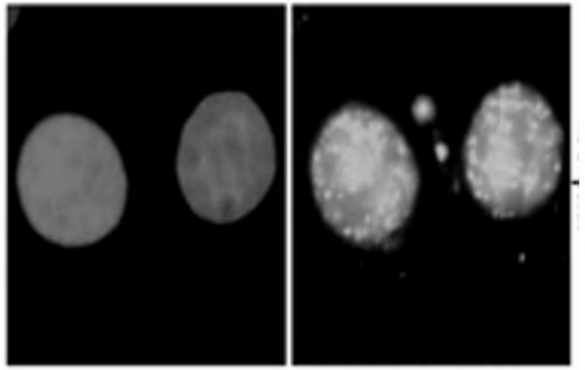
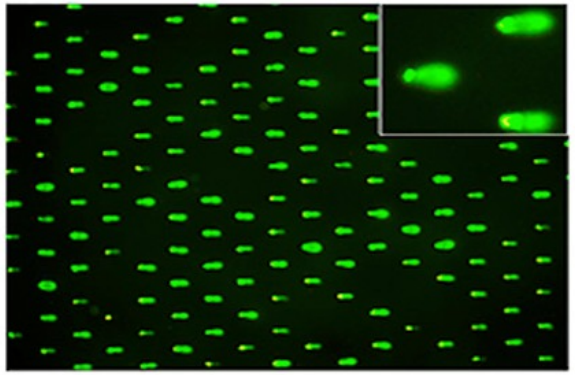
M1D4: Treat cells and perform high-throughput genome damage assay

1. Quiz
2. Prelab
    1. Review H2AX analysis
3. Perform CometChip experiment

Mod 1 Overview





1. Use repair foci experiment to measure DNA breaks

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

2. Use high-throughput genome damage assay to measure DNA damage

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

damage & repair

SSB & DSB

Notes on fluorescence imaging and analysis

- Imaging set up:
  - Experimental condition (presumably the most damage/H2AX foci)
  - Set exposure time for each channel with this condition (we did 50ms)
    - Prevents saturation in the image (i.e. “signal blow out”) and allows for cleaner analysis
  - Images from all 4 conditions are collected under these parameters to ensure comparability in analysis

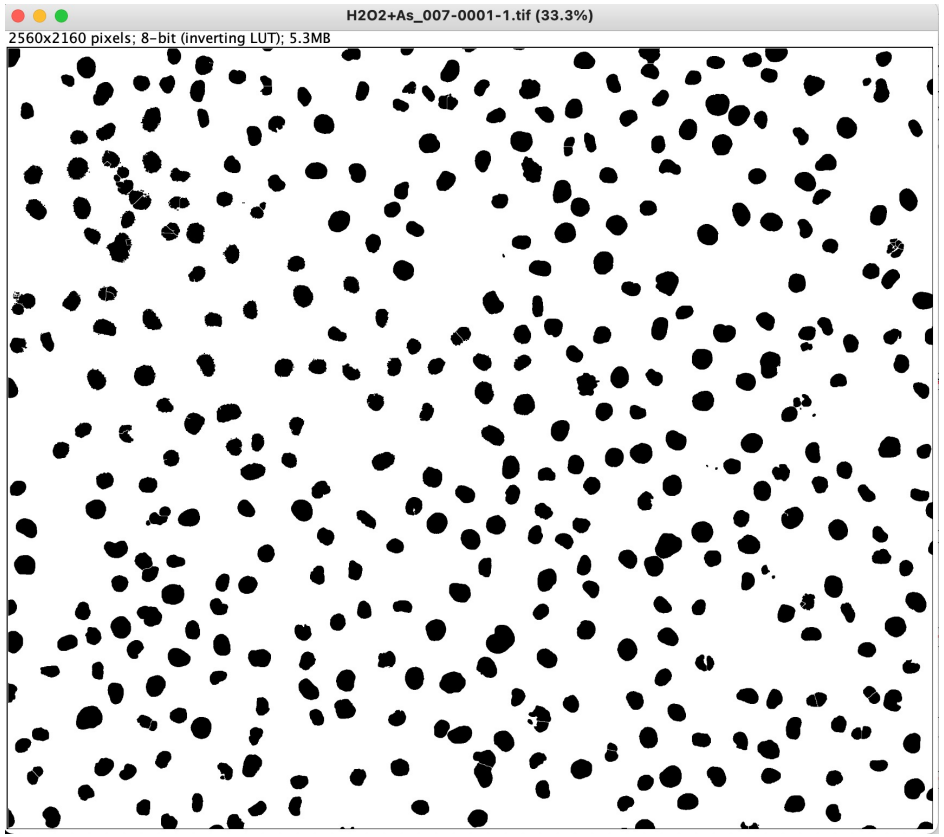
low signal green → not saturate
→ low background

- Image Presentation:
  - Images kept well below saturation threshold can be difficult to visualize by eye
  - The signal intensity can be adjusted manually to provide more contrast
    - Be sure to keep adjustment parameters as the same range so that your images can still be compared

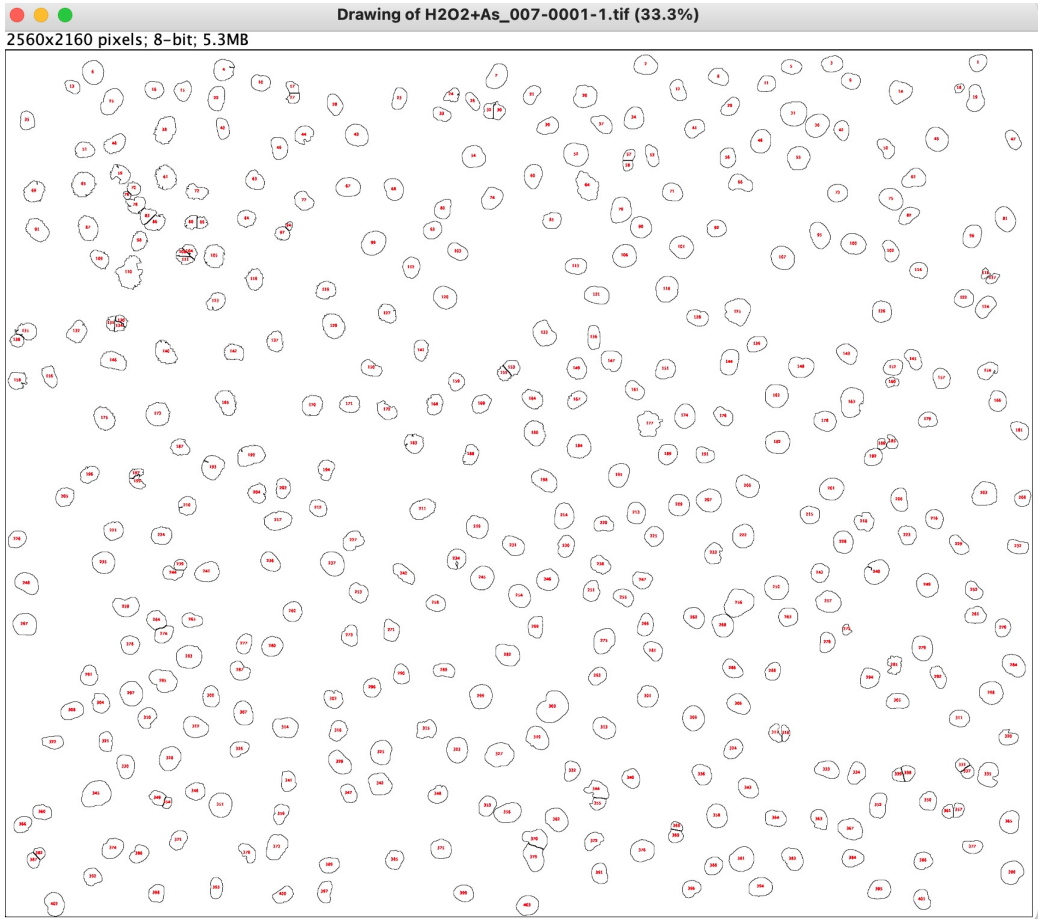
* applied equally across all images!

Fun with foci maxima…

- My nuclei were masked just fine…

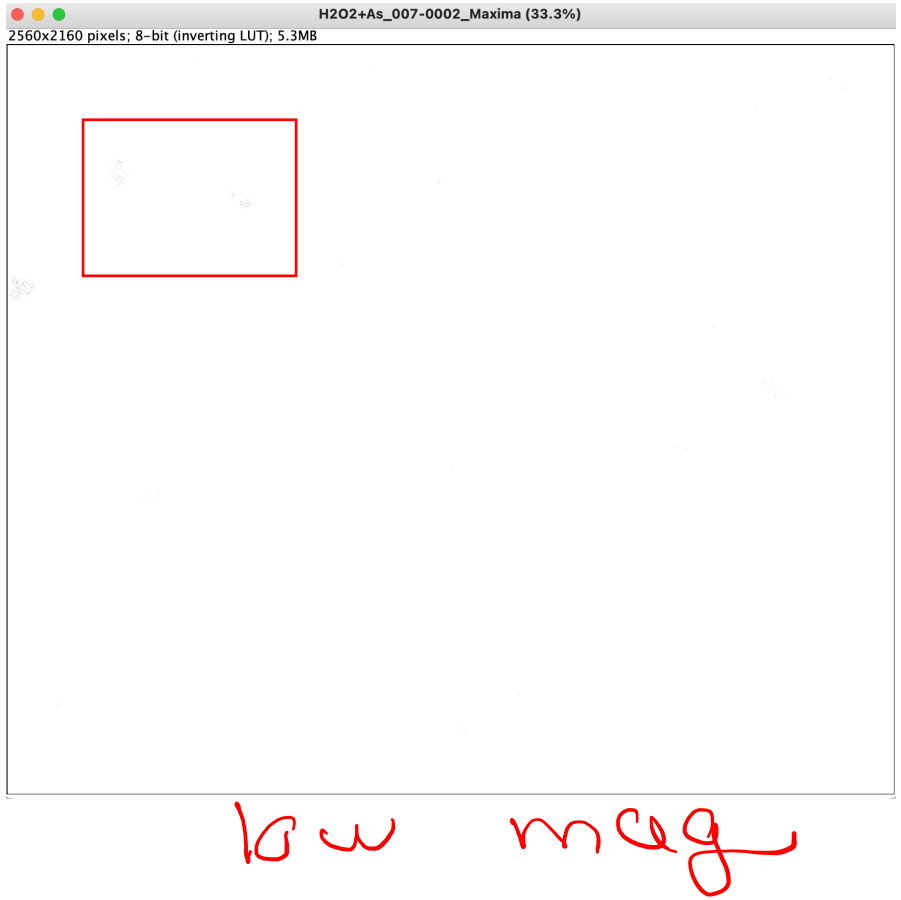


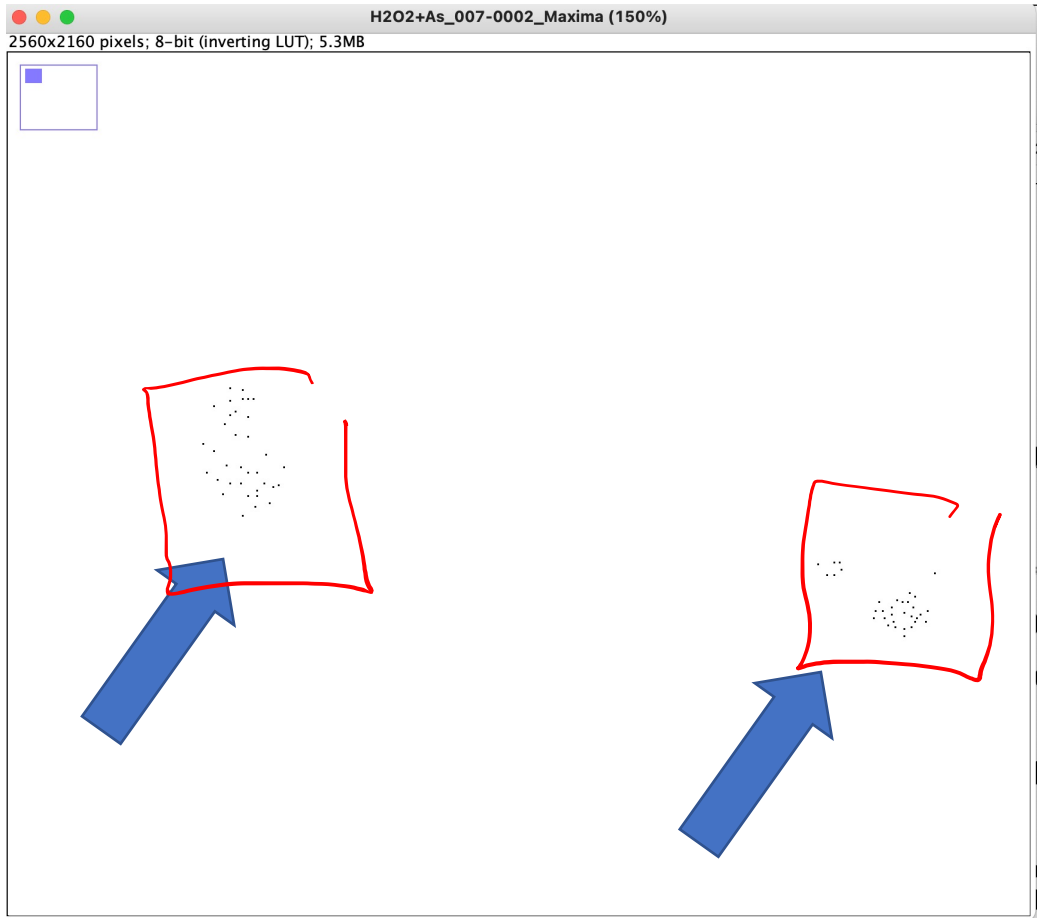
- The nuclei outlines seem accurate…



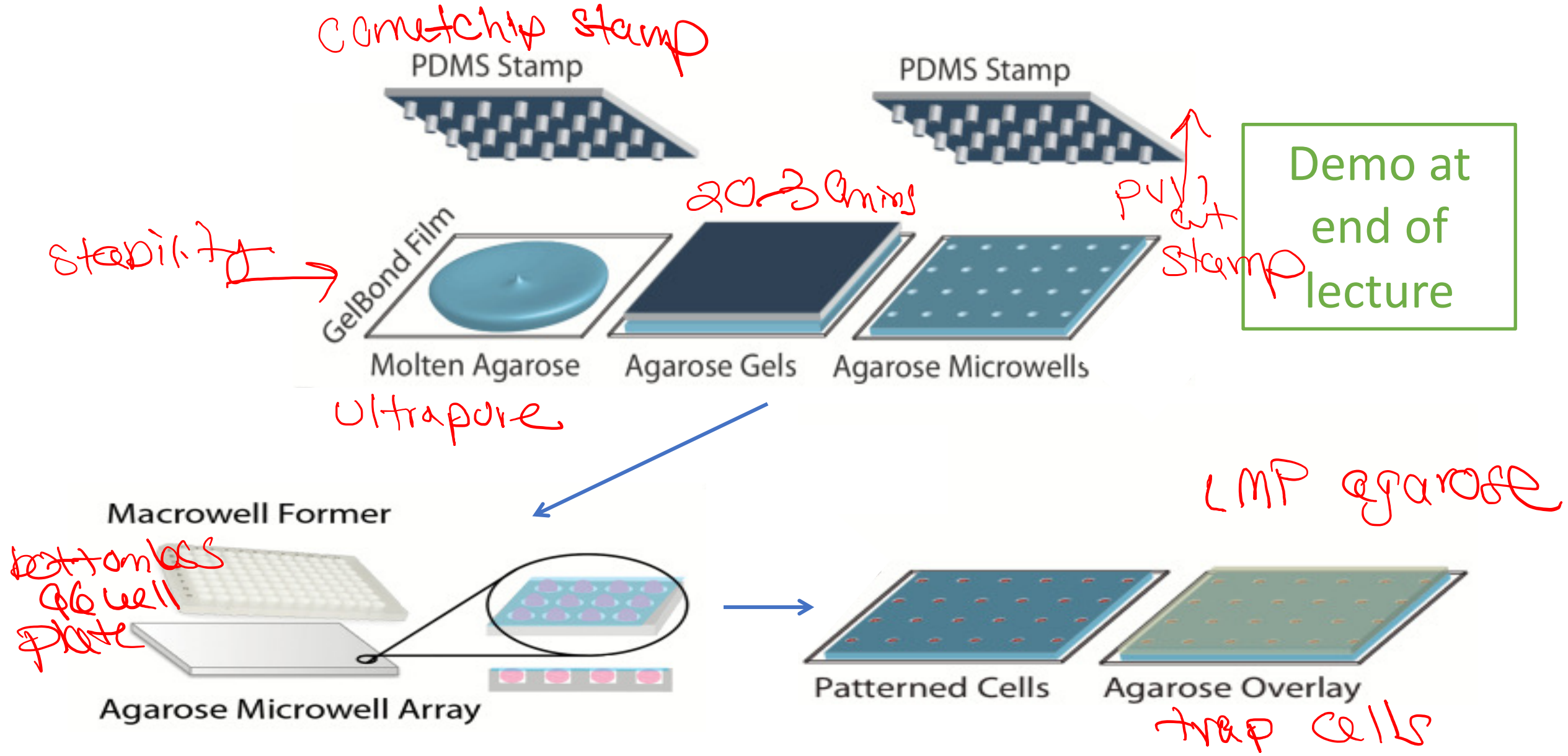
Fun with foci maxima…

- But the foci analysis is giving me a white screen.





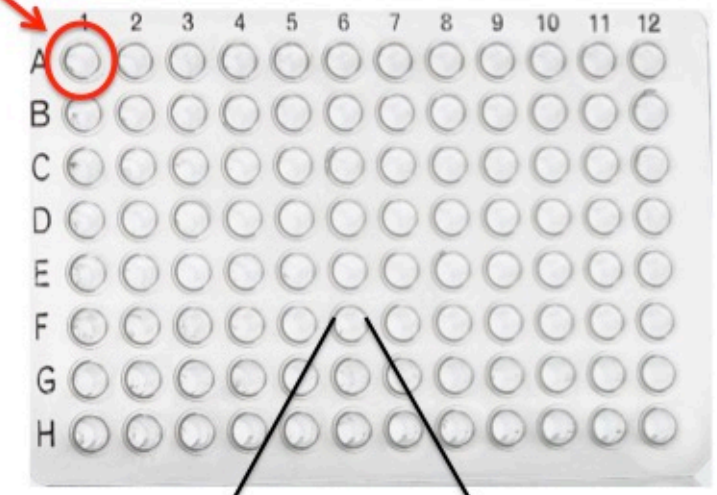
Overview of the CometChip assay: pouring and loading cells



Demo at end of lecture

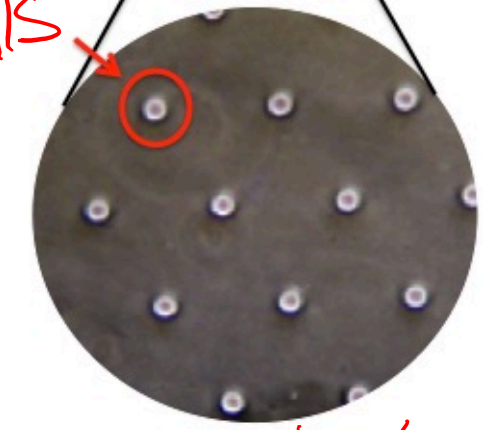
Loading cells into CometChip wells

macrowells



microwells

~40μm



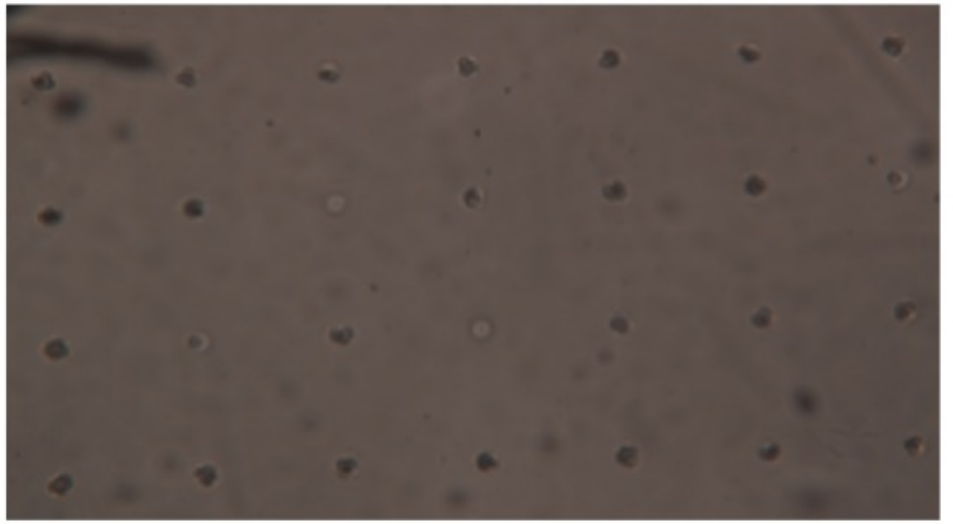
~300 microwells/macrowell

- How many cells are in a microwell?

1-2 cells

- How many cells are in a macrowell?

~25K cells



empty microwell

2 cells

Overview of the CometChip assay: treating cells

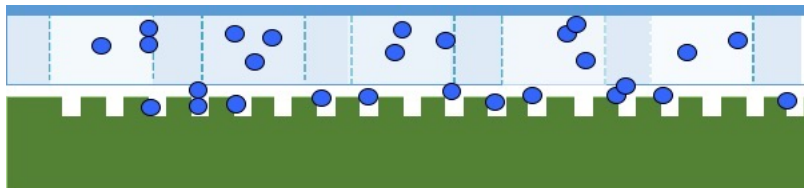
Treat with As for 24hrs

(+) As

(-) As

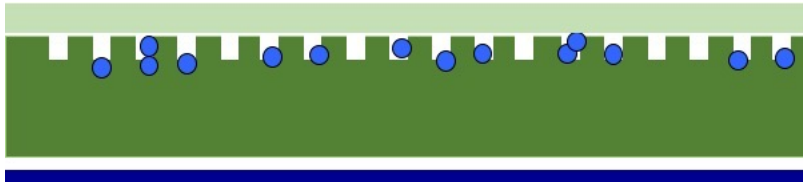
load cells

96 well plate



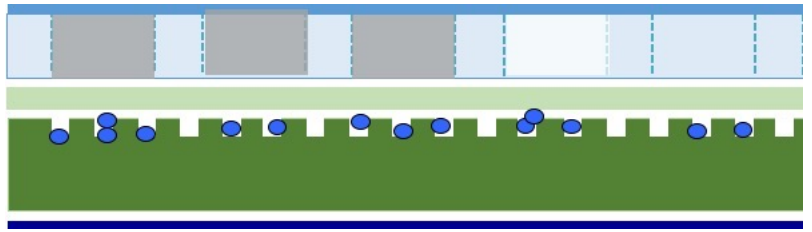
Wash, Add 1% LMP agarose

trap cells



Treat with H_2O_2

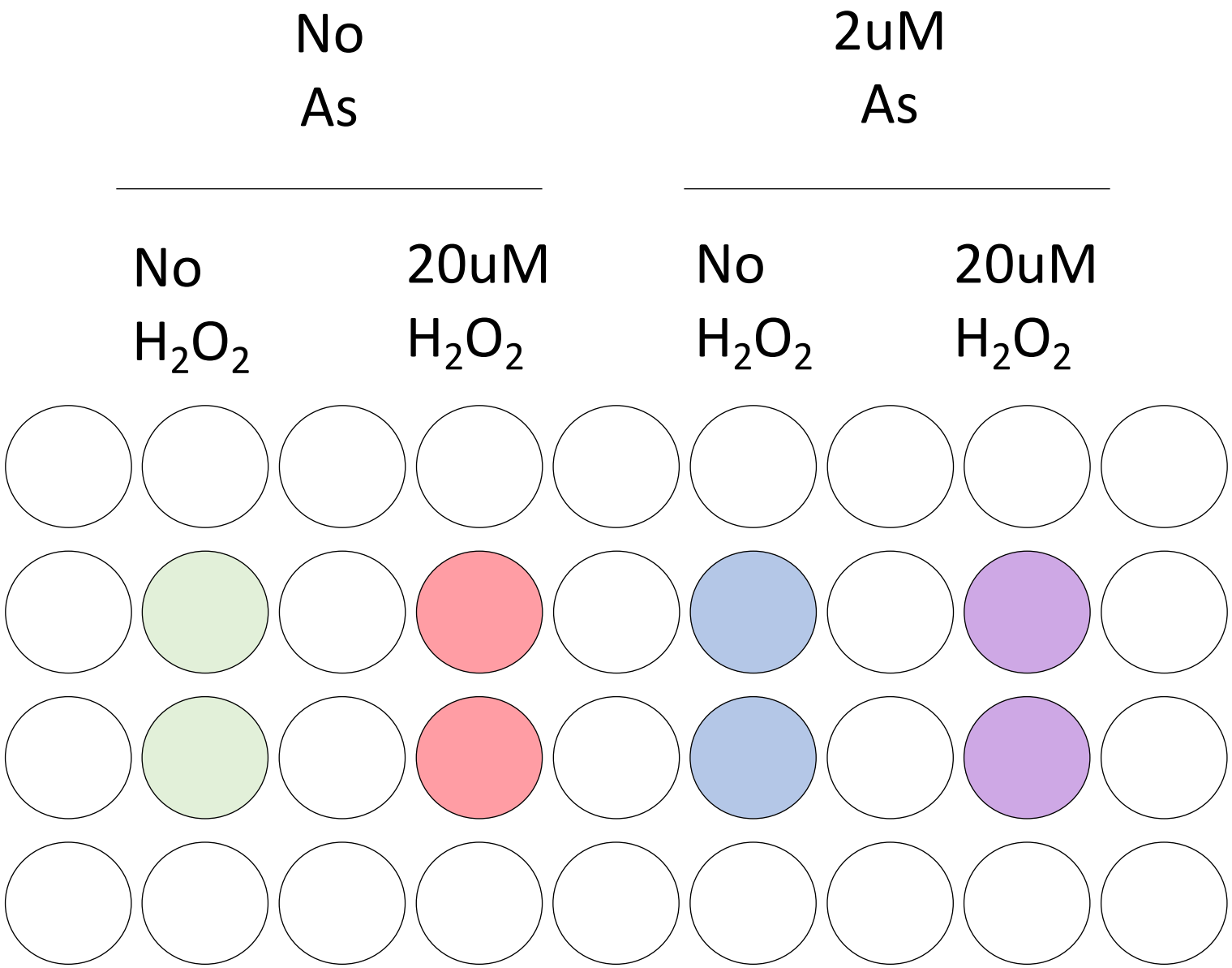
H_2O_2 penetrate



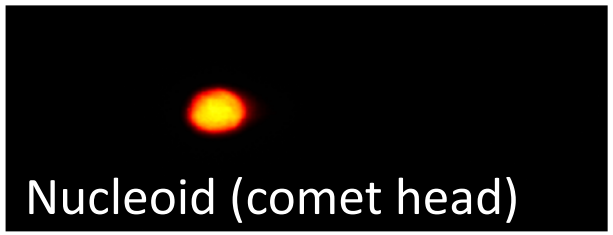
0min recovery

Place directly in lysis buffer

Macrowell layout



Output of the alkaline CometChip assay



No Damage

- Supercoiled nucleoid
- Little or no migration

High Damage

- SSBs, abasic sites, alkali labile sites, sites of incomplete excision repair
- forms a "comet tail"

* Nuclear DNA normally supercoiled
    * DNA breaks and fragmentation releases tension
        * Unwound DNA will migrate in response to electrical current to create comet

For Today

- Perform CometChip experiment
- With any extra time, continue H2AX analysis
- At 4:30pm, Demo of CometChip Electrophoresis

For M1D5

Group

- Revise methods and add in M1D3 (I'm uploading Noreen's comments to Stellar)

Individual

- Read paper linked on M1D5 and prepare for group discussion
- Write summary for BE Comm Lab visit