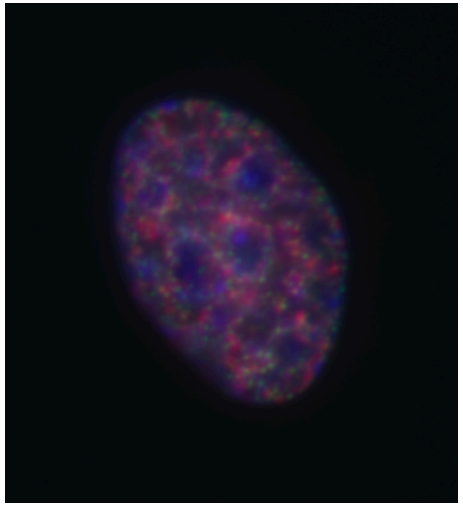
M1D4: Image H2AX and perform CometChip

9/25/19

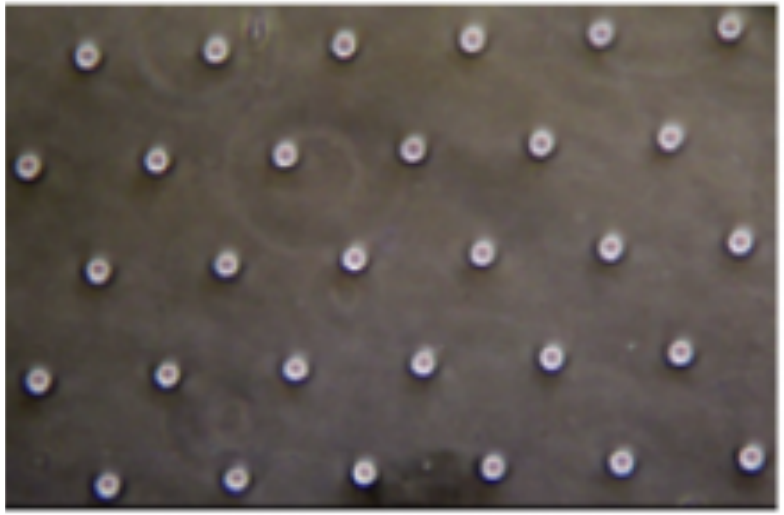
1. Quiz…
2. Prelab discussion Part 1
3. Load and treat cells for MMS
4. Prelab Part 2
5. As Treatments in CometChip
6. Finish H2AX staining
7. Lyse CometChip

Overview of Module 1: Measuring Genomic Instability

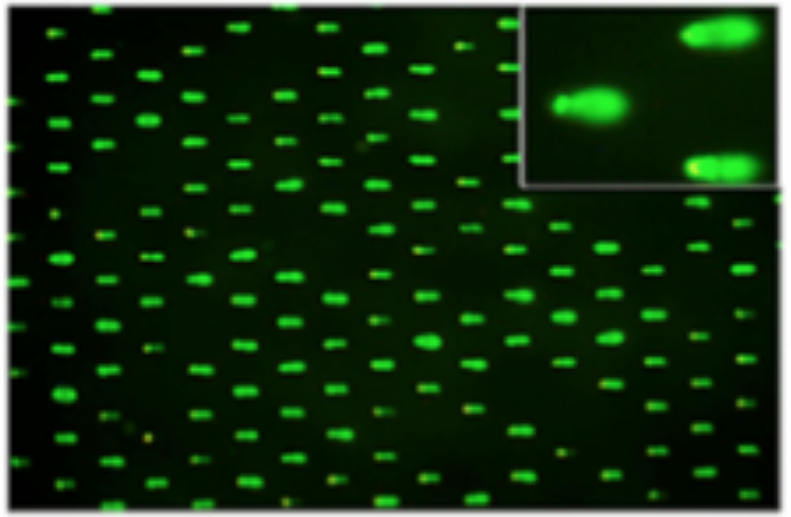
Aim: Evaluate effect of Arsenic exposure on methylation induced base excision repair (BER)



γH2AX assay



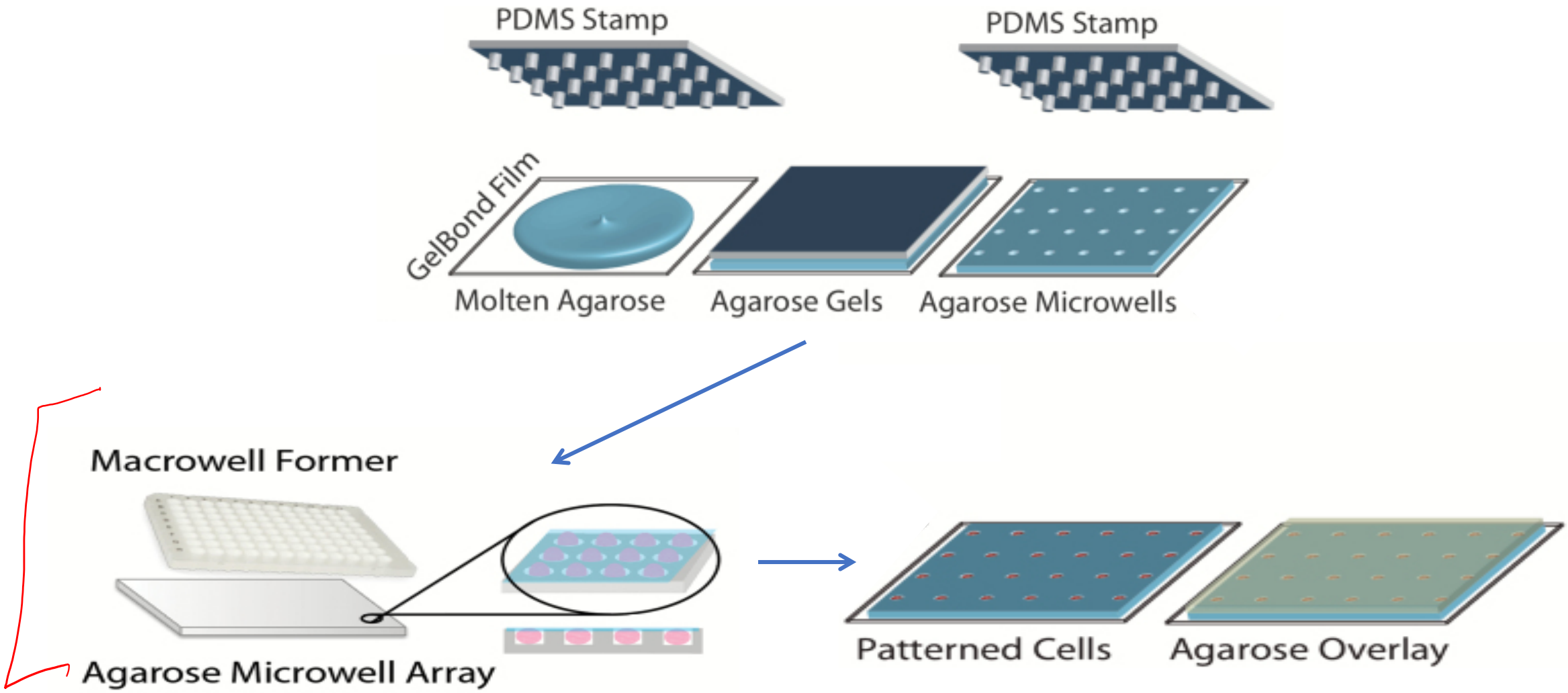
Optimize CometChip loading



CometChip assay

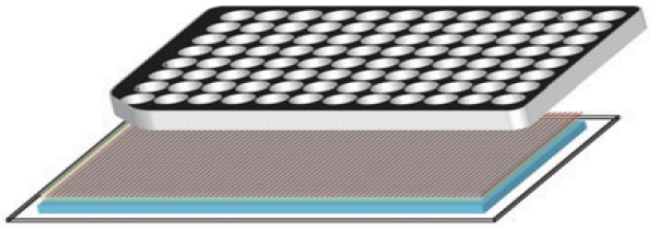
2°

Overview of the CometChip assay: pouring and loading cells



Overview of CometChip Assay: chemically treating cells and visualization

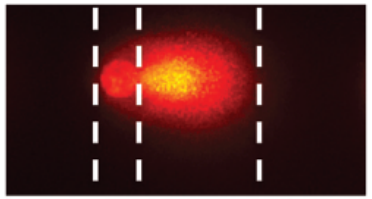
Treat captured cells in comet chip with MMS and As



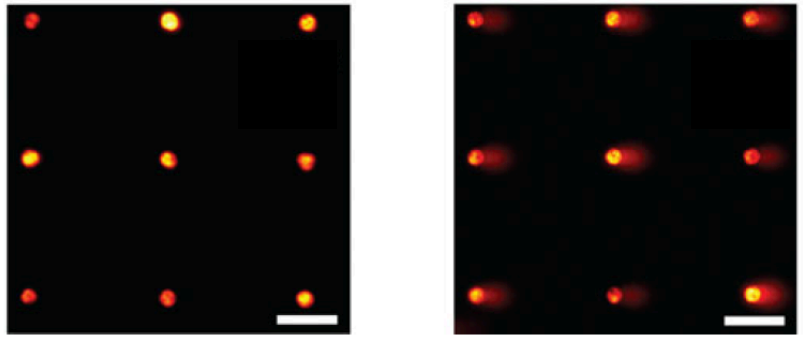
Today End

Lyse cells & unwind DNA
(DNA still captured agarose in overlay)

Agarose Electrophoresis

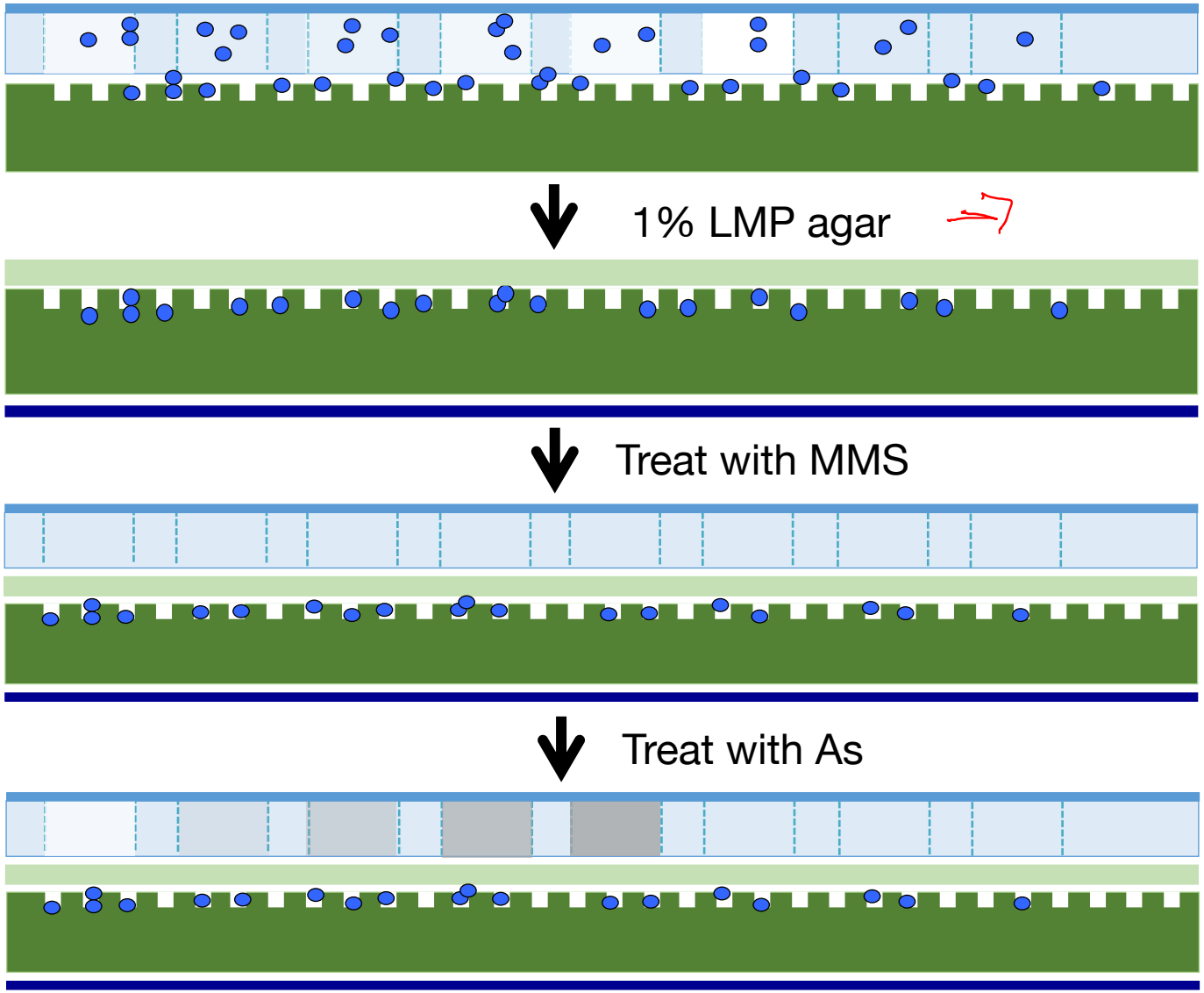


Analysis via Matlab



Stain DNA and image via fluorescence microscopy

Experimental Overview



Load _____cells per macrowell

Be sure to mix your cells right before loading!!!

Replace

Wash off mutagen (carefully)

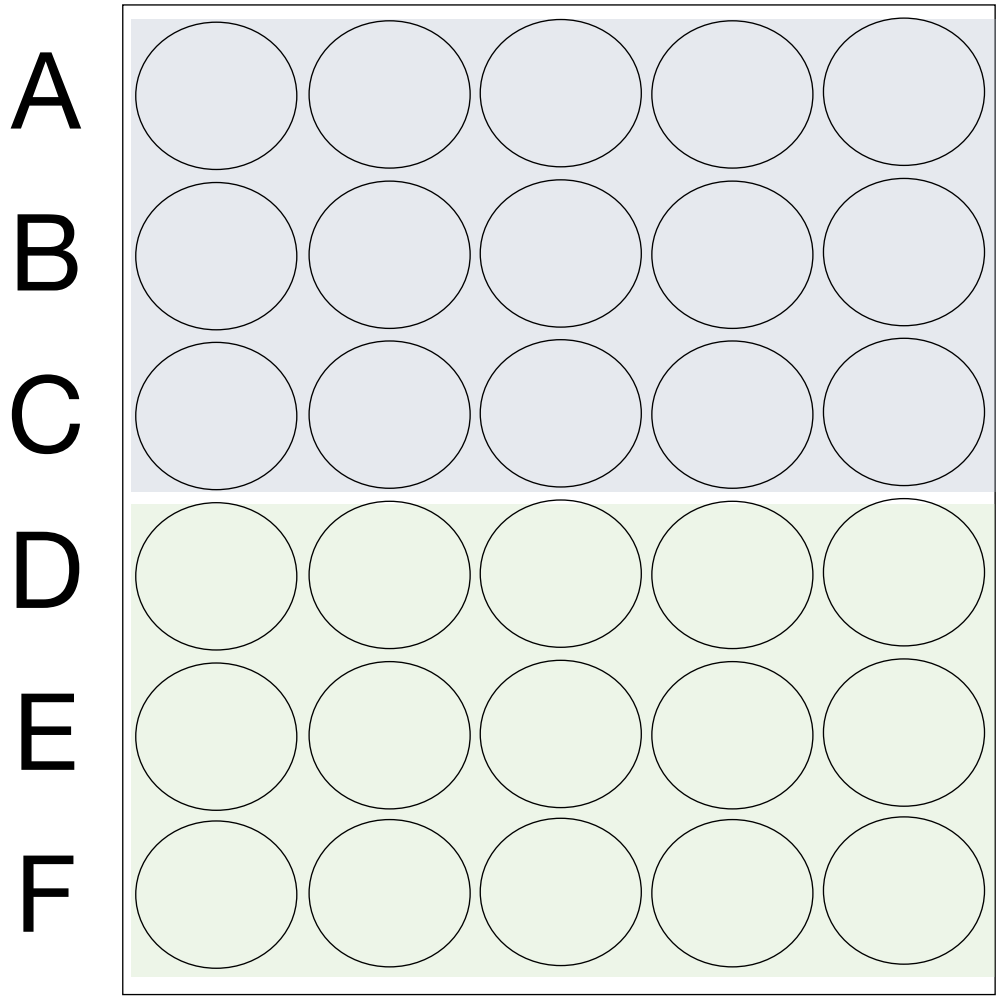
Wash off arsenite (carefully)
Leave in lysis buffer

Keep track of the wells

Handling tips:

- MMS stock should be left on front bench, dilutions made in DMEM
- Minimize waste and collect all MMS!
- Must wear green flocked gloves, goggles

MMS 1hr, 37C in TC



No MMS
(media only)

Control

0.4mM MMS
(why is this condition on the bottom?)

0.2mM
0.8mM

Preparing MMS dilution

Treat with: 0 or 0.4mM MMS

- 37°C for 1hr
- Add 100µl of drug dose to each macrowell
- Triplicate: each concentration will have three macrowells for each cell line
  - Make 2 ml of each concentration

Stock: 12 mM

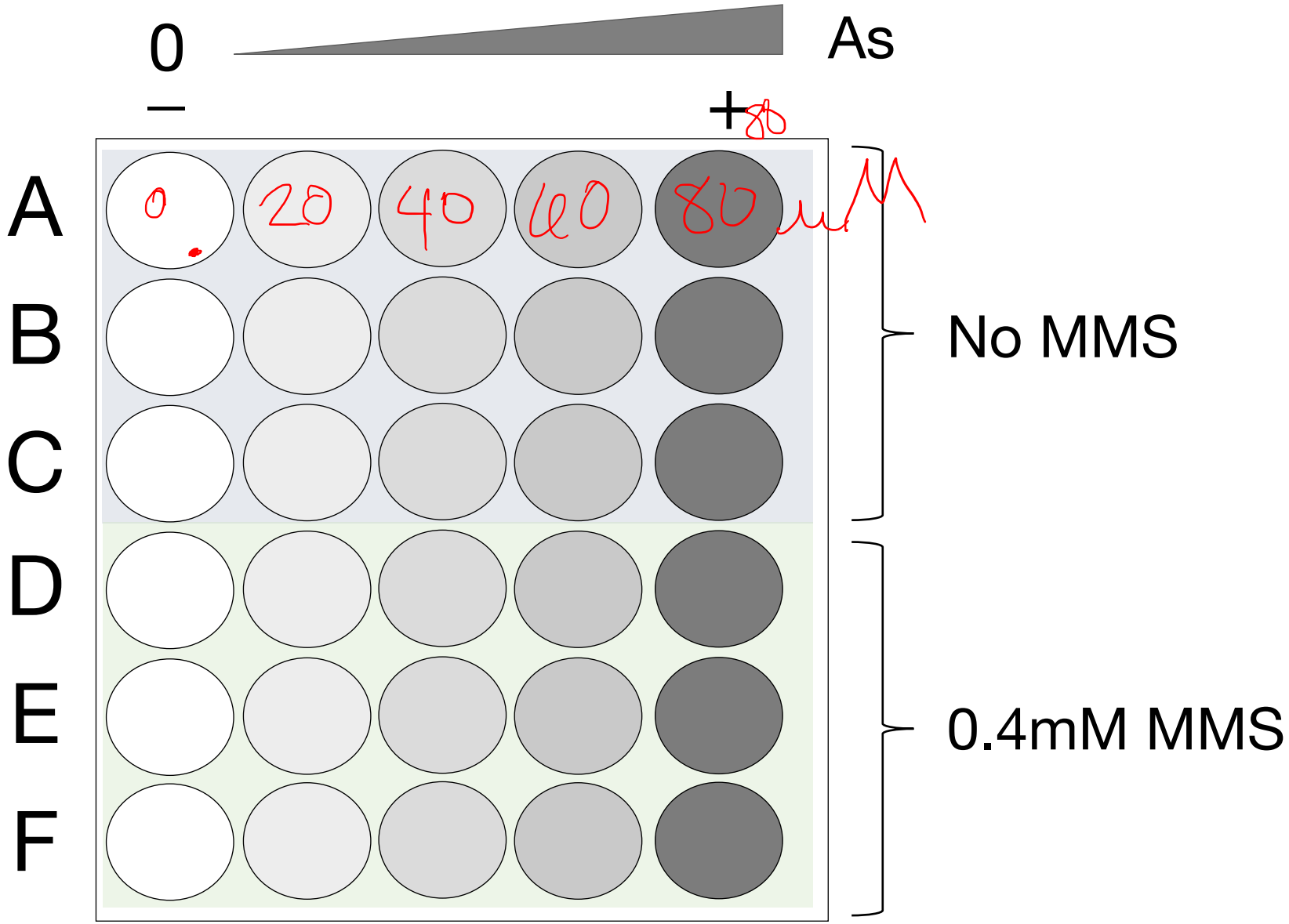
[Final]: 0.4mM

$$C_1V_1=C_2V_2$$

1) Cell # / macrowell

2) [MMS]

Keep track of the wells– 5 concentrations



Preparing As dilution series:

Treat with: 0, 20, 40, 60, 80 µM As

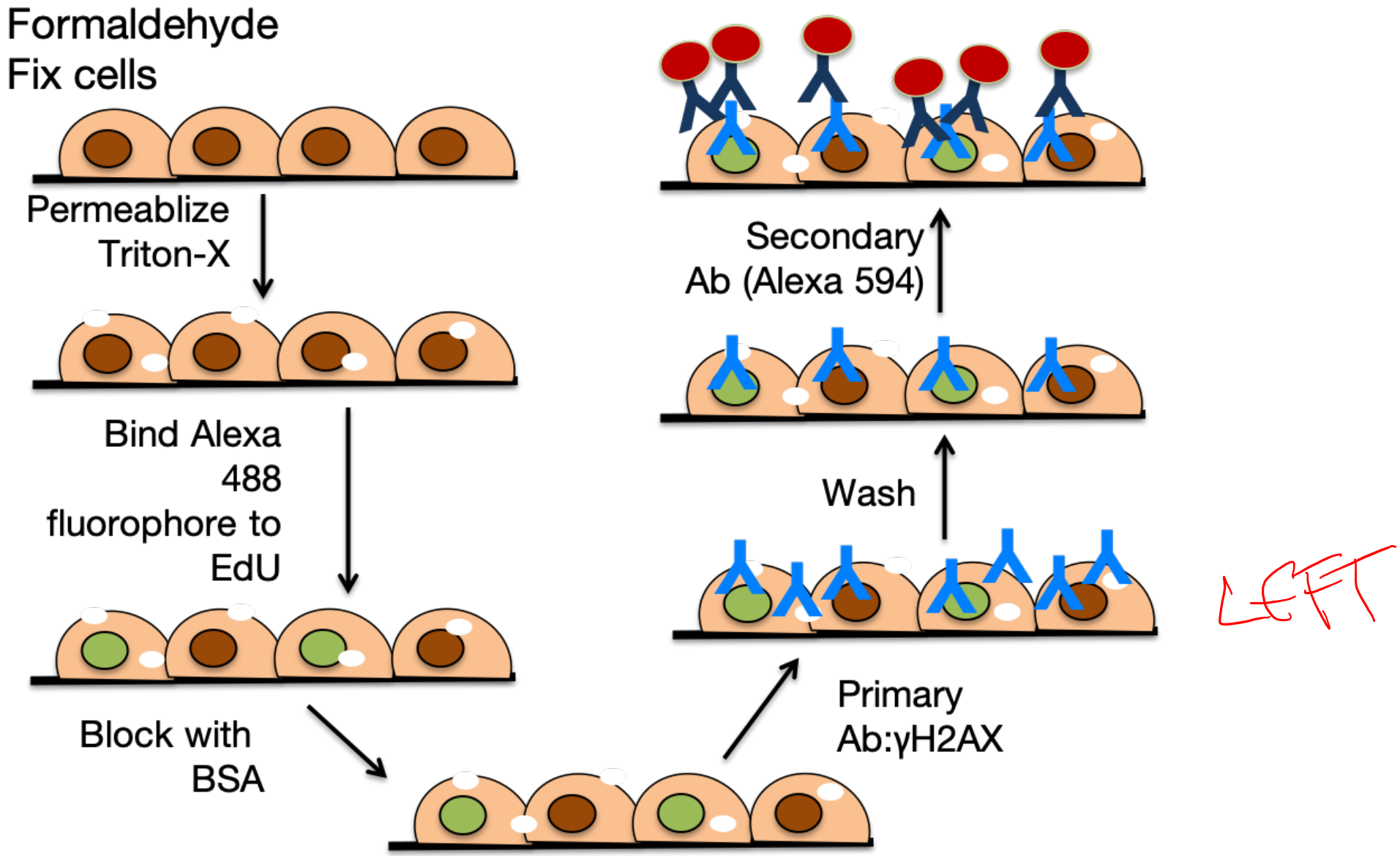
- 37°C for 2hr
- Add 100µl of drug dose to each macrowell
- Triplicate: each concentration will have three macrowells for each cell line
  - Make 1000 µl of each concentration

1mL

Stock 1: 100 mM
Stock 2: 1:1000 dilution of Stock 1, 100µM

	0	20	40	60	80uM
Stock 2	0ul				
Media	1ml				

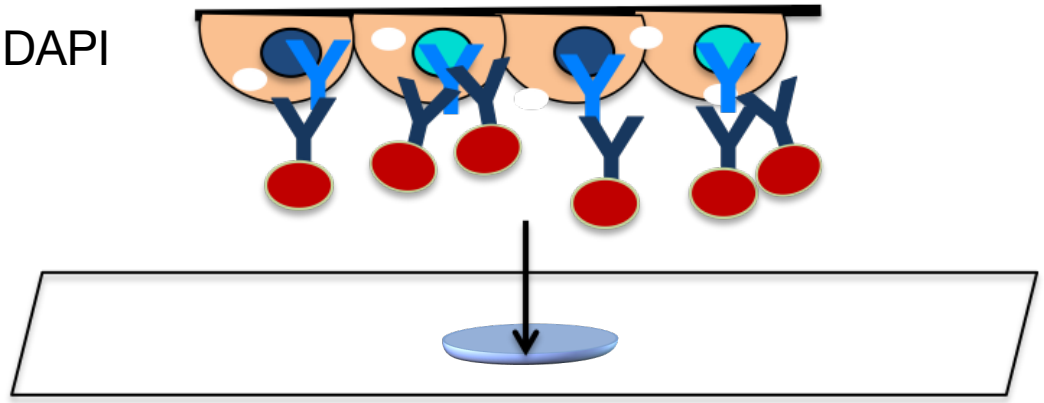
Using immunofluorescence (IF) to detect DSBs



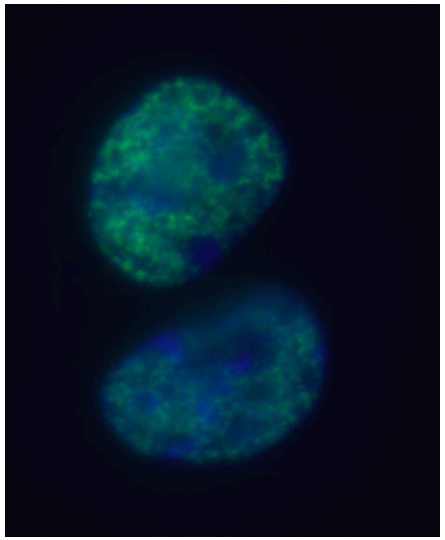
Imaging DSBs with fluorescent microscopy

Wash TBS 1X
DAPI incubation 10 min
Wash TBS 1X

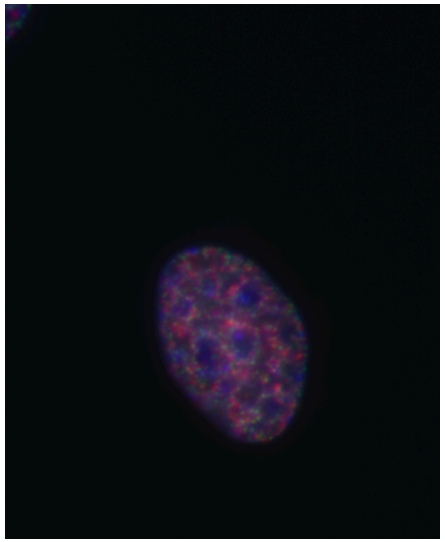
Add DAPI



Mount coverslip on glass slide
with mounting media



DAPI + EdU
(no γH2AX)



DAPI + EdU
+ γH2AX

Notes on Data Summary and data slides…

Data Summary to be completed using PowerPoint

Each figure should relay one message

- Subpanels should be related to single conclusion
- Rather than multiple microscopy panels consider representatives and / or quantified representation of the data
- Remember the title and caption!!

Text should be related to results in the figure

- See guidelines in homework description
- Write in bullets!!

Results slide example

- Image **should not be** the entire page
  - Only needs to be large enough to be clear / visible
- Title **should be** conclusive
  - Don’t include what you did, rather state what you found
- Caption **should not** detail the methods
  - Define abbreviations, symbols, etc.

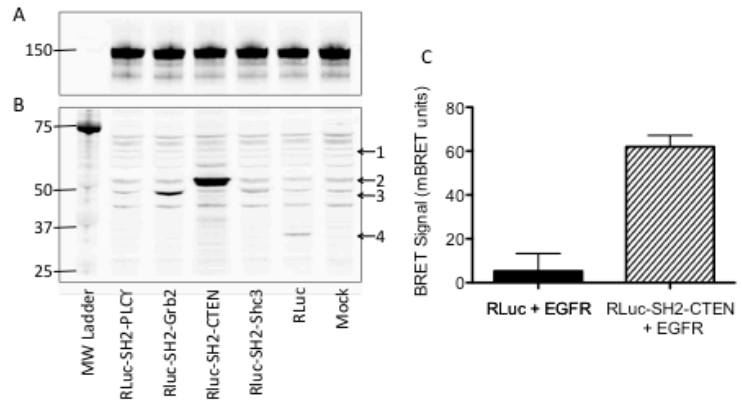


Figure 1: Development of BRET assay to monitor EGFR and SH2 domain interactions. CHO-K1 cells were transfected with Citrine-EGFR (A) and renilla luciferase (RLuc)-tagged SH2 domains from PLCg, Grb2, CTEN, and Shc3 (B). Western blots of CHO-K1 lysates were probed with anti-EGFR (A) or anti-RLuc (B) antibodies. Arrowheads indicate the expected molecular weight of the RLuc-tagged proteins; (1) RLuc-SH2-PLCg, (2) RLuc-SH2-CTEN, (3) RLuc-SH2-Grb2 and RLuc-SH2-Shc3, and (4) RLuc alone. Mock indicates no cDNA was utilized during transfection. (C) For CTEN only, BRET signal was quantified using a luminometer after stimulation of CHO-K1 with 100 ng/mL EGF for 15 min.

BRET system effectively measures EGFR activation:

- To determine if the BRET system could be used to monitor EGFR activation, CHO-K1 cells were transfected with fluorescent EGFR and luciferase-tagged SH2 domains and a BRET assay was performed after growth factor stimulation.
- CHO-K1 were transfected with Citrine-EGFR in all conditions as indicated by correct molecular weight band at 150 kDa (Figure 1A).
- Several protein bands are present in Mock transfection lane suggesting off-target binding of the RLuc antibody (Figure 1B).
- RLuc alone, RLuc-SH2-Grb2, and RLuc-SH2-CTEN were successfully transfected as indicated by correct molecular weight bands (Figure 1B).
- RLuc-SH2-PLCg and RLuc-SH2-Shc3 did not appear by Western blot analysis -- bands different from those in the Mock lane are not identifiable. This outcome could be due to protein expression levels below the detection limit by Western blot or to unsuccessful transfection of cDNA.
- BRET signal increased in cells transfected with Citrine-EGFR and RLuc-SH2-CTEN versus Citrine-EGFR and RLuc alone after EGF stimulation. This difference suggests that the BRET signal is specific for an SH2-EGFR interaction versus randomly localized RLuc.
- In sum, these data suggest that the RLuc-SH2 constructs can be utilized to monitor EGFR phosphorylation, as SH2 domain-EGFR association occurs only at sites of EGFR tyrosine phosphorylation. Next, we determined the dynamic range of the BRET assay.

In lab today

1. Load cells onto comet chip, start treatment with MMS
2. Complete staining of gamma-H2AX assay
3. Remove MMS and incubate comet chip with Arsenite 2 hours, followed by lysis

HW due M1D5 (both individual)

1. Use the data from your cell loading experiment to create a figure, figure title and figure caption
   - Consider how you will represent the data and the size of the figure
2. Write a short summary of your communication lab appt. (1-2 paragraphs)