

M1D2: Design experiment to optimize cell loading variables

09/14/18

1. Pre-lab Discussion
2. Instructor Check-in: design parameters
3. Load CometChips: start in tissue culture
4. During downtime research the M059J and M059K cell lines



Office Hours

Noreen

Monday 2pm-5pm
in 16-317

Leslie

Thursday 2-3pm
Friday 12-1pm
in 56-341c

Josephine

Wednesday 12-1pm
Friday 2-3pm

Announcements

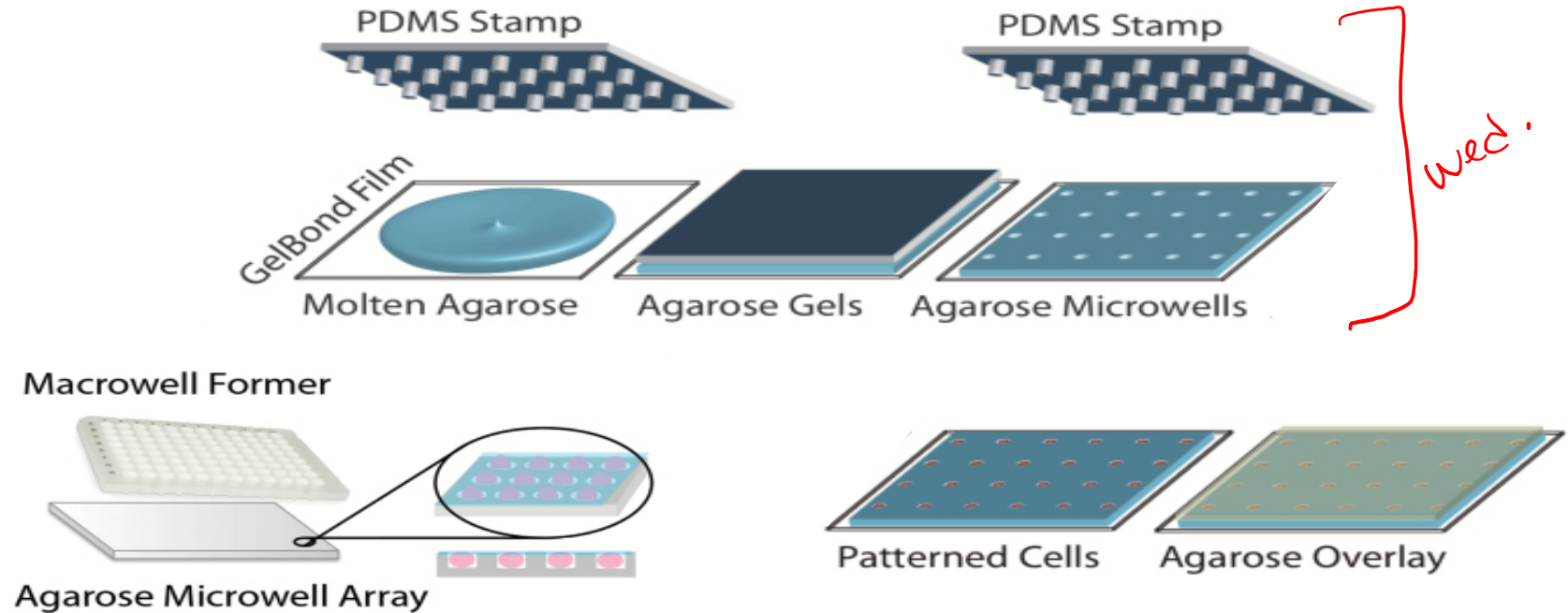
- *Next time meet in 16-220 at 1:05pm for Comm Lab workshop (bring a copy your figure HW)
- *Remember to spray & wipe benches with 70% ethanol before and after work

M1 major assignments—

- Data summary (15%)
 - In teams, submit on Stellar
 - Draft due 10/8, final revision due 10/20
 - Bullet points, .PPTX
- Mini-presentation (5%)
 - Individual, submit video via Gmail
 - Due 10/13
- Lab quizzes –be on time!
 - M1D4 and M1D7
- Notebook* (part of 10% Homework and Notebook)
 - Due **10/6** at 10pm, graded by Jai
- Blog: <https://be20109f18.blogspot.com> (part of 5% Participation)
 - by 10/9

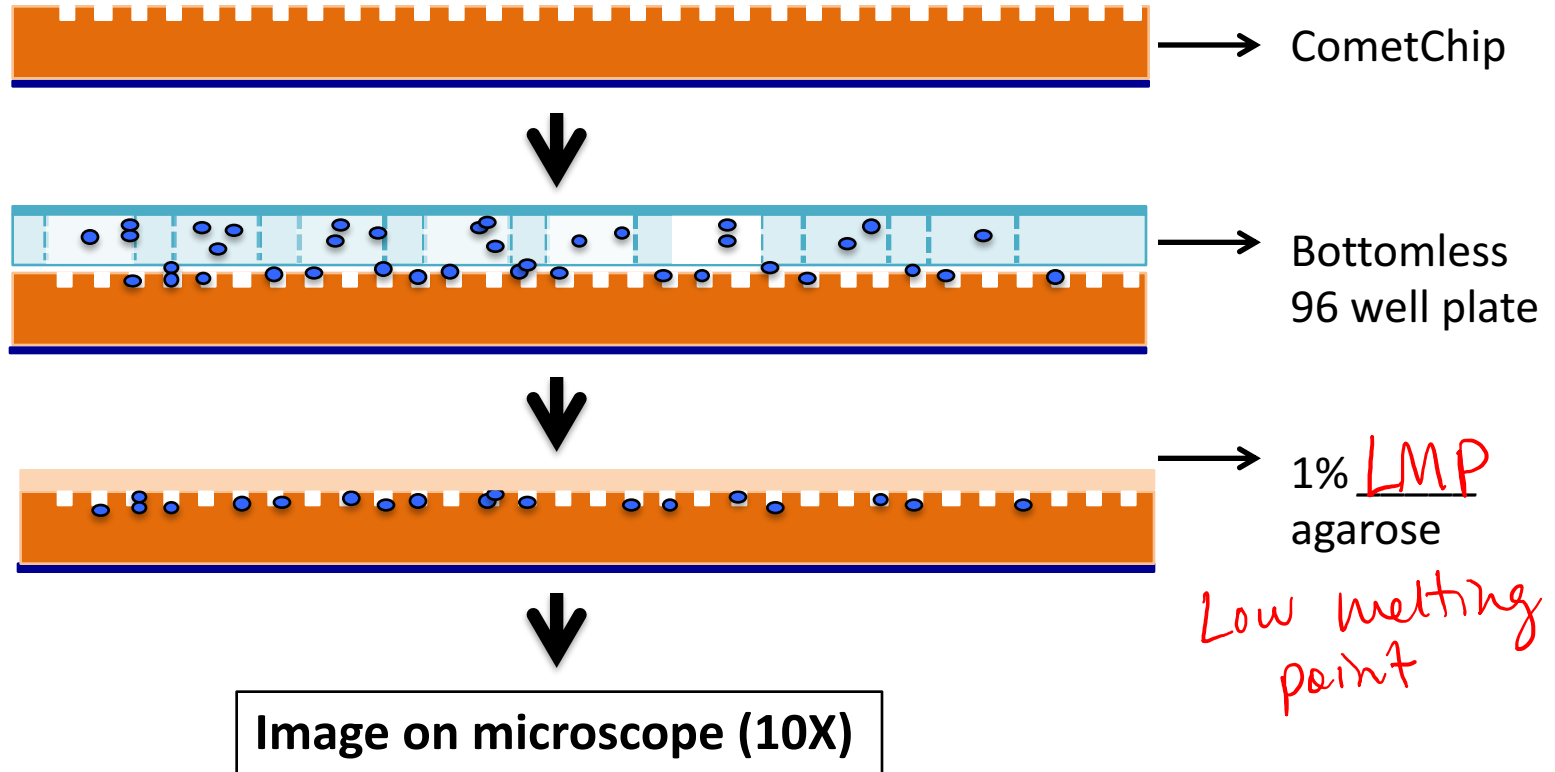
*Notebooks will be graded at 10 pm the day following the final day of each module (i.e. at 10 pm on the day after M1D7, M2D8, and Research proposal presentations).

This week: Create a CometChip & optimize cell loading



What is the minimum number of cells needed in each macrowell to obtain efficient loading?

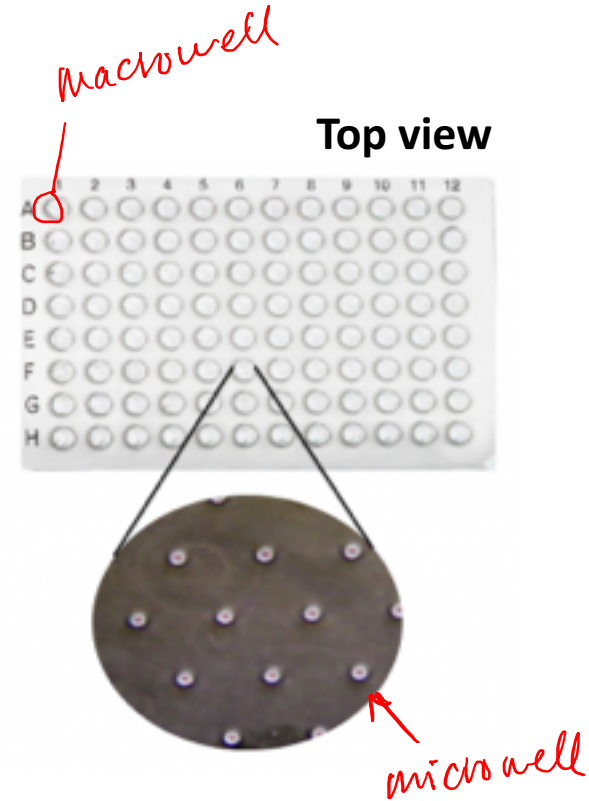
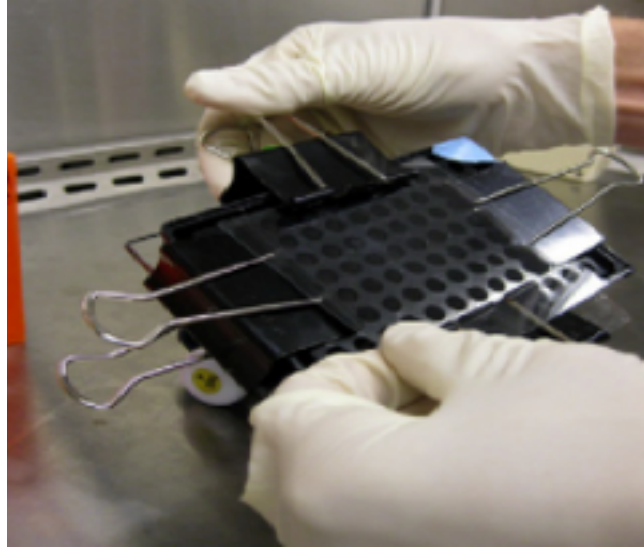
Today: Load cells onto the CometChip



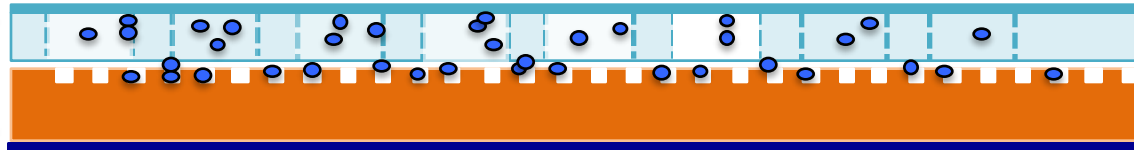
side view

What this looks like in real life

- Glass plate
- Bottomless 96-well plate
- 4 binder clips
- 37°C incubator



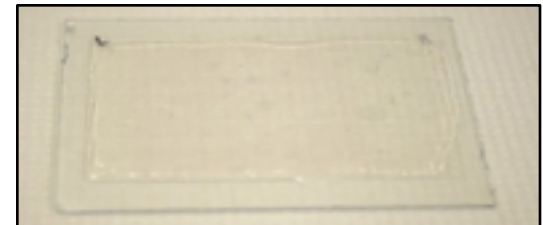
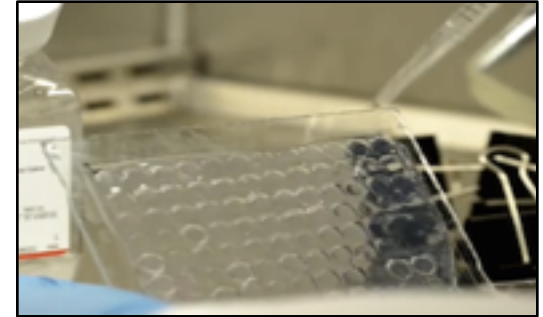
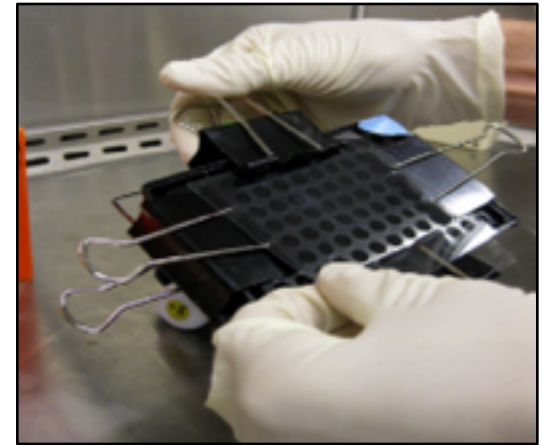
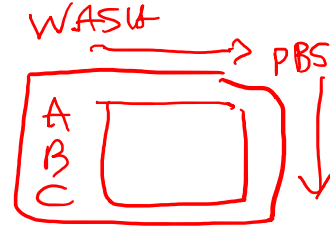
Clamp with
binder clips



Side view

Critical steps:

- Cell loading
 - Line up macrowells carefully within the pattern drawn on gel bond
- Washing
 - Not too much!
 - Across the top of the glass plate
 - Wash from low to high concentration
 - Don't mix cell types!
- 1% LMP agarose gels *quickly*
 - Leave glass plate under comet chip
 - Dispense it drop-by-drop with P1000
 - Leave it undisturbed for 3 min then move to 4°C for 3 min



Designing the cell loading experiment

Experimental question: What is the minimum number of cells needed in each macrowell to obtain efficient loading?

300 microwells / macrowell

Considerations:

Volume: 50 μ l \rightarrow 350 μ l

Size of well: 40 μ m, distance between wells 250 μ m

Size of cell: \approx 20 μ m

Variable:

of cells loaded / macrowell

Control: \rightarrow outcome known

negative control: no cells

Repeatability:

triplicate = 3 macrowells / condition

Designing the cell loading experiment

A

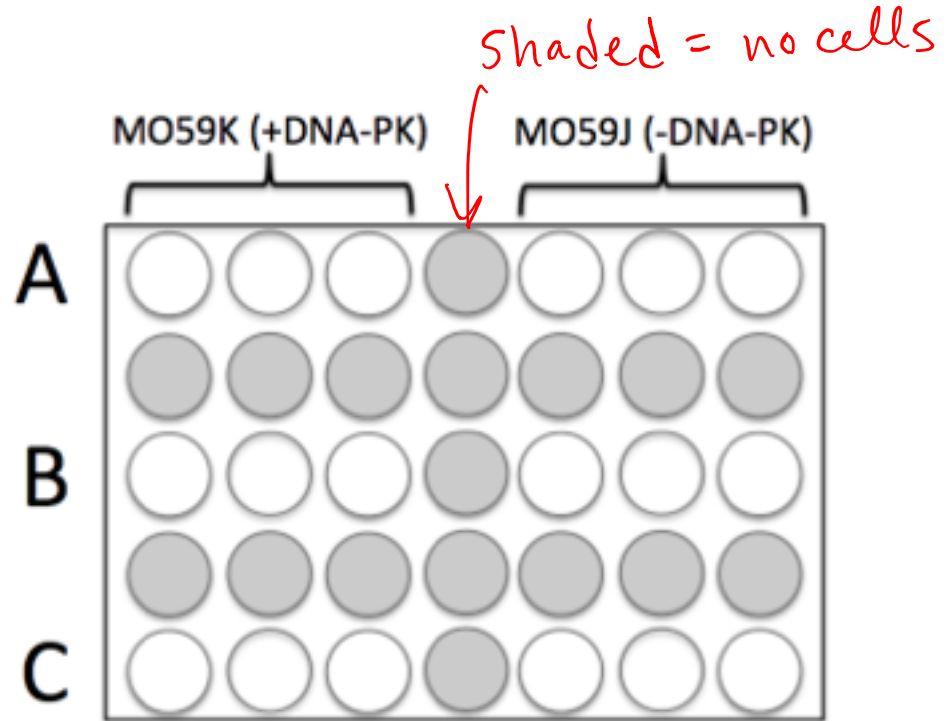
Condition A will be 'no cells loaded' control

B

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

C

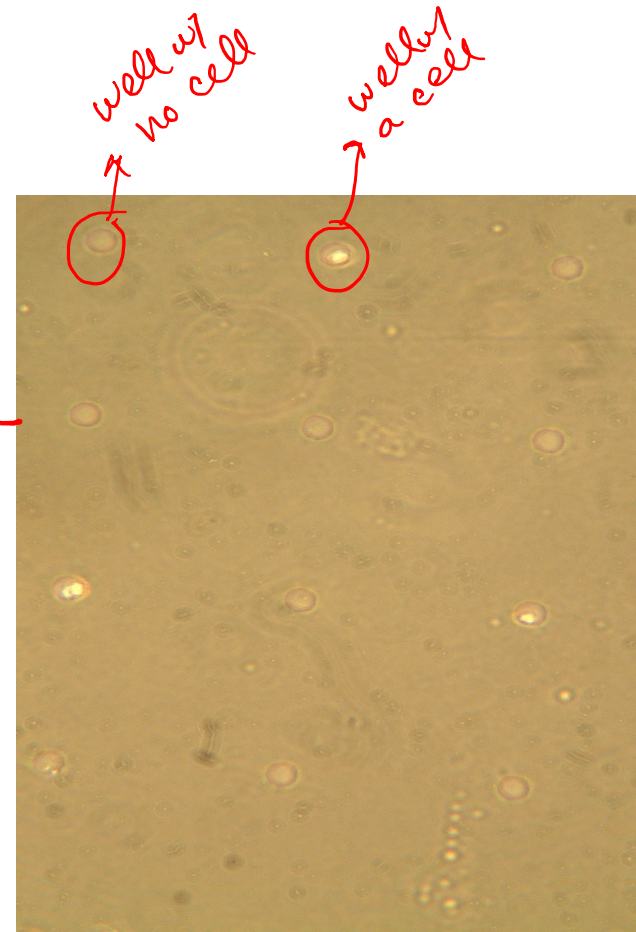
Condition C will be the higher cell number decided by your team



Incubate cells at 37°C for 15min

Homework and analysis due 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 away message from figure*
 - Caption: *specific information necessary to describe the image or 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. All teams can go to tissue culture room when ready to prepare cells
3. Make sure to get .jpeg images from loading experiment before you leave! (You need them for homework)