M1D1:
Prepare CometChip
Cell culture

09/14/2016

Today in the lab

- Hand in your homework
- Lab orientation quiz
- Pre-lab discussion

- 2 teams prepare a CometChip
- 2 teams split cells in the tissue culture room

Office hours

Noreen Lyell

- Mondays 1pm
- Mondays 5pm
- in 16-317

Leslie McClain

- Mondays 4pm
- Wednesdays 9am
- in 16-429b

Maxine Jonas

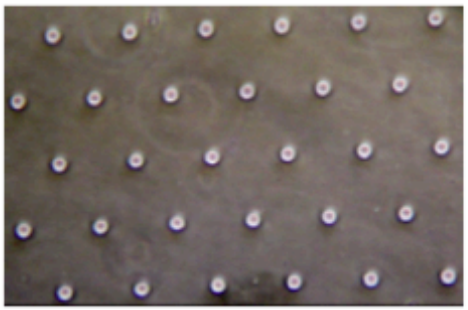
- Mondays 2pm
- Fridays 9am
- in 16-239

by appointment: nllyell@, lesliemm@, jonas_m@

M1 major assignments

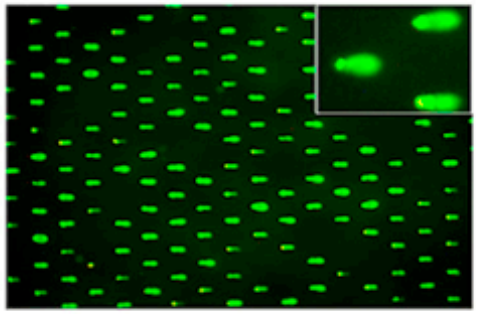
- Data summary (15%)
  - in teams, on Stellar
  - draft due 10/12, final revision due 10/24
  - bullet points, .PPTX
- Mini-presentation (10%)
  - individual, video via Gmail
  - due 10/15
- Lab quizzes (extra credit)
  - M1D3, M1D4, and M1D6
- Notebook (5% total)
  - one day will be collected and graded by Emily on M1D7
- Blog: http://be20109f16.blogspot.com/ (participation: 5% total)
  - by 10/25

Overview of
“M1: Measuring Genomic Instability”



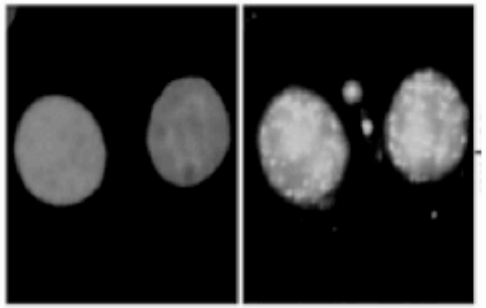
1. Optimize comet chip assay

- Test loading variables



2. Use comet chip assay to measure DNA damage / repair

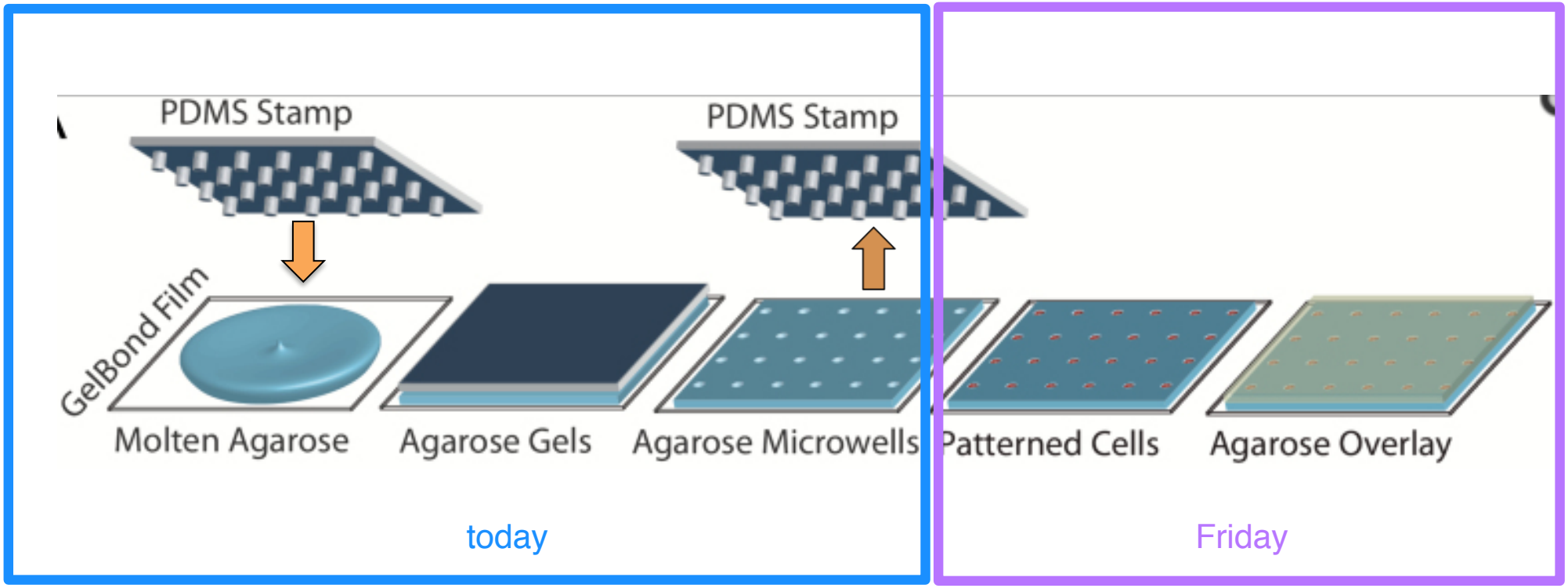
- Measure effects of MMS and H_2O_2 on BER
- Assess repair variability in healthy individuals



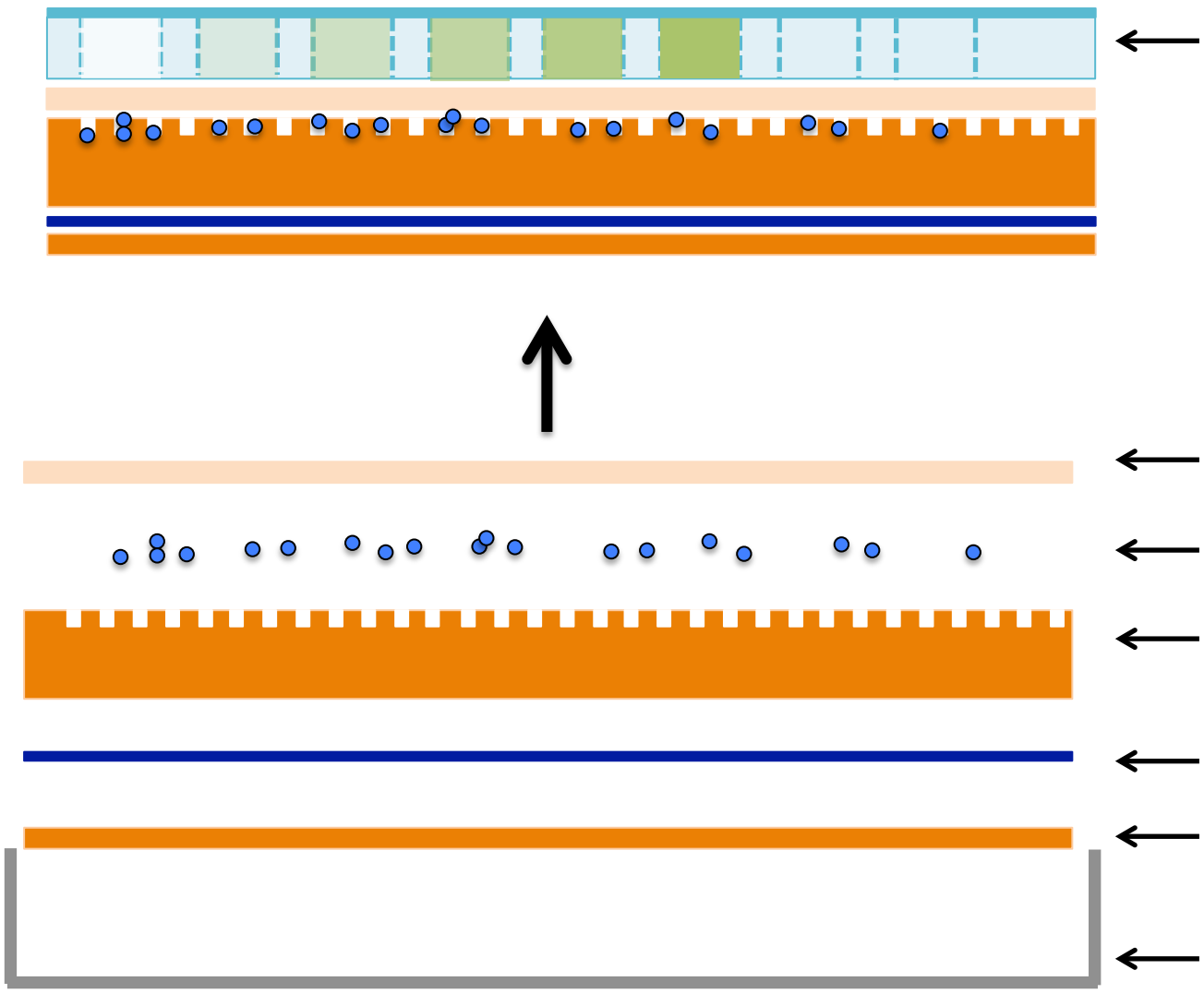
3. Use immuno-fluorescence assay to visualize DNA repair

- Examine effect of H_2O_2 on DSB abundance

Making a CometChip



The CometChip layers



drug treatment
(in macrowells of
96-well plate)

~1 mL of 0.3%
(or 1%) LMP agar

TK6 cells

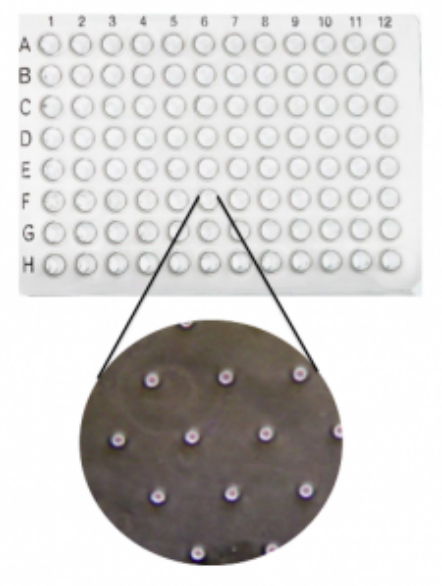
11.5 mL of 1% NMP
agarose, *stamped*

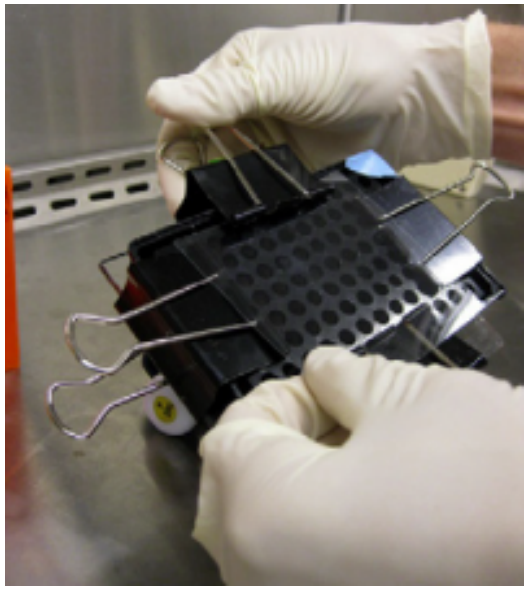
GelBond film

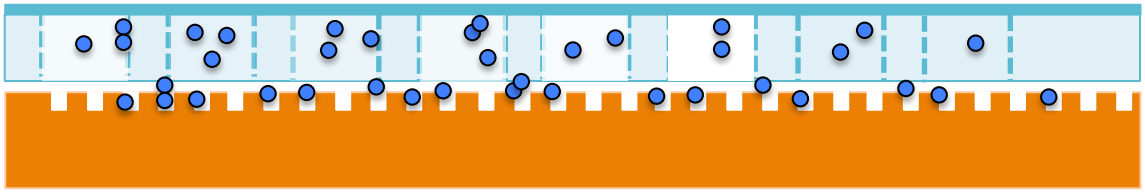
2.5 mL of 1% NMP
agarose

plastic dish

Loading the CometChip

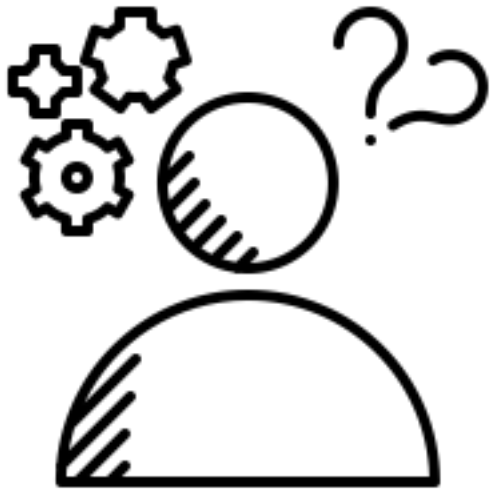






DNA in agarose wells…

Does this ring a bell?



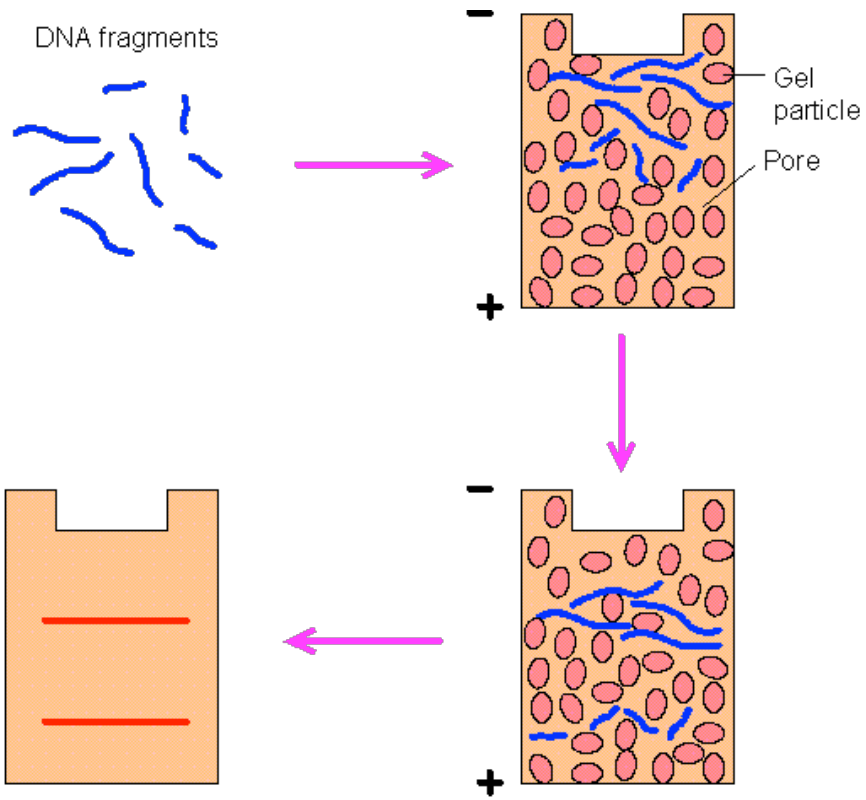
Separate DNA by gel electrophoresis

- Agarose gel electrophoresis
  – driving force:

    charge

  – separates DNA by:

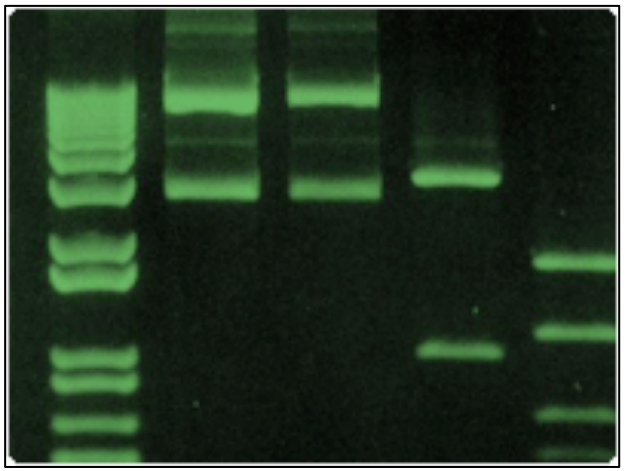
    size



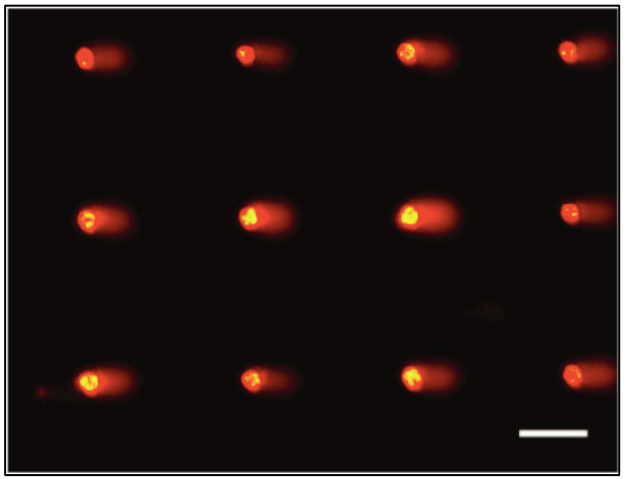
Visualize DNA: SYBR Gold

- DNA stain

  intercalates
  excited by UV/blue
  > 25 pg of DNA (ss or ds)



damaged DNA in tails
intact DNA in heads
of comets



➢ Safety : wear nitrile gloves

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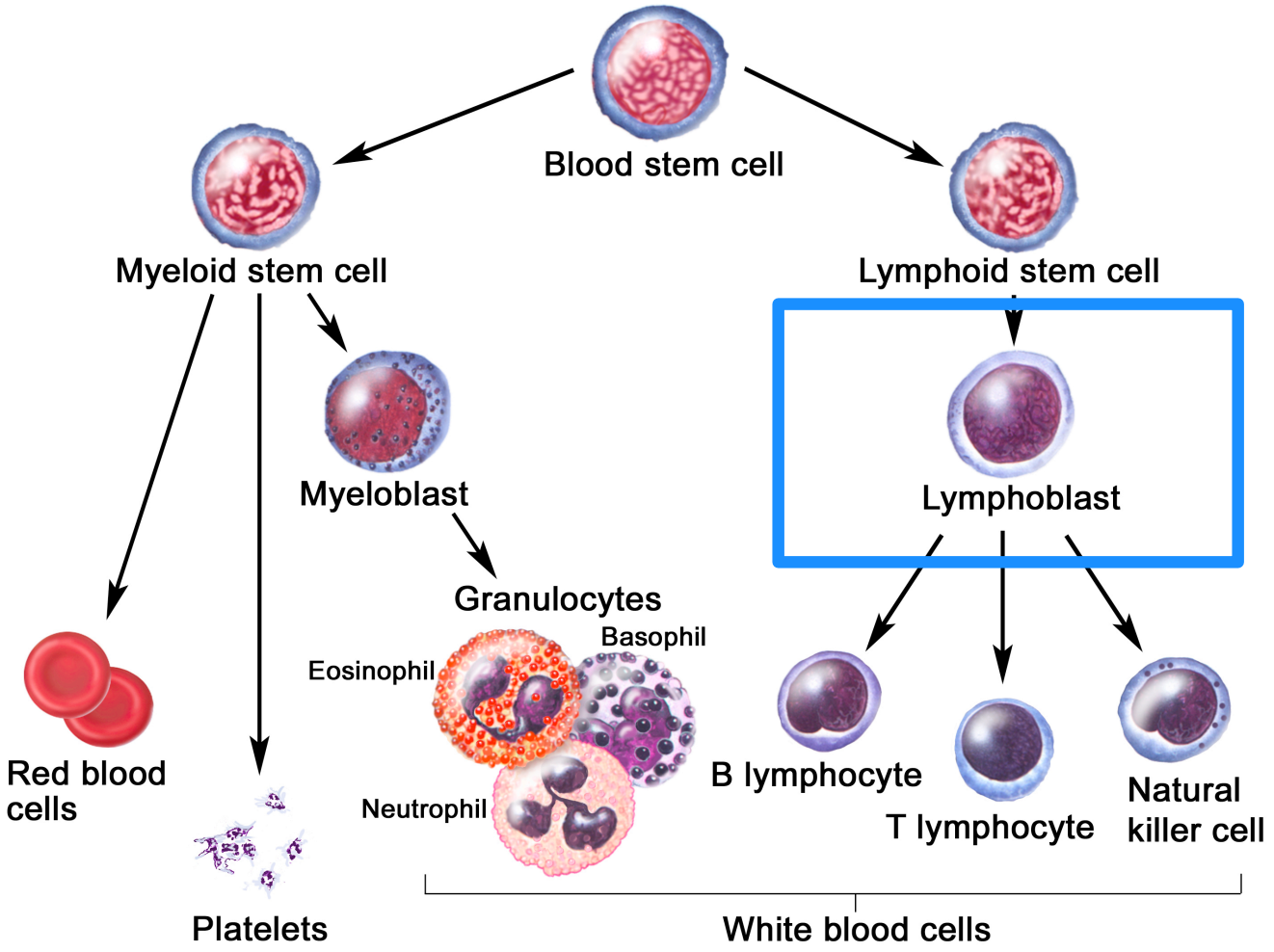
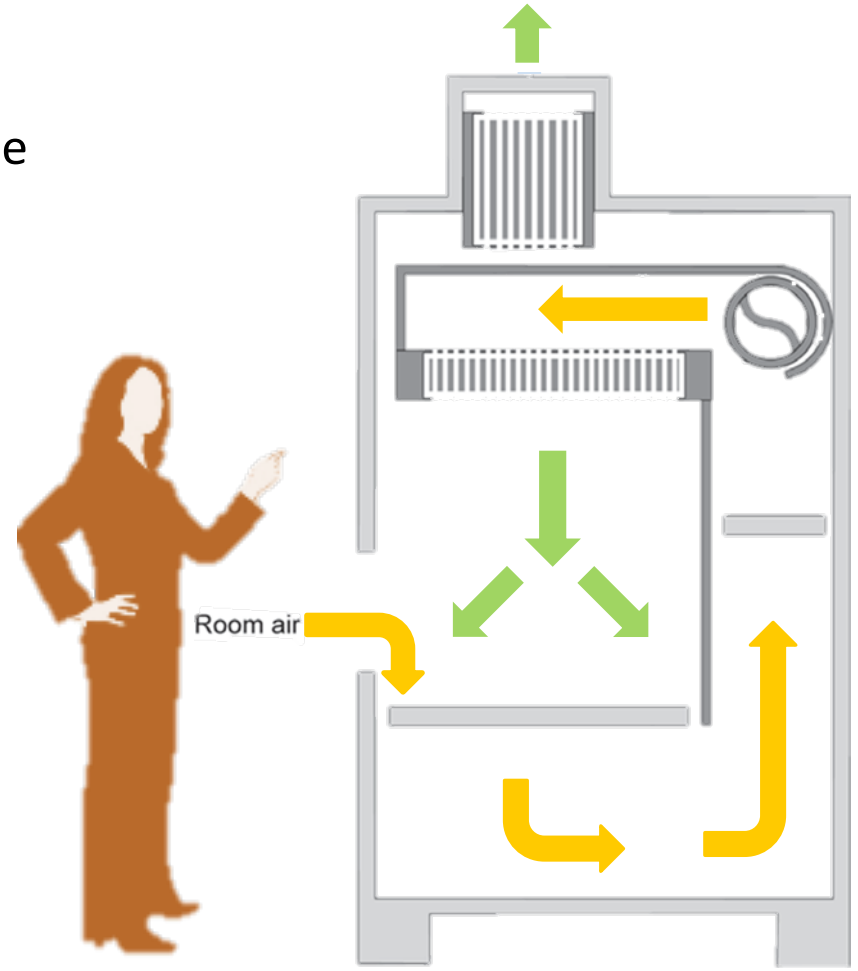
- 2 teams split cells in the tissue culture room

TK6 are human lymphoblast cells



Tissue culture sterile technique

- **70% ethanol** is your BFF:
  - wipe cabinet before and after use
  - wipe everything that enters the cabinet
- **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*
- Do not talk into incubator!
- Only open in hood



Mammalian cell culture medium

Food:

- RPMI 1640 (Roswell Park Memorial Institute) defined

  glucose, salt, amino acids, vitamins
  osmotic pressure is physiological
  phenol red is pH indicator

10%

- FBS: fetal bovine serum undefined

  albumin and other proteins
  cytokines, growth factors
  lipids, cholesterol

Non-food:

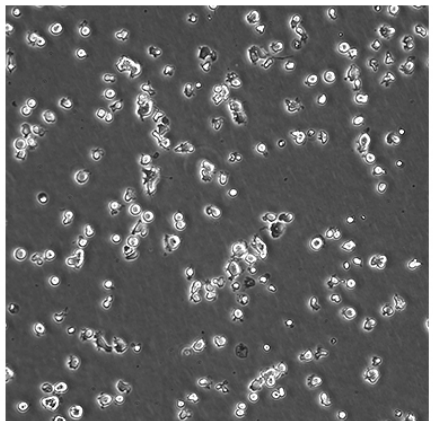
- antibiotics:
  - penicillin
  - streptomycin

1%

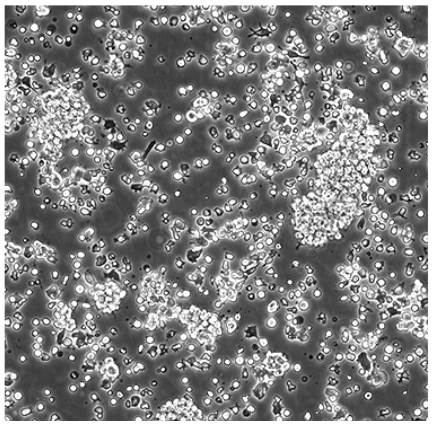
Splitting cells (and other “TC” jargon!)

1. Look at cells
   - estimate density
   - confirm absence of contamination
2. Count cells with hemocytometer
3. “Seed” new culture vessel

T75 flask

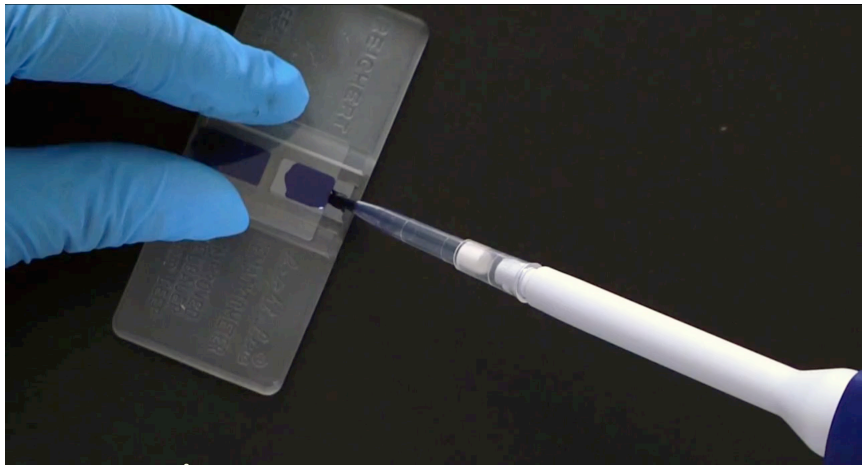


low density



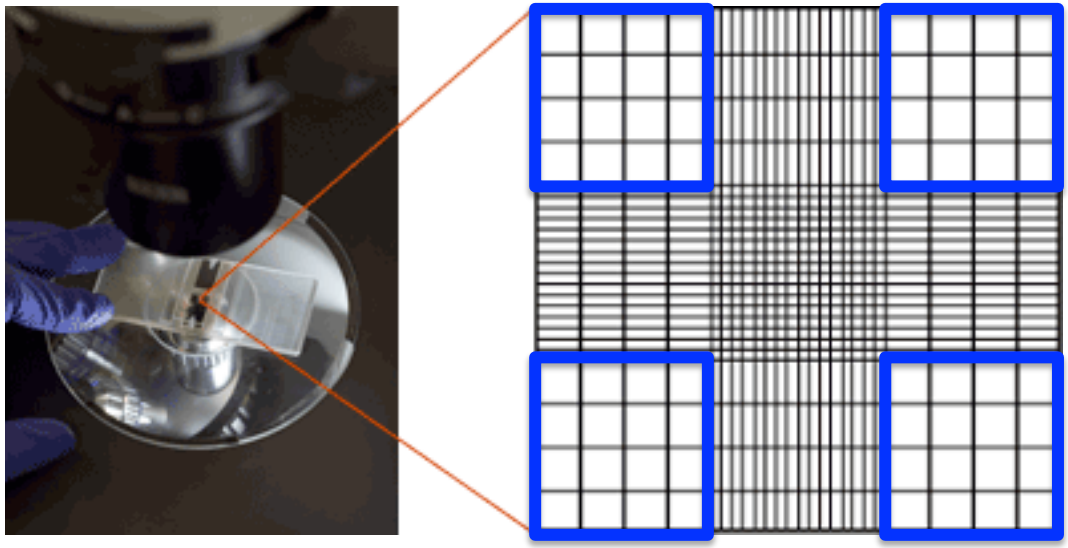
high density

Calculating number of cells



- Hemocytometer
- Trypan blue

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



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- 2 teams prepare a CometChip blue green
- 2 teams split cells in the tissue culture room red purple
- Make sure to keep notes in Benchling