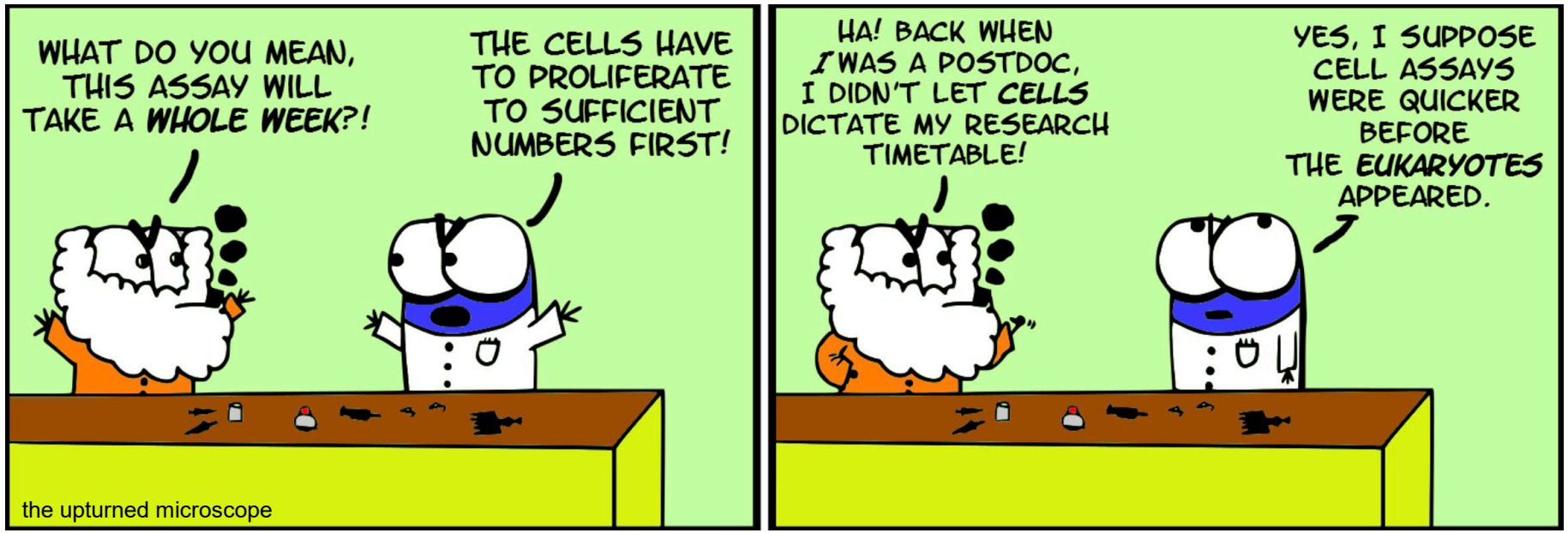

Katie K.
Jordan D.
Isabella G.
Sofia F.
Victory Y.

M1D1: Learn best practices for mammalian cell culture

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

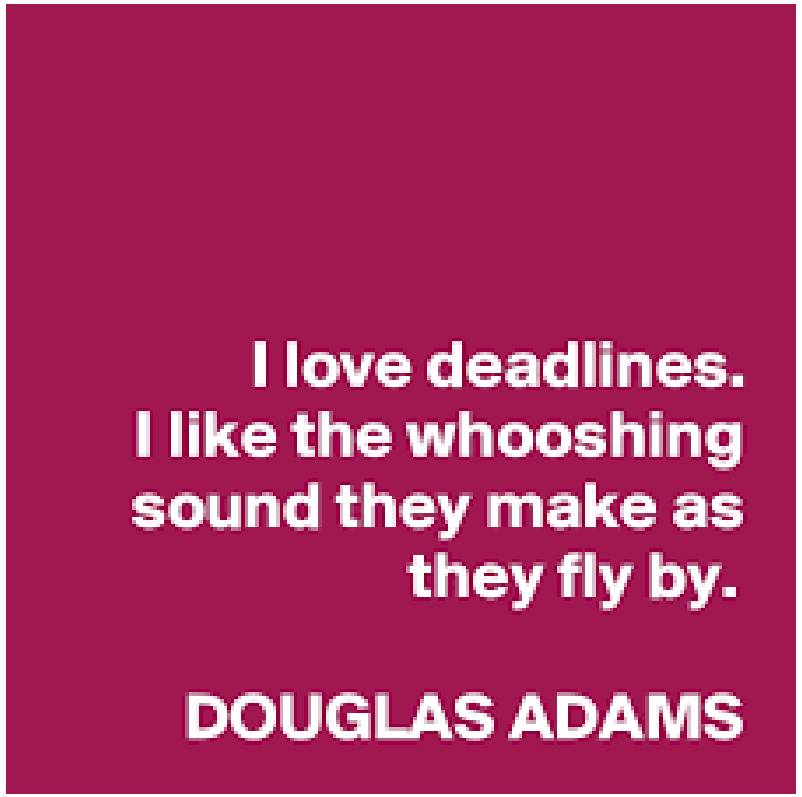
Reminder

Lecture attendance will only be taken for the first 15 minutes of class



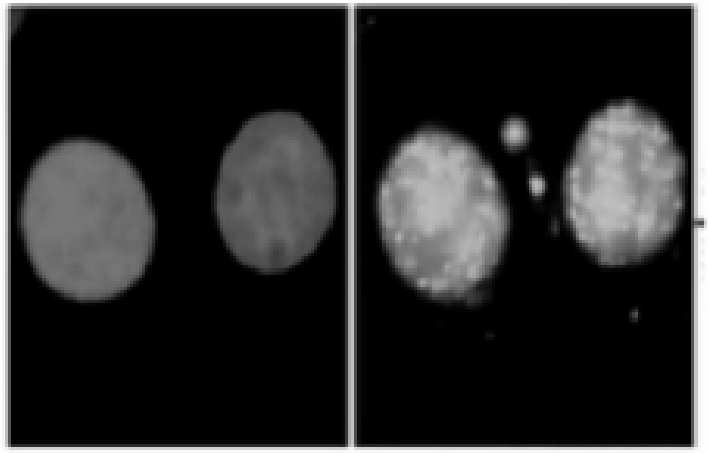
Mod 1: Major Assignments

- Data summary (15%)
  - In a team
  - Draft due 10/11, final revision due 10/21
  - Format: Bullet points, .PPTX
- Research Talk (5%)
  - Individual, submit video via gmail
  - Due 9/30 by 10pm
- Lab quizzes (5% collectively)
  - Individual (orientation quiz is exception)
- Notebook (5% collectively)
  - Due 10/6 at 10pm, graded by Simone
- Blog (part of 5% Participation)
  - Due 10/12 at 10pm



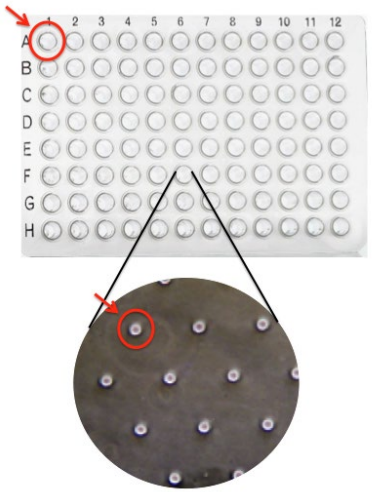
Overview of Module 1: Measuring Genomic Instability

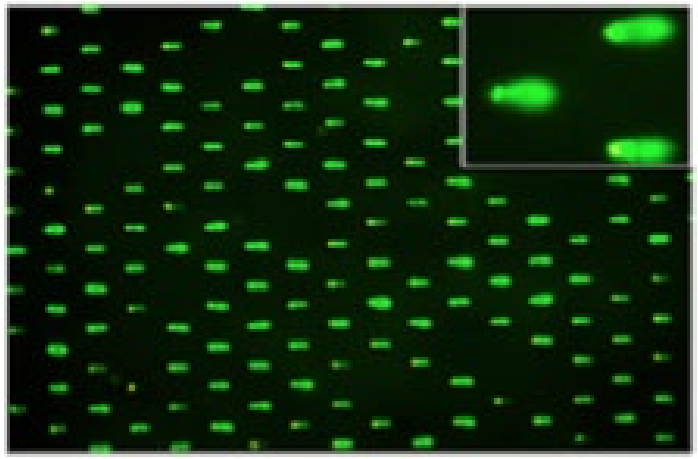
Research question: Does exposure to As inhibit, or decrease, repair of H_2O_2-induced DNA damage, raising the possibility that combined exposure is an important risk to public health?



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



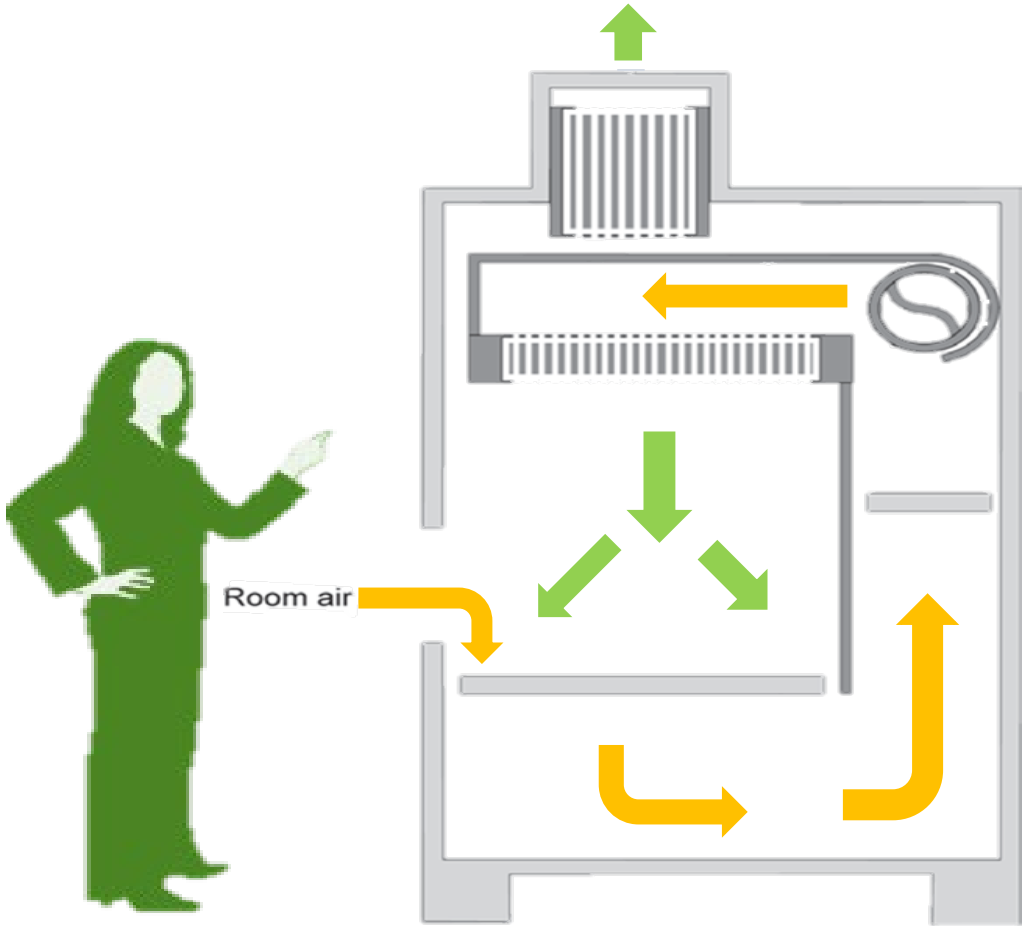


Measure the effects of H_2O_2 +/- As on DNA damage by measuring DNA migration in agarose matrix

- 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



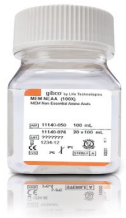
Mammalian Cell Culture Medium

We are using MEF cells

Food:

- DMEM (Dulbecco's Modified Eagle Media)
  - Defined

- FBS (fetal bovine serum)
  - Undefined



Non-food:

- antibiotics:
  - penicillin
  - streptomycin

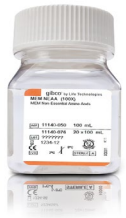
Mammalian Cell Culture Medium

We are using MEF cells

Food:

- DMEM (Dulbecco's Modified Eagle Media)
  - Defined

- FBS (fetal bovine serum)
  - Undefined



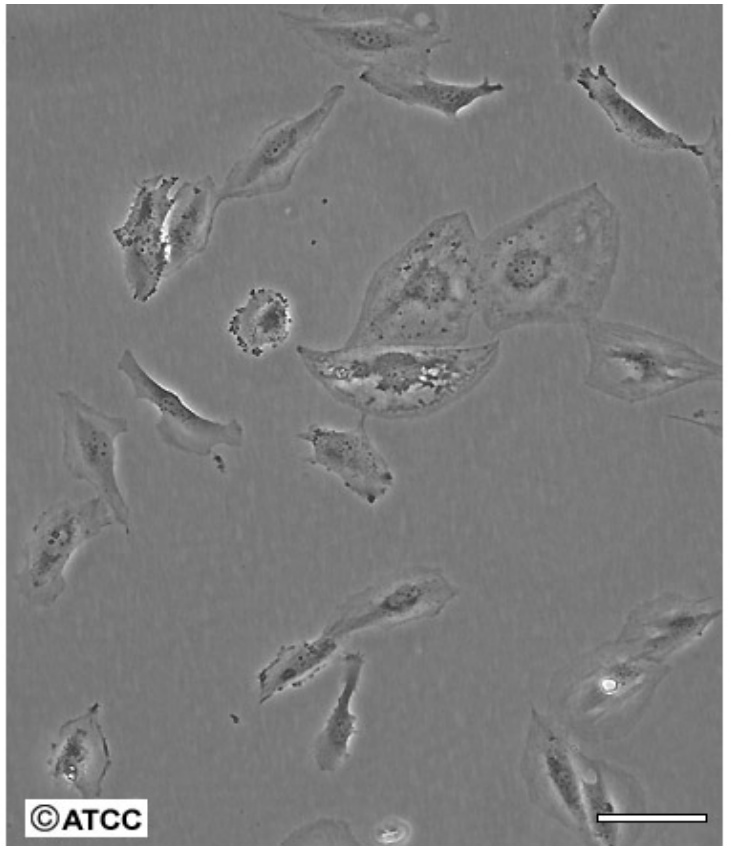
Non-food:

- antibiotics:
  - penicillin
  - streptomycin

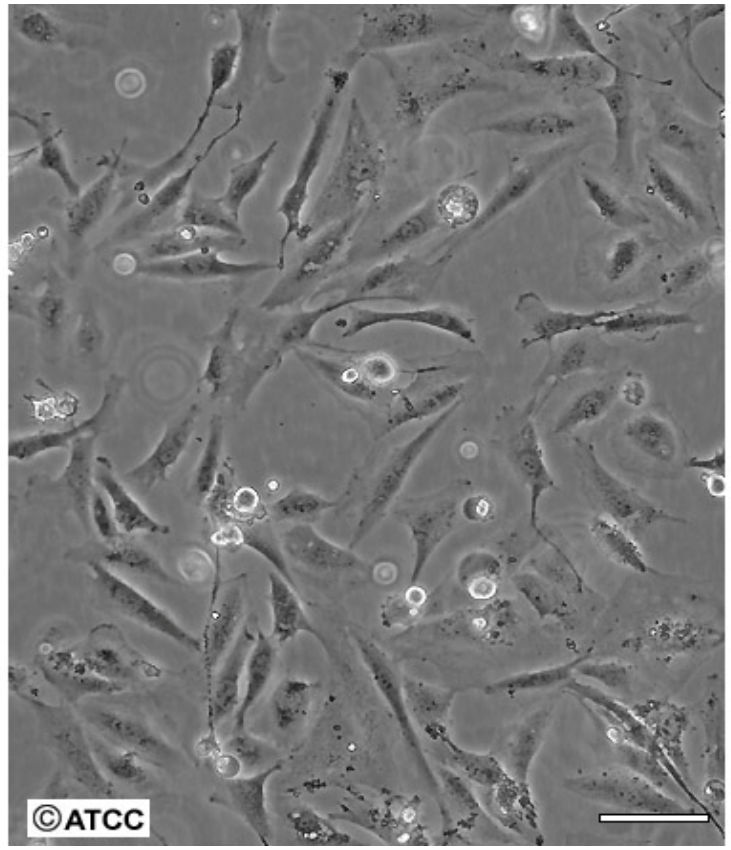
Mammalian Cell Culture Terminology

- Confluence
- Adherent / Non-adherent
- Splitting / Passaging
- Seeding

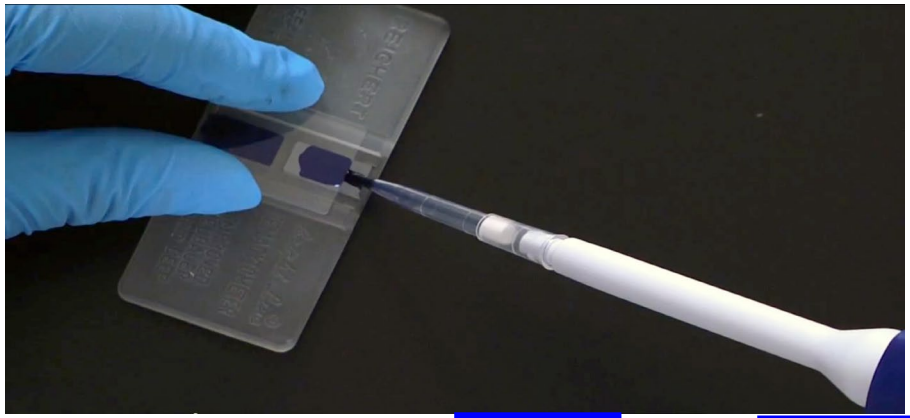
Low Density

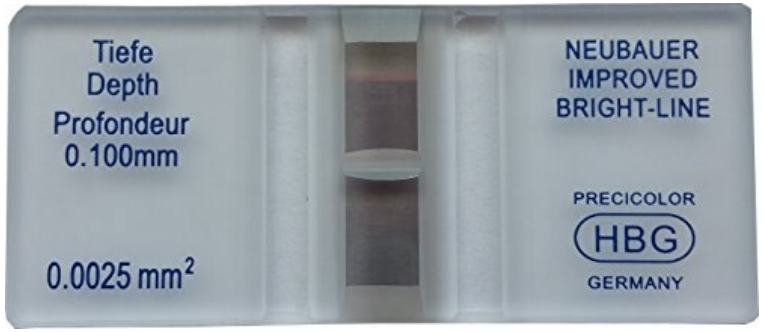


High Density



Counting cells





- Hemocytometer
- Trypan blue

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

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>

- Make sure Bishal has seen your Benchling notebook

1. He will check to see that you have written more than just copying the template and writing a sentence or two
2. He 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
   - Please watch video on wiki to prep for procedure. TC technique will also be demonstrated.

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!