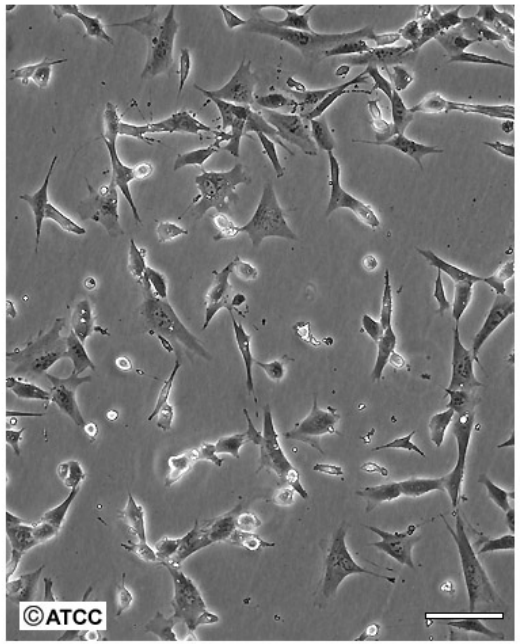
- confluence
  density

split ~80% confluent

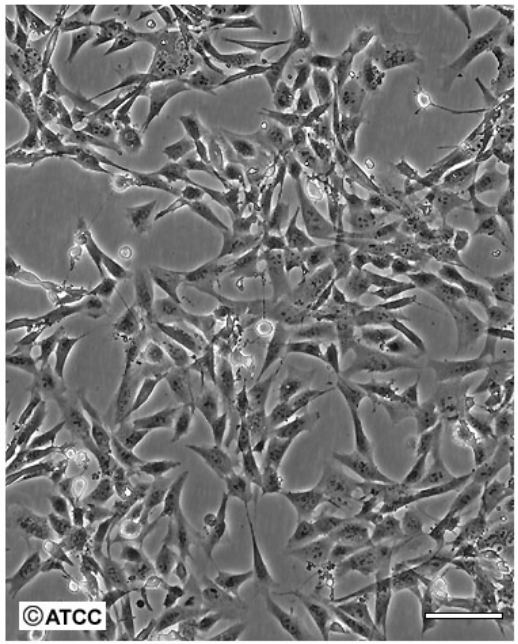
- splitting
  sub culturing

put cells on new dish

Low Density



High Density



- seeding

20-40% confluent culture and putting on new flask

General steps for splitting cells +WHY?

1. Look at cells, estimate confluence

2. Rinse with PBS

   wash away debris, dead cells, removing anti-trypsin agents

3. Detach cells with trypsin(enzyme)

   break substrate adhesions

4. Count cells

   seed specific number in new vessel

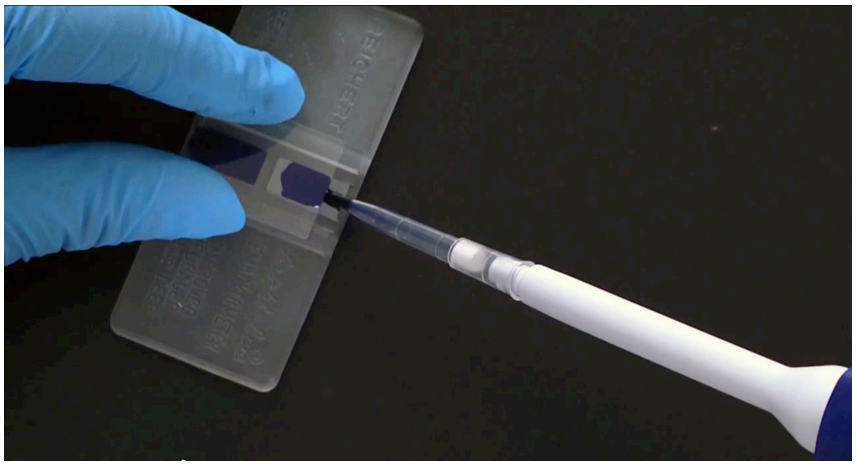
5. "Seed" new culture vessel

   give them room to divide and grow

T25 flask

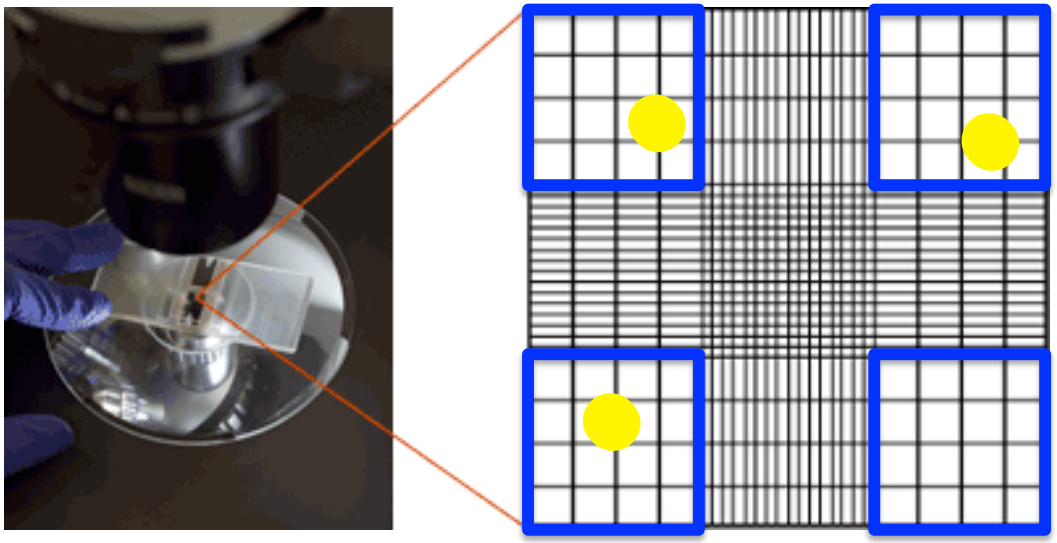
T75 flask

Counting Cells



- Hemocytometer:
- Trypan blue:

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



16/4 = 4

4 x 10,000 =

40,000 cells/mL

Today in lab:

1. 4 teams into tissue culture room to split MEFs (Red, Orange, Yellow and Green)
2. 4 teams start preparing CometChip (Blue, Pink, Purple and White)
3. Make sure to keep notes in Benchling!

- Watch Engleward lab JOVE video during downtime (I will email link.) On the wiki: Mod1 landing page
- M1D2HW: Create a M1D2 template for your benchling notebook and turn in a printed copy.

Adding collaborators to Benchling