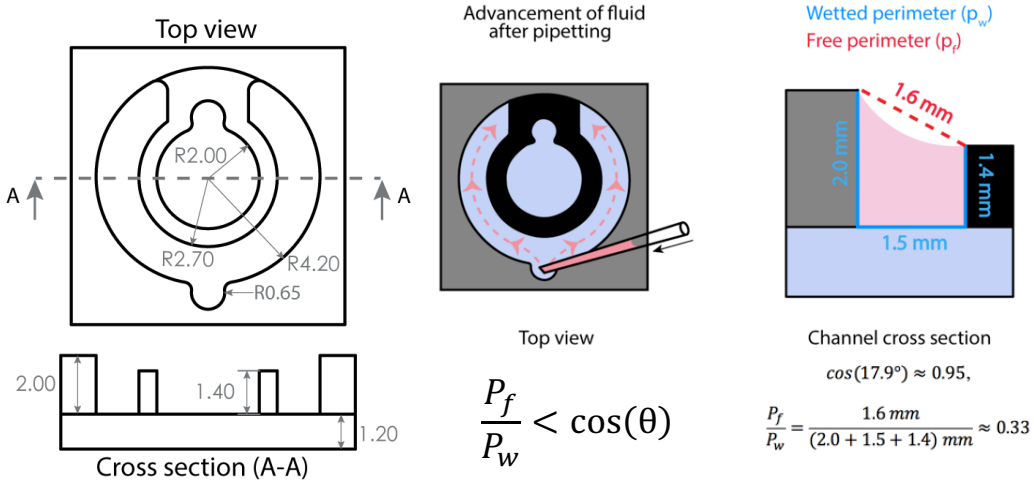
Polystyrene coculture device loading information

- *Large device dimensions (1uL = 1 mm^3)*
  Inner chamber seeding area: 13.69 mm^2 (radius: 2.0 mm)
  Inner chamber loading volume: 16 uL
  Outer chamber seeding area: 31.14 mm^2 (width: 1.6 mm)
  Outer chamber loading volume: 40 uL
  Ridge thickness: 0.7 mm
  Ridge height: 1.4 mm



To connect both chambers: remove old media and re-add 100uL

- *Small Device dimensions*
  Inner chamber seeding area: 7.54 mm^2 (radius: 1.3 mm)
  Inner chamber loading volume: 6 uL
  Outer chamber seeding area: 16.59 mm^2 (width: 1.2 mm)
  Outer chamber loading volume: 16 uL
  Ridge thickness: 0.5 mm
  Ridge hight: 1.4 mm

- The device should be plasma treated and UV sterilized right before using.

- Coat the bottom of the device with hydrogel (i.e. collagen) if needed.

- The cell seeding process should be completed as soon as possible as the loaded cell media will evaporate fast.

- The loaded plate should be stored inside an omni tray and surrounded with 1 mL of PBS droplets.

- The omni tray should be kept inside a bio-assay dish. Use soaked Kim wipe to wrap around the perimeter of the omni tray. Keep the dish in the back on the lowest level of the incubator.

- After both cell types adhere (recover overnight), old media should be gently pipetted out from the chambers, and 100 uL of fresh media should be loaded in the well to make the chambers connected.

- Strictly follow the ***evaporation management protocol*** during the incubation process.

Microscale cell culture device evaporation management protocol

This protocol applies to any experiment that requires incubation at 37C or even at room temp. Once you get an experimental design working, then you can decide to test if a bioassay dish is actually needed or not, ***but please assume that a bioassay dish is needed as a "default" from the beginning, which will save lots of time in the long run.***

Even "invisible" amounts of evaporation (5-10% of the volume) can be extremely detrimental to cell culture. Any visible evaporation would cause big problem.

- **Sacrificial water in the primary containment** (primary containment=omnitray, petri dish, or well plate used to contain the culture): Use 50:50 water:PBS (sterile if doing sterile work). If omnitray or petri dish put sacrificial drops of water (typically at least 1 mL total volume) - space the drops evenly around the perimeter - symmetry of the drop placement is important (you don't want lots of liquid on one side but not on another). If working with well plate, fill all of the wells in the perimeter of the well plate nearly to the top with 50:50 water: PBS.

- **Soaked kim wipes in the secondary containment**: Place the primary containment into a secondary containment (secondary containment=bioassay dish). "bioassay dish"= large plastic square tray with lid that is approximately 30 cm x 30 cm - ask if you don't know). Put kim wipes around the perimeter of the bioassay dish and soak the kim wipes with 50:50 water:PBS.

- **Water in the tray at the bottom of the incubator**: In the non-sterile incubator adding water into a large petri dish at the bottom of the incubator works well - please dispose of the water when you turn off the incubator at the end of the experiment, so it doesn't start to grow mold, etc.

- **Bioassay dish stored towards the back corner of the incubator**: the humidity maintains better away from the door. If you take out your bioassay dish, and then return it to the incubator, place it ***below*** the other bioassay dishes in the incubator (if you put a cold bioassay dish on top of a stack of bioassay dishes in the incubator it will cause condensation on the other bioassay dishes, ruining the experiments below).