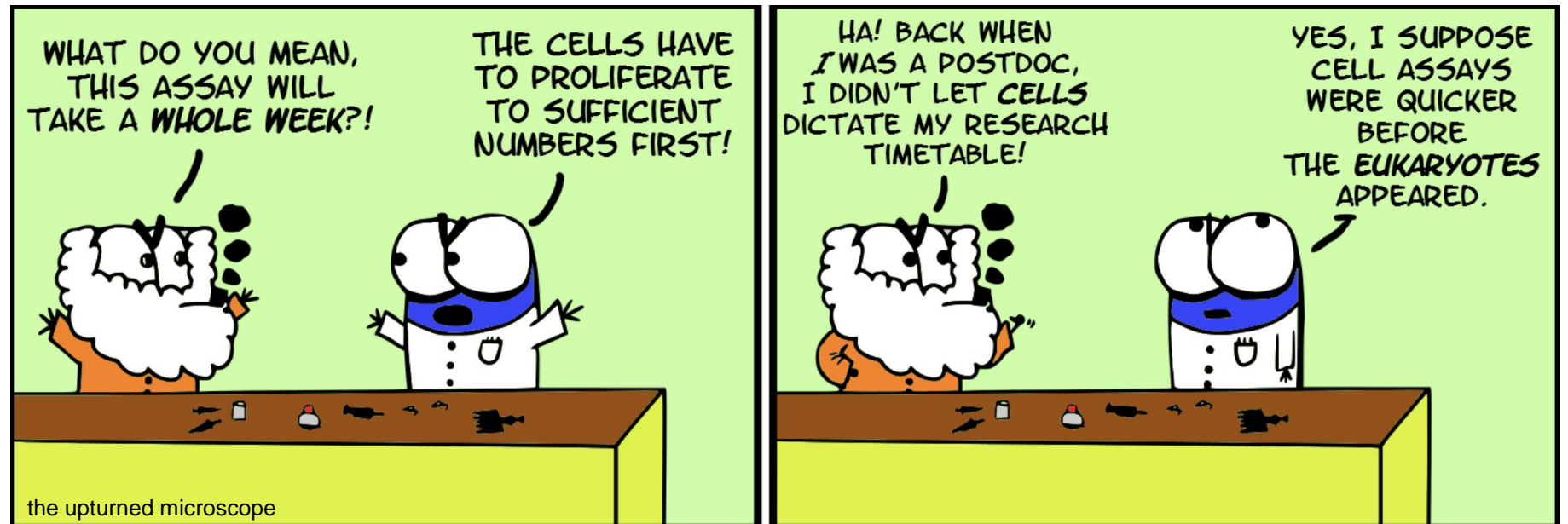




M1D1: Learn best practices for mammalian cell culture

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



Mod 1: Major Assignments

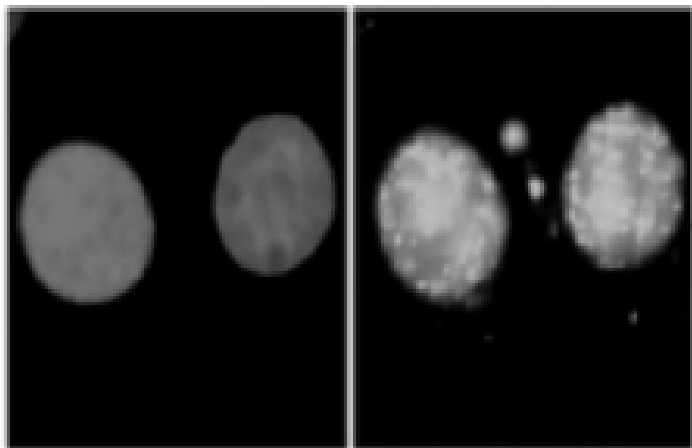
- **Data summary (15%)**
 - In a team
 - Draft due 10/12, final revision due 10/22
 - Format: Bullet points, .PPTX
- **Research Talk (5%)**
 - Individual, submit video via gmail
 - Due 10/1 by 10pm
- **Lab quizzes (5% collectively)**
 - Individual (orientation quiz is exception)
- **Notebook (5% collectively)**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

**I love deadlines.
I like the whooshing
sound they make as
they fly by.**

DOUGLAS ADAMS

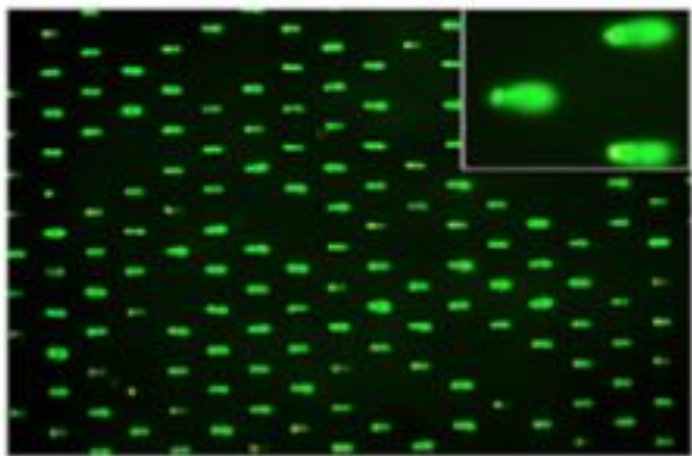
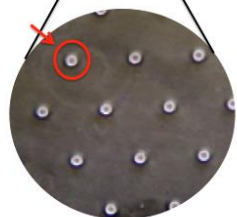
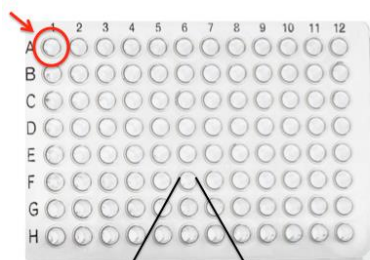
Overview of Module 1: Measuring Genomic Instability

Research question: Does exposure to As inhibit, or decrease, repair of H₂O₂-induced DNA damage, raising the possibility that combined exposure is an important risk to public health?



Examine effect of H₂O₂ +/- As on double and single strand DNA breaks by measuring γ H2AX foci formation

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

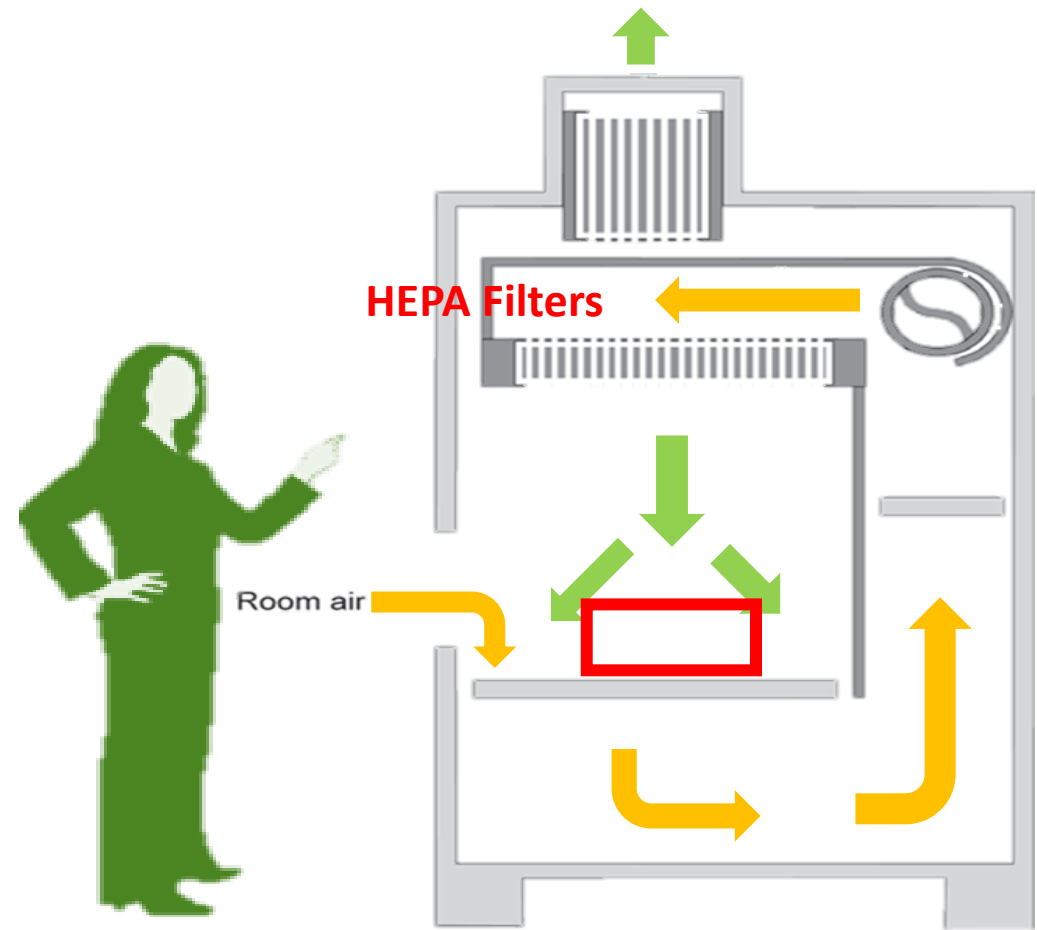


Measure the effects of H₂O₂ +/- As on DNA damage by measuring DNA migration in agarose matrix

- 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** items in hood (Media, coverslips, forceps – anything that contacts cells)



Mammalian Cell Culture Medium

We are using _____ cells



Food:

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

**Glucose, salts, amino acids, vitamins
Phenol Red (pH indicator)**



- FBS (fetal bovine serum)
 - **Undefined**

**BSA, proteins, Growth factors, cytokines,
lipids, cholesterol, nonessential amino
acids**



Non-food:

- antibiotics:
 - penicillin
 - streptomycin

Mammalian Cell Culture Terminology

- Confluence

What % of the coverslip/flask is filled?

- Splitting

Culturing your cells by moving them from one tube/flask to other flasks

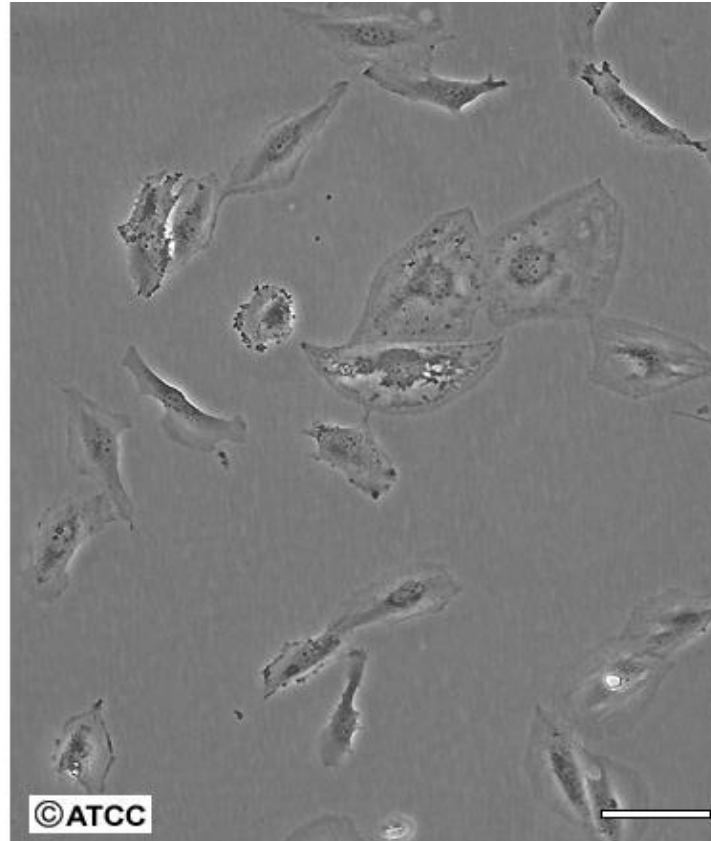
- Seeding

Adding a known # of cells to a vessel in preparation for a specific experiment

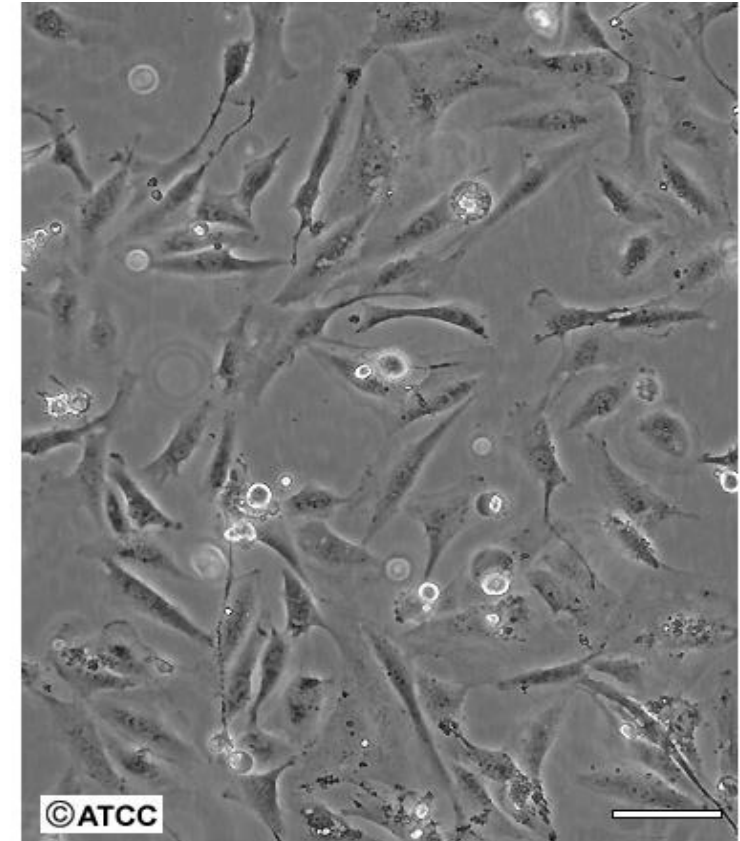
- Adherent/Nonadherent

Cells that like growing on stuff (like glass) vs prefer growing in suspension

Low Density **10-20%**

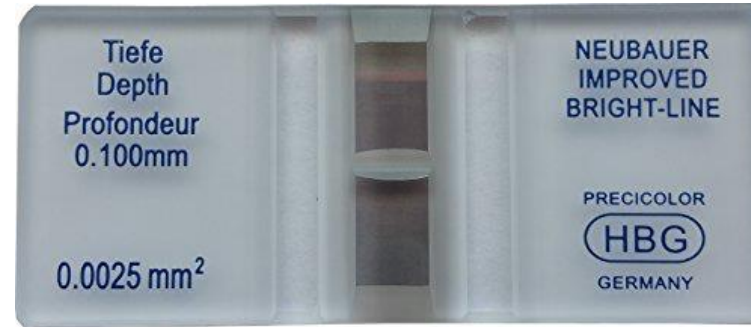
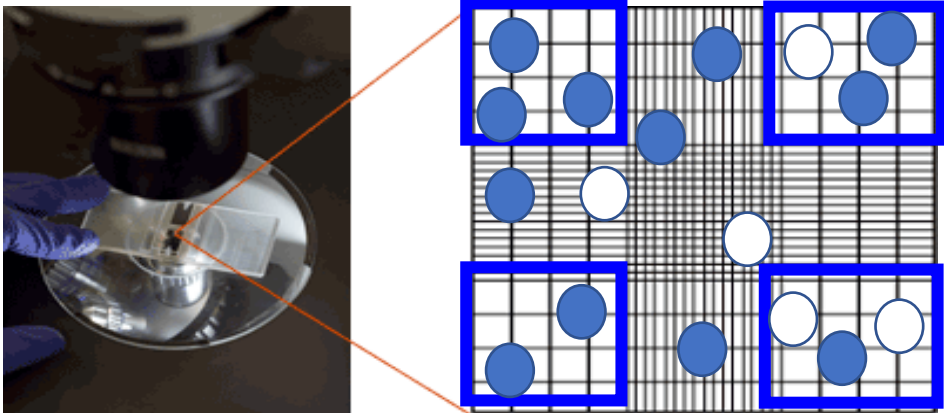
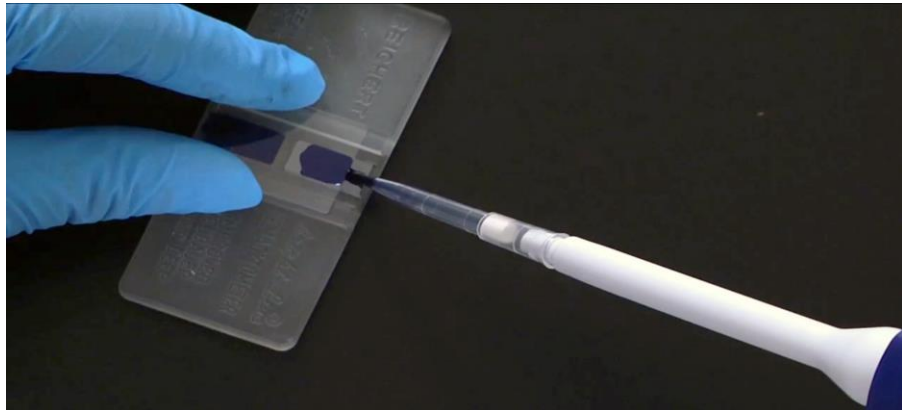


High Density **80-90%**



Counting cells

Weighted Glass



$$(8 \text{ cells}) / 4 \text{ corners} * 10,000 = 20,000 \text{ cells/ml}$$

- Hemocytometer

Specialized Glass slide

- Trypan blue

Cell Exclusion Dye – dead cells appear BLUE

$$\# \text{ cells / mL} = 10,000 \times \text{average of 4 corners}$$

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

Before you leave each day

- physically show Alex 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
3. Research MEF cells

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!