M1D2:

Develop experiment to optimize loading variables

- 1. Pre-lab discussion
- 2. Experimental design
- 3. Benchwork
 - Prep cells in TC
 - Load CometChip in main laboratory
- 4. Statistics practice

How many cells will you load?

- Considerations:
 - Size of MEF cells
 - Number of MEF
 cells / microwell
 - Size of microwells
 - Surface area of CometChip

Α

В

С

Condition A will be 'no cells loaded' control

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

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

Loading cells into the CometChip

Macrowell Former



Add Cells





Let's take a closer look



How does this actually look?



Take note of these critical steps!

- Loading
 - align wells within box on GelBond
- Washing
 - not too much!
 - waterfall from above CometChip
 - keep 'no cell' control at top
- Trapping
 - agarose (1% LMP) solidifies quickly
 - dispense drop-by-drop





Hints for homework due M1D3

Draft figure with title and caption

- Use light microscope image data
- Represent microwell loading efficiency and cells per microwell information

Title:

conclusive take home message

Caption:

information needed to interpret / understand figure NOT an interpretation of the figure



Today in lab...

- 1. Carefully consider design parameters and discuss with teaching faculty before cell prep
- Be sure you have your .jpeg images before you leave
- If you are unable to complete all steps of the cell loading protocol during class time, the teaching faculty will finish
 - Tell us where you are in the procedure!