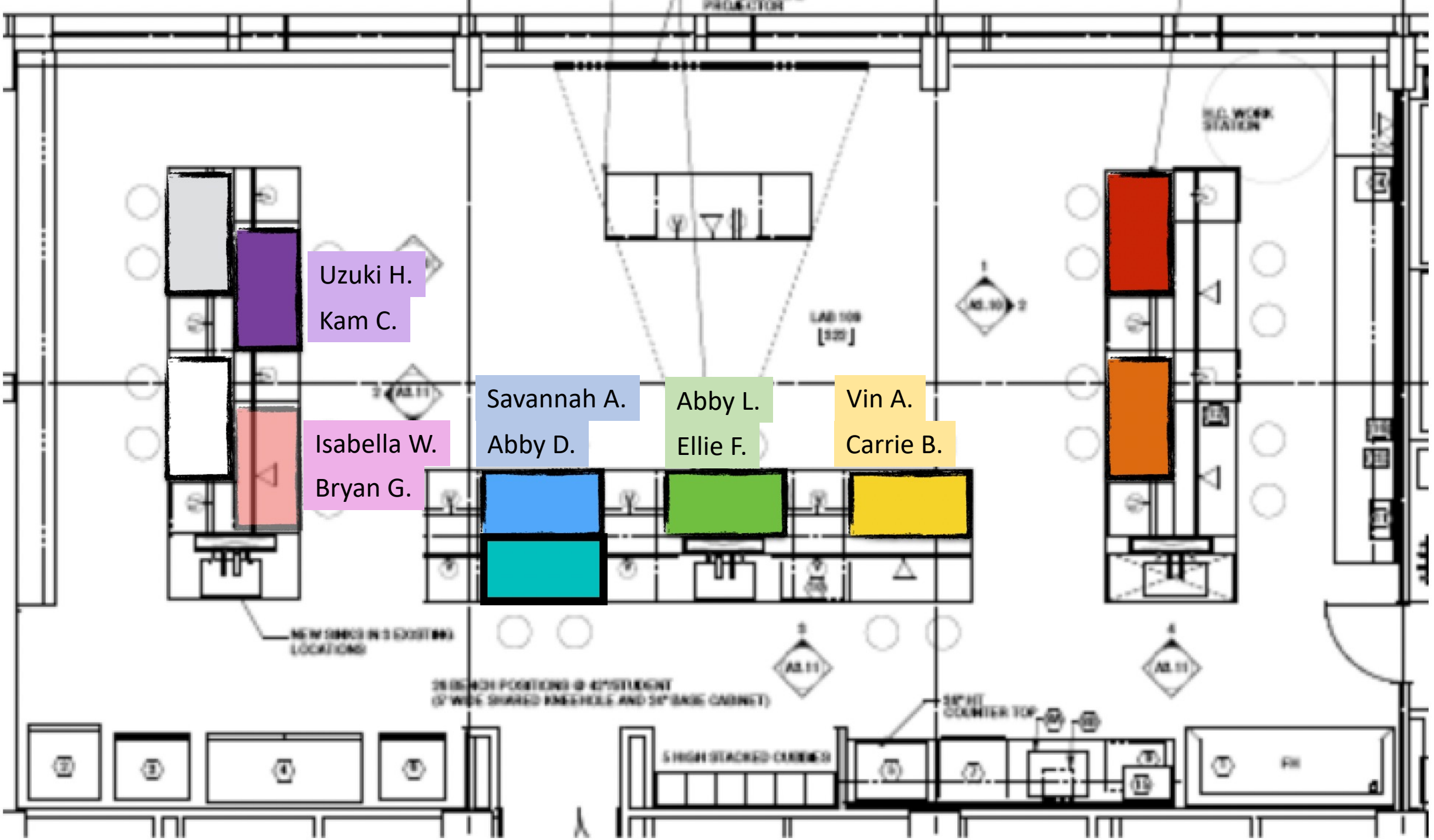
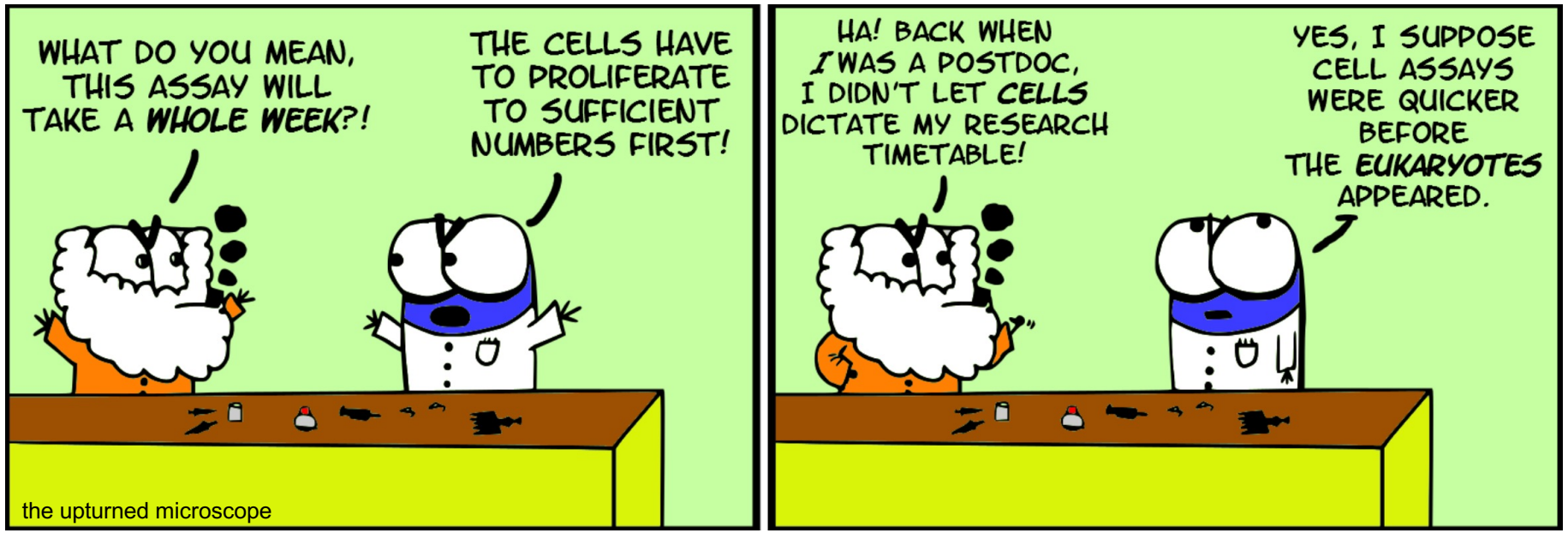

Uzuki H.
Kam C.
Isabella W.
Bryan G.
Savannah A.
Abby D.
Abby L.
Ellie F.
Vin A.
Carrie B.

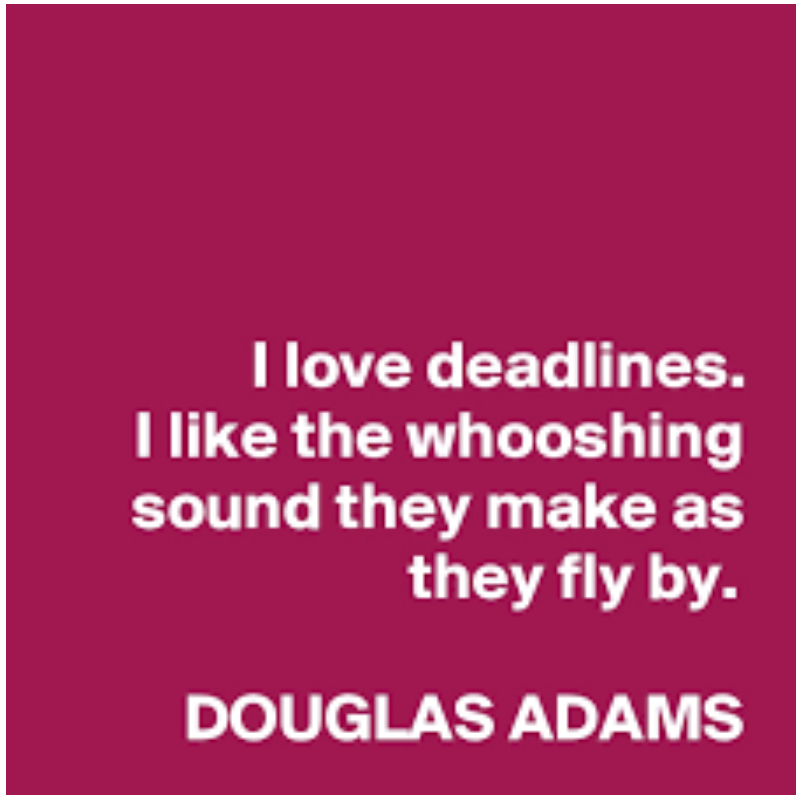
M1D1: Learn best practices for mammalian cell culture

1. Orientation Quiz
2. Prelab discussion
3. Learn about cell culture in the lab



Mod 1: Major Assignments

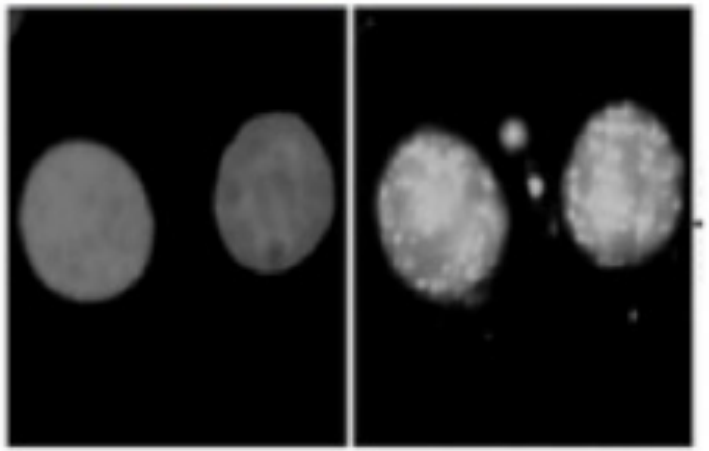
- Data summary (15%)
  - In a team
  - Draft due 10/12, final revision due 10/22
  - Format: Bullet points, .PPTX
- Research Talk (5%)
  - Individual, submit video via gmail
  - Due 10/1 by 10pm
- Lab quizzes (5% collectively)
  - Individual (orientation quiz is exception)
- Notebook (5% collectively)
  - Due 10/7 at 10pm, graded by Chyna
- Blog (part of 5% Participation)
  - Due 10/13 at 10pm

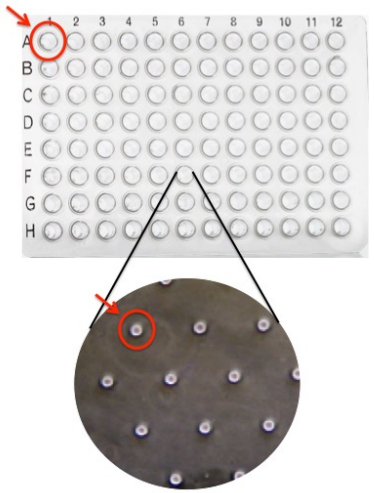


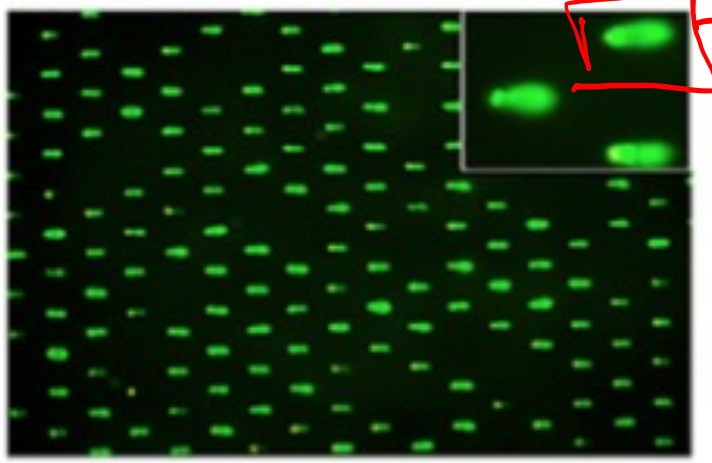
Overview of Module 1: Measuring Genomic Instability

Arsenic ROS

Research question: Does exposure to As inhibit, or decrease, repair of H_2O_2-induced DNA damage, raising the possibility that combined exposure is an important risk to public health?







Examine effect of H_2O_2 +/- As on double and single strand DNA breaks by measuring γH2AX foci formation

- Immunofluorescence (IF)
  - Cells attached to glass coverslips
- Cellular response to DNA damage

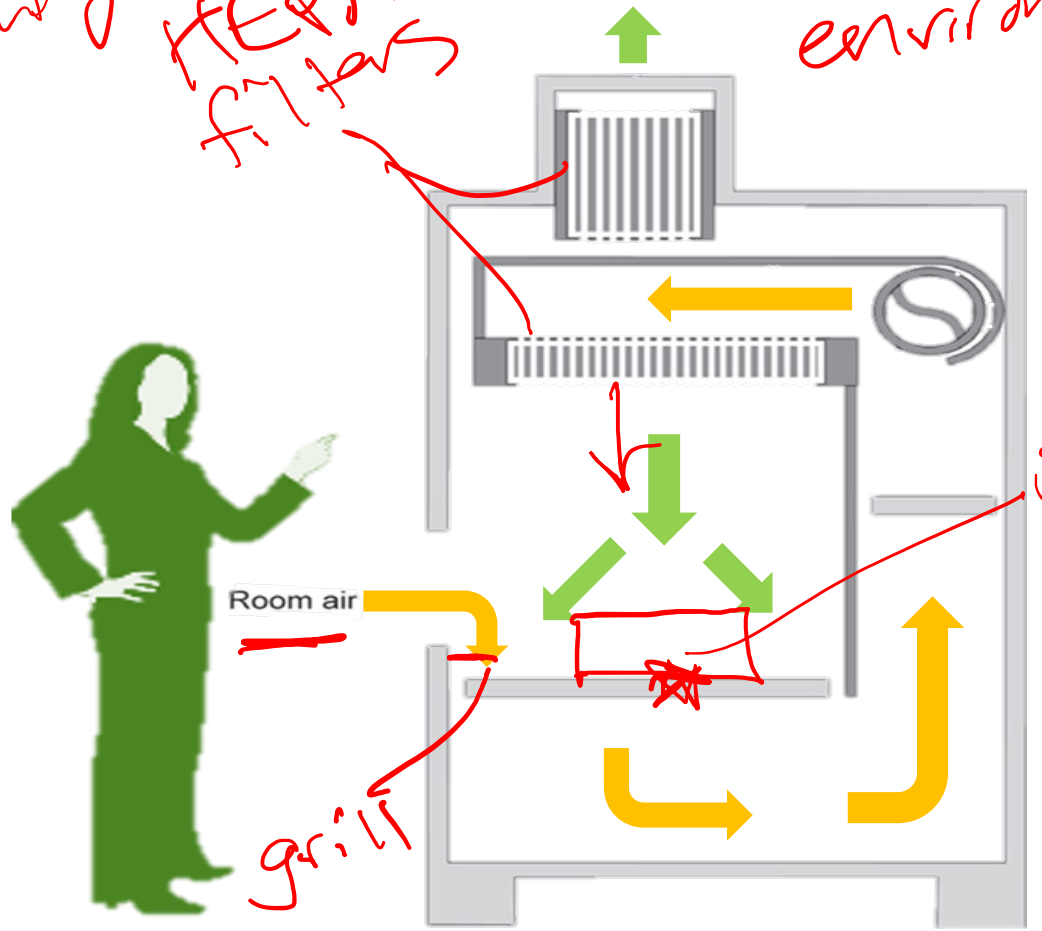
DNA Comet

Measure the effects of H_2O_2 +/- As on DNA damage by measuring DNA migration in agarose matrix

- CometChip assay
  - single cell gel electrophoresis in 96 well format
- Directly visualize stained DNA

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 not 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

We are using 1° MEF cells

Food:

- DMEM (Dulbecco's Modified Eagle Media)
  - Defined – exact measurement – consistent
    amino acids, vitamins, sugar/glucose, salts
    H_2O
- FBS (fetal bovine serum)
  - Undefined – varies between stock/lot
    lipids, growth factors, cytokines

Non-food:

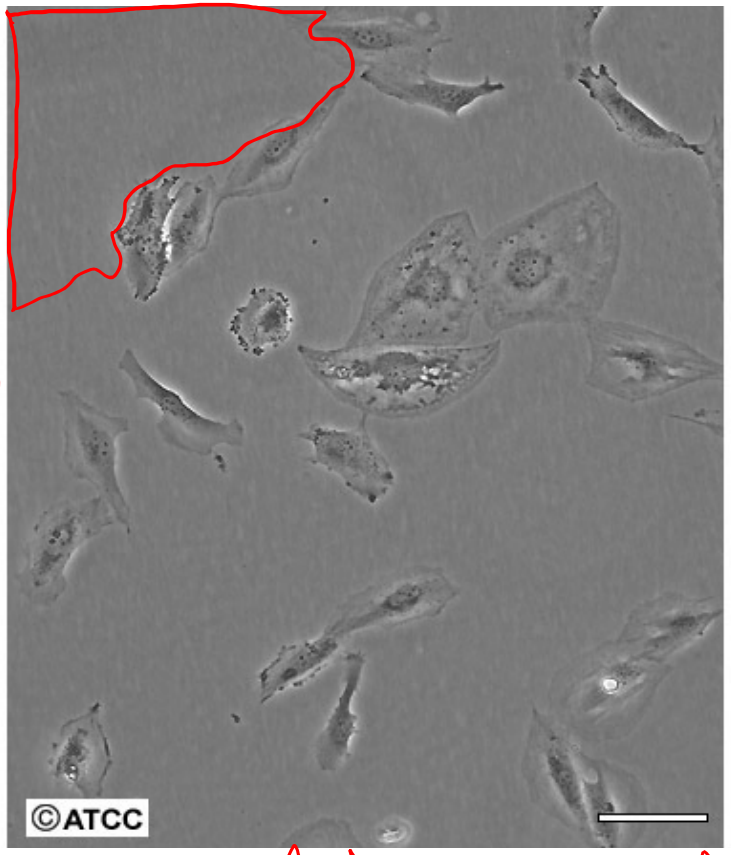
- antibiotics:
  - penicillin
  - streptomycin

→ PS prevents bacteria growth / contamination

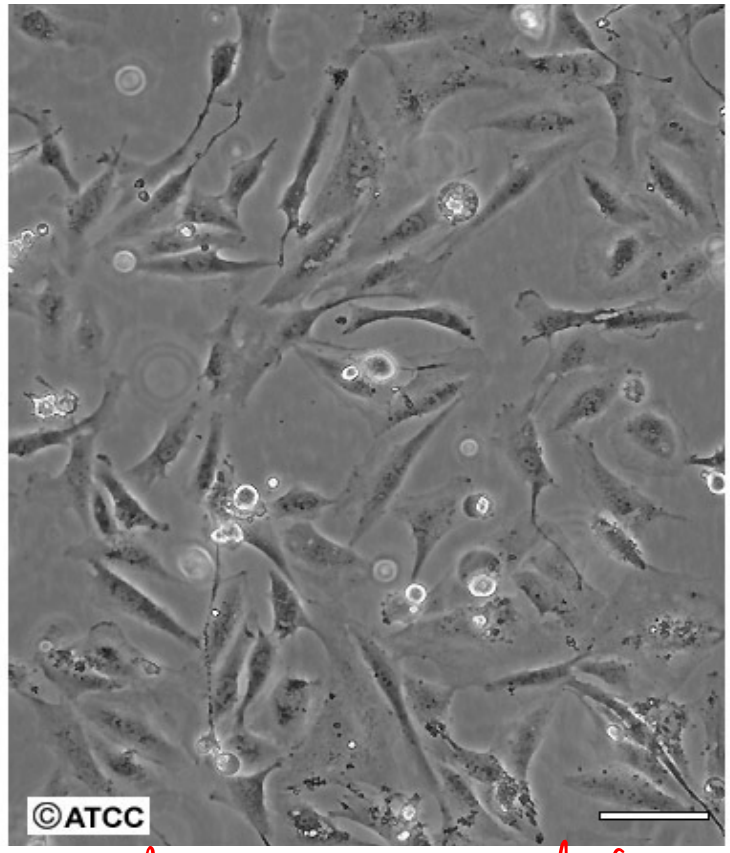
Mammalian Cell Culture Terminology

- Confluence - density of culture
  - 70-90% confluence split cell culture
- Splitting / subculturing
  - divide up cells in flask & move to new vessels - keep culture going
- Seeding - specific type of subculturing
  - experiment
  - specific vessel | count # of cells

Low Density 10-20%



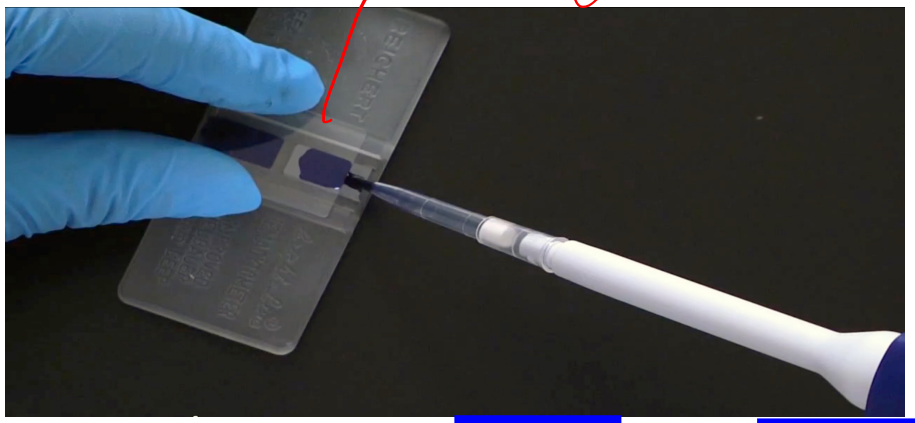
High Density 80-90%

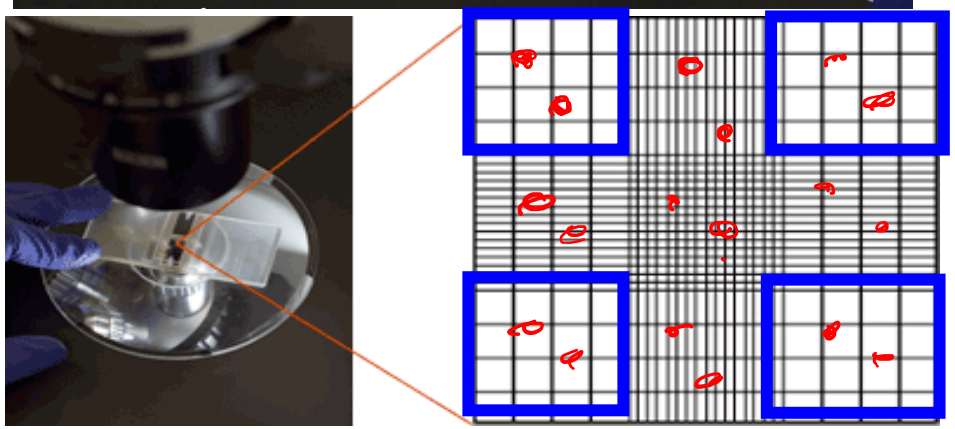


- Adherent vs. Non-Adherent / Suspension cells
  - ↳ trypsination

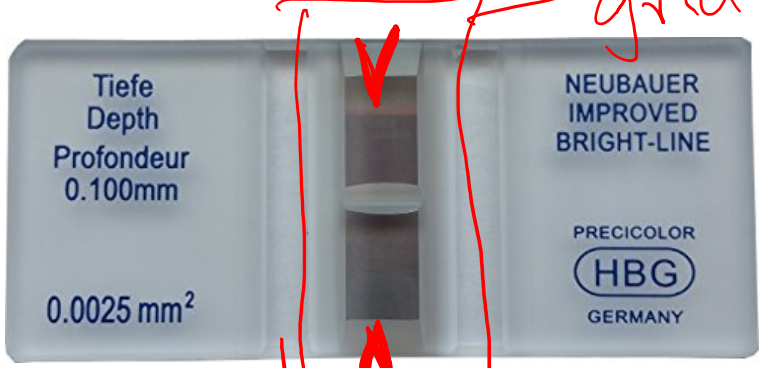
Counting cells

weighted coverslip





count



grid section

specialized glass slide

- Hemocytometer
  - cells for counting
- Trypan blue - stains dead cells

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

$\frac{8}{4} = 2 \times 10^4 = 20{,}000$ cells/mL

What should go in your notebook?

Laboratory notebook entry component:	Points: Complete	Partial	Missing
Date of experiment (include Module#/Day#) and Title for experiment	1	0.5	0
Hypothesis or goal / purpose	2	1	0
Protocols (link to appropriate wiki sections)	1	0.5	0
Answering questions embedded in wiki sections	5	3	0
Observations from demonstrations and video tutorials	3	2	0
*Visual details			
*Qualitative information			
*Raw data			
Data analysis	3	2	0
*Calculations			
*Graphs and Tables			
Summary and interpretation of data	3	2	0
*What did you learn?			
*How does this information fit into the larger scope of the project?			
Information is clear	2	1	0
All days represented	5	3	0
OVERALL /25			

Notebook entries for module are graded the day after the module ends.

- One entry (selected by instructors) will be graded according to this rubric
- The remaining entries will be checked for completeness.

Daily Notebook Check = participation points

<u>Before you leave each day</u>

- physically show Chyna your Benchling notebook

1. She will check to see that you have written more than just copying the template and writing a sentence or two
2. She will record that you are making adequate progress through the laboratory exercises
3. You will get participation points!

For today:

1. Complete Orientation quiz with lab partner

2. Practice cell culture and seed cells for H2AX assay

3. Research MEF cells

TC - printed protocols

- pink/purple team - adding coverslips to plate

For M1D2:

Answer wiki questions in homework tab to begin to outline your Background and Motivation section

- You will discuss the structure of the Background and Motivation section during the next class

Must visit the Comm Lab before M1D5!