M1D2: Develop experiment to optimize loading variables

09/14/17

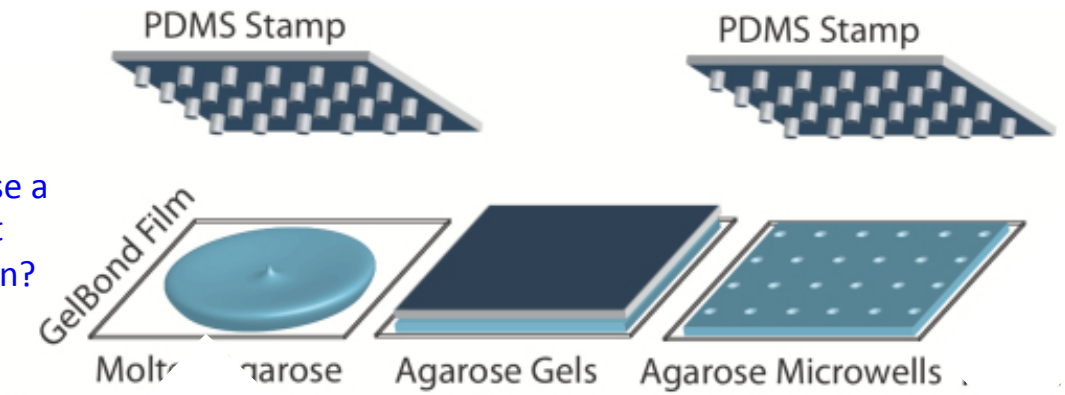
Next time meet in 56-614 at 1:05pm

1. Pre-lab Discussion
2. Instructor Check-in: design parameters
3. Load CometChips: ½ go to TC to prep cells
4. ½ in main lab start statistics exercise

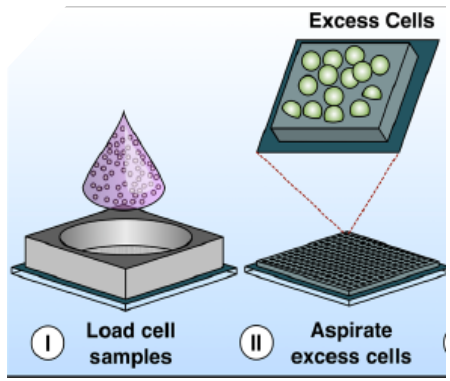
Creating a CometChip and optimize loading

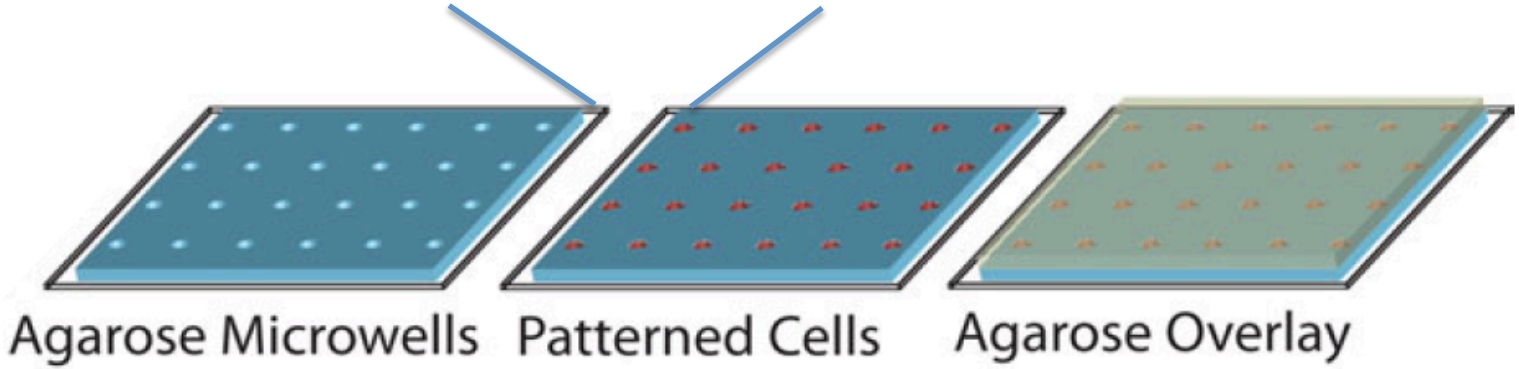
Tuesday

**Did you use a detergent resistant pen?



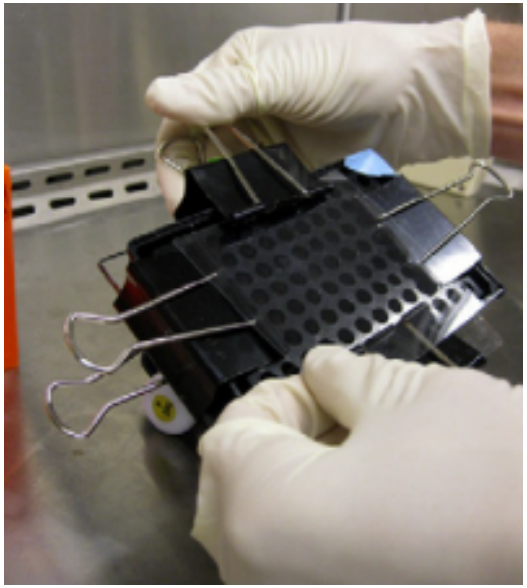
TODAY

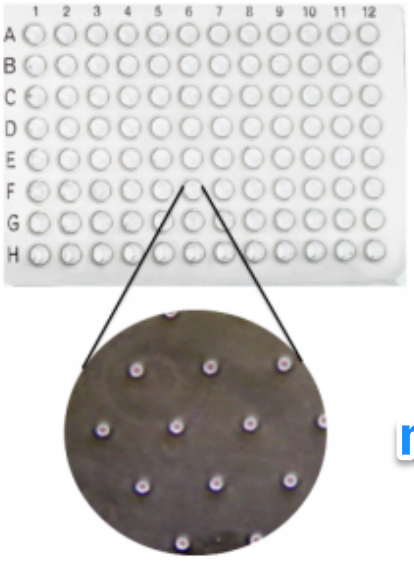




Loading the CometChip

- glass plate
- bottom less 96 well plate
- 4 binder clips
- 37°C incubator

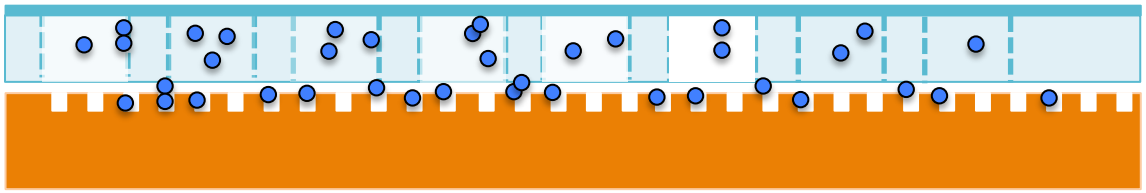




macrowells

microwells

side view



Outline for today

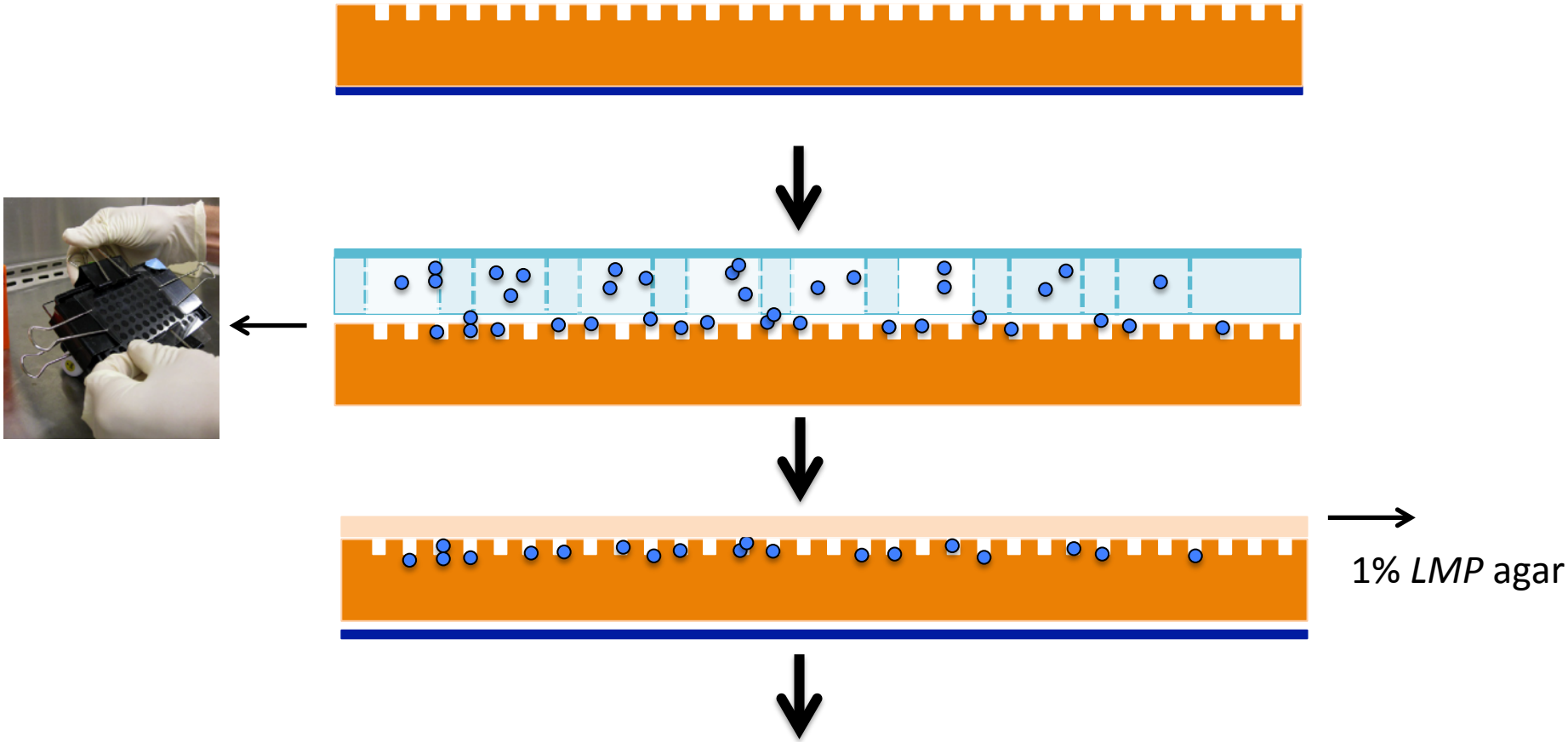
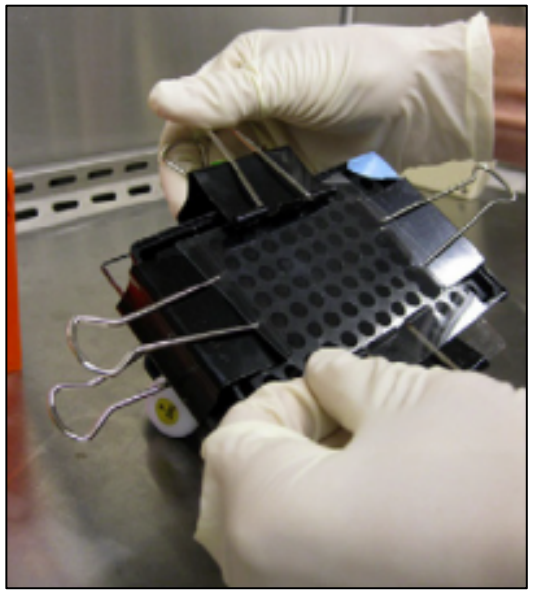
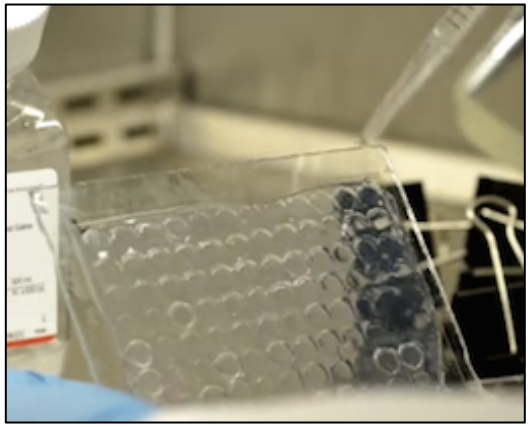


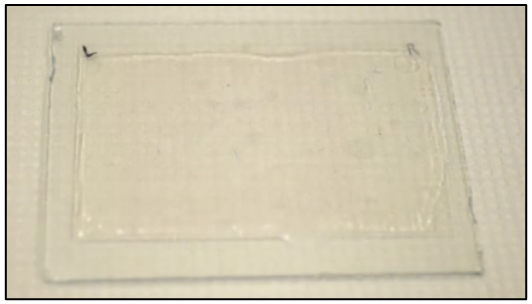
image on microscope (10X), lyse captured cells and dye DNA with Sybr Gold

Critical steps:

- Cell loading
  - line up the macrowells carefully within the pattern drawn on gel bond
- Washing
  - not too much!
  - across the top of the glass plate
- 1% LMP agarose gels *quickly*
  - leave glass plate behind comet chip
  - dispense it drop-by-drop with P1000
  - leave it undisturbed for 3min then move to 4°C for 3min







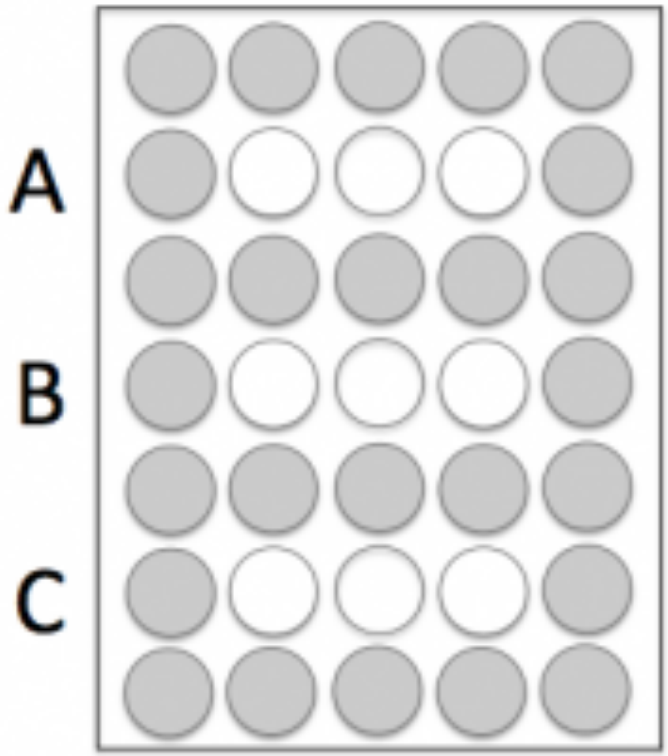
Designing your loading experiment

What size are MEFs?
What is the size of a microwell and macrowell?

A Condition A will be 'no cells loaded' control

B Condition B will be the lower cell number decided by your team

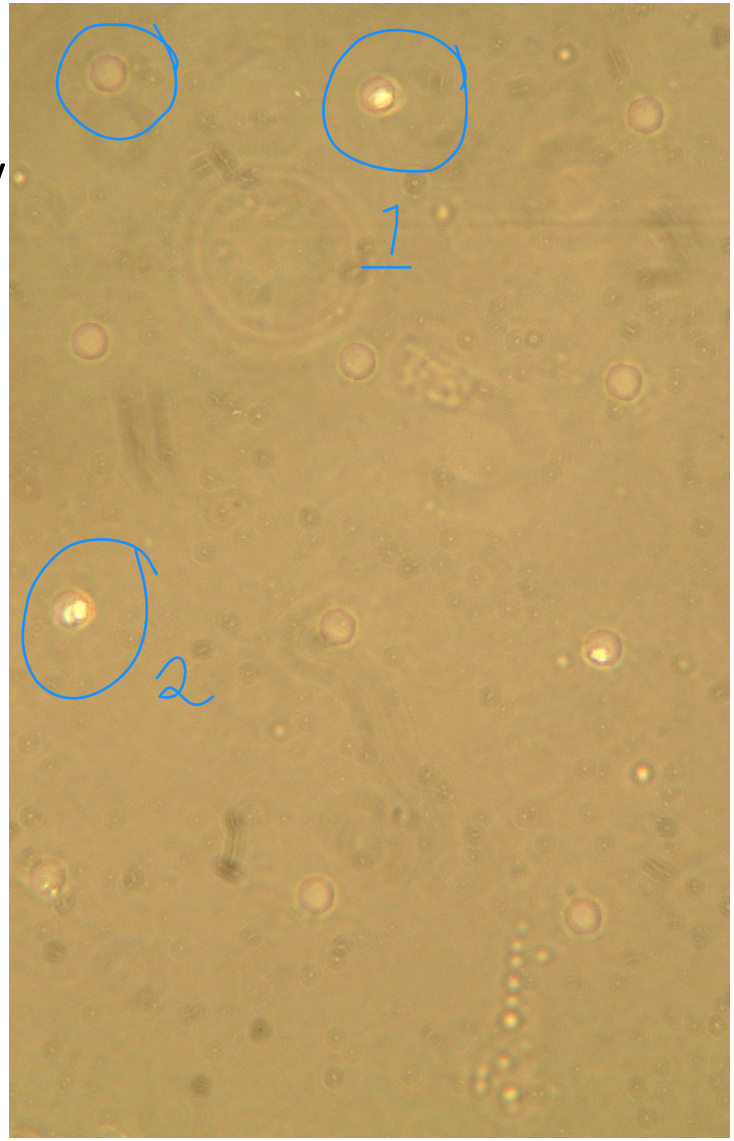
C Condition C will be the higher cell number decided by your team



Incubate cells at 37°C for **15min**

Homework and analysis M1D3

- Make a *figure & caption*
  - You will receive light microscope images today for your experimental conditions
  - All figures **must include a title and a caption**.
  - Title: **take home message**
  - Caption: **info. necessary to describe the image/data**
- Receive homework credit for visiting Comm. Lab before M1D5!
- Which loading parameters are ideal?
  - *Row B or Row C? Keep this info in your lab notebook. We'll discuss next time.*



Today in lab:

1. Carefully consider your design parameters and check with an instructor before starting your experiments.
2. Blue, Pink, Purple and White teams to the tissue culture room first to prepare cells
3. Red, Orange, Yellow and Green teams can start the statistics exercise
   - **IMPT for everyone's future analysis**
4. Everyone should leave lab today with .jpeg images from their loading experiment.