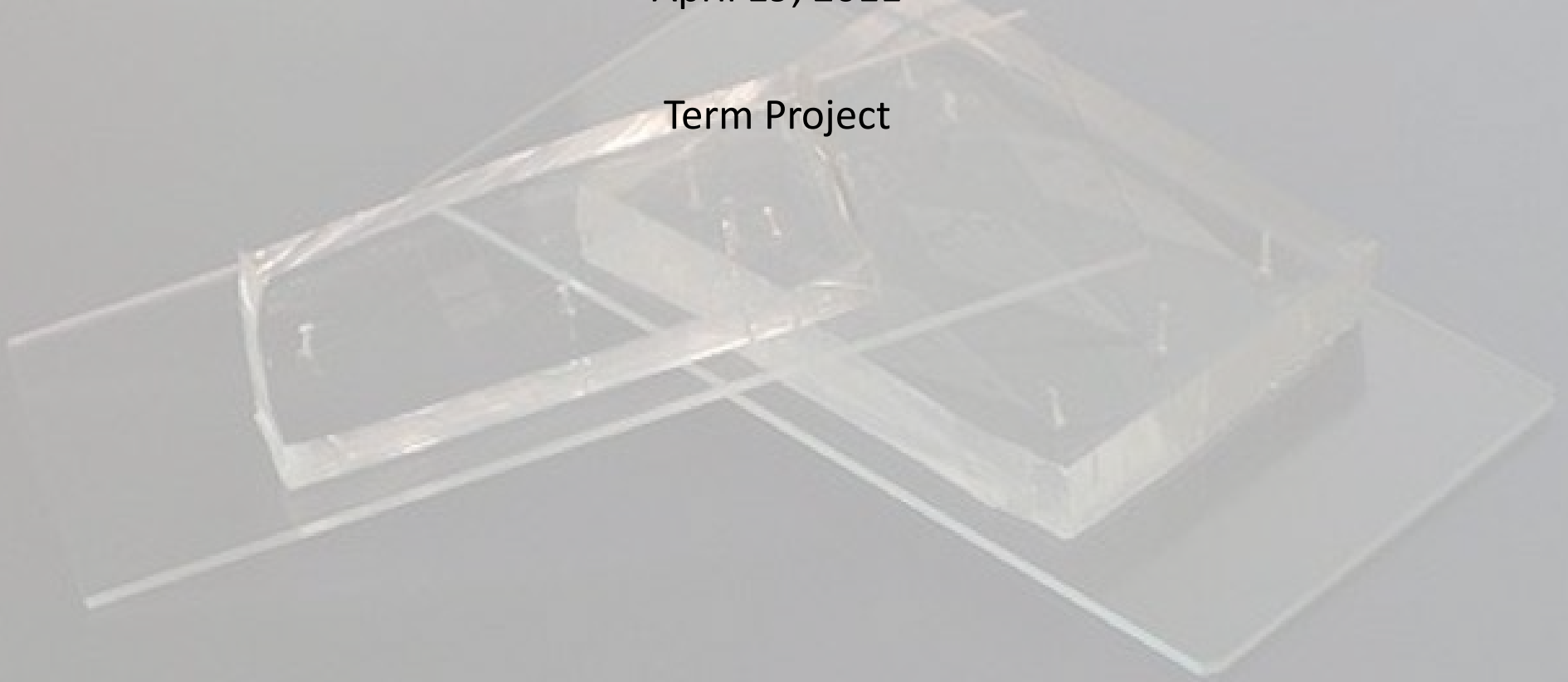
BME: Diffusion Screening Device

April 19, 2011

Term Project



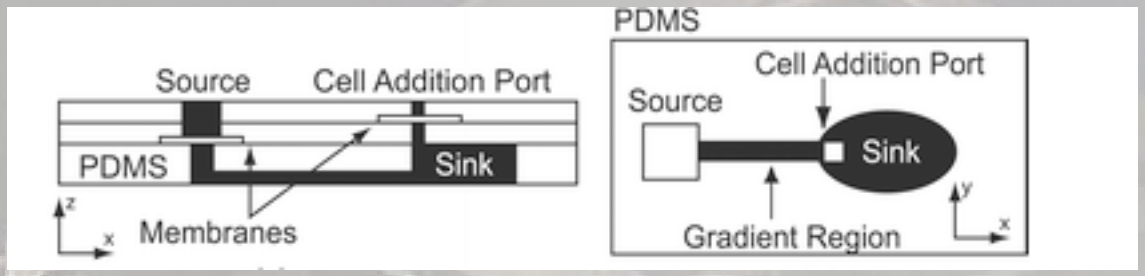
Diffusion Screening Device

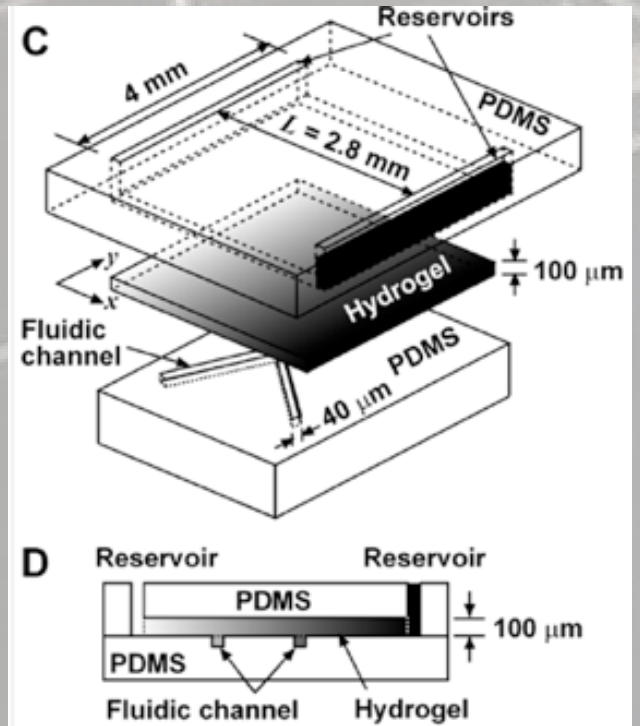
- **Goal**:

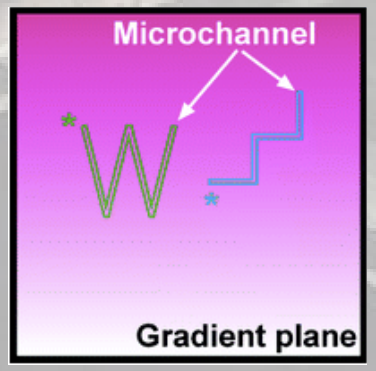
  Create a concentration gradient that varies the concentration of pathogen and antibiotic presented to a cell
- **Why**:
  Discover how the amount of antibiotic and pathogen can aid or hinder a cell's activity in a controlled environment that can be easily varied to one's liking

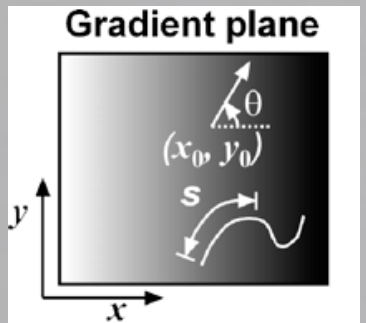
Current Studies and Their Limitations

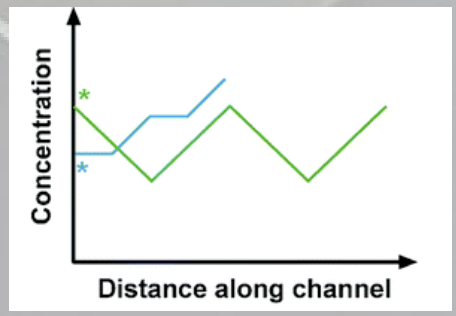
Diffusion-Based Diffusion





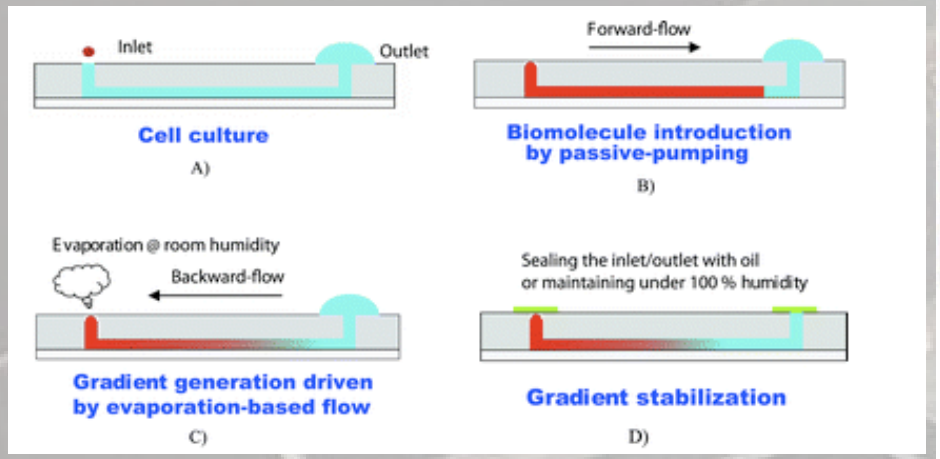


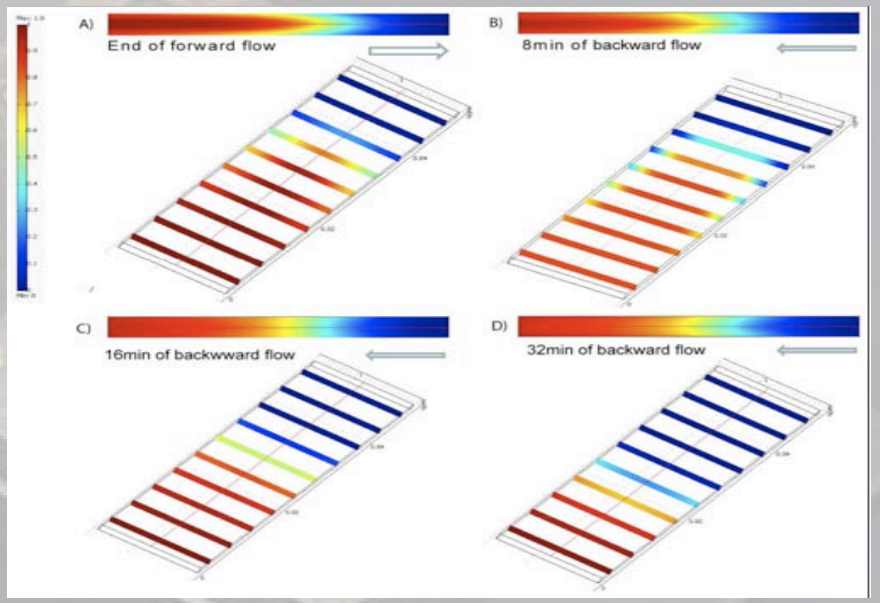


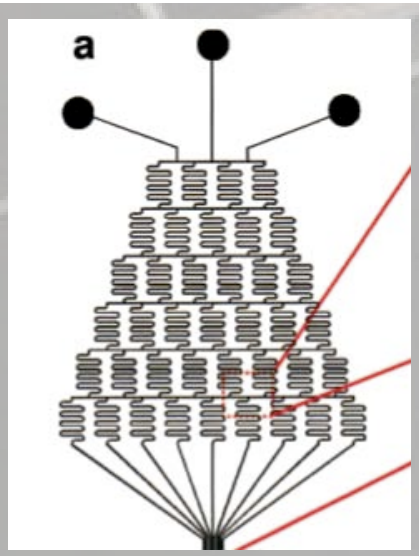


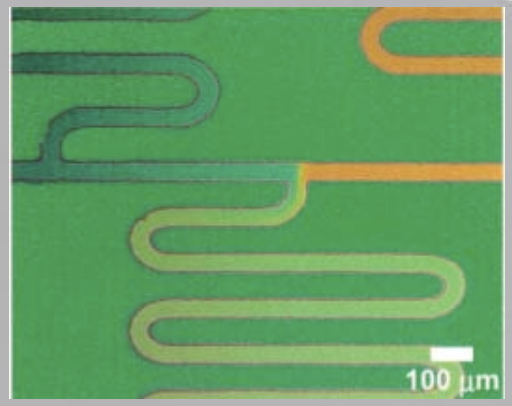
Current Studies and Their Limitations

Flow-Based Diffusion





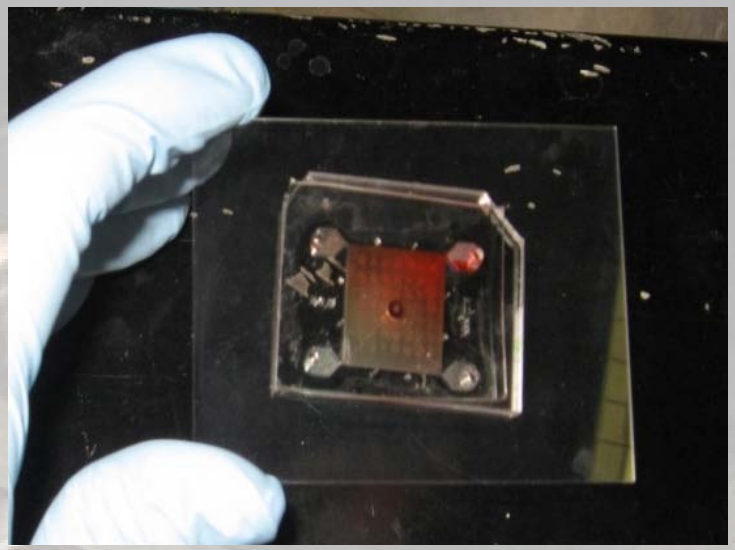


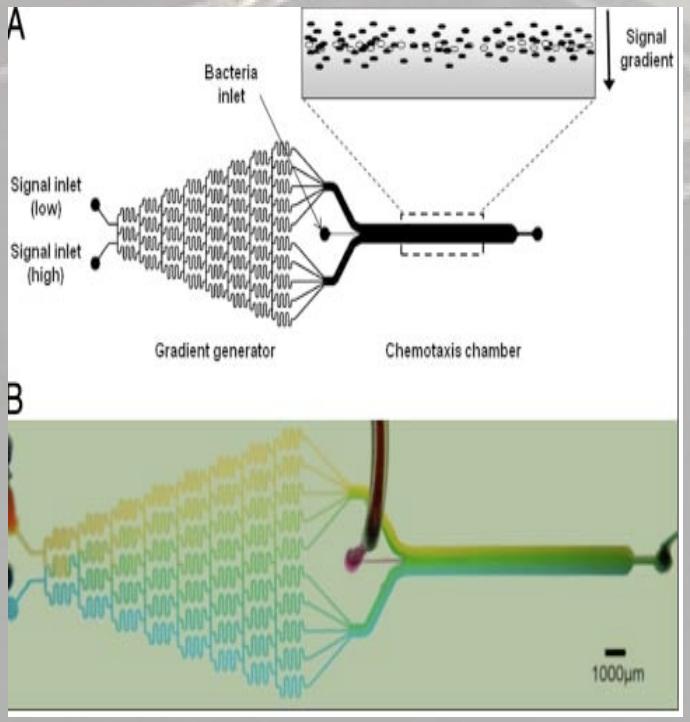


Current Studies of Pathogens Using Diffusion

Example 1 – Diffusion-Based

-Four different antibiotics inserted into corner wells to create various gradients





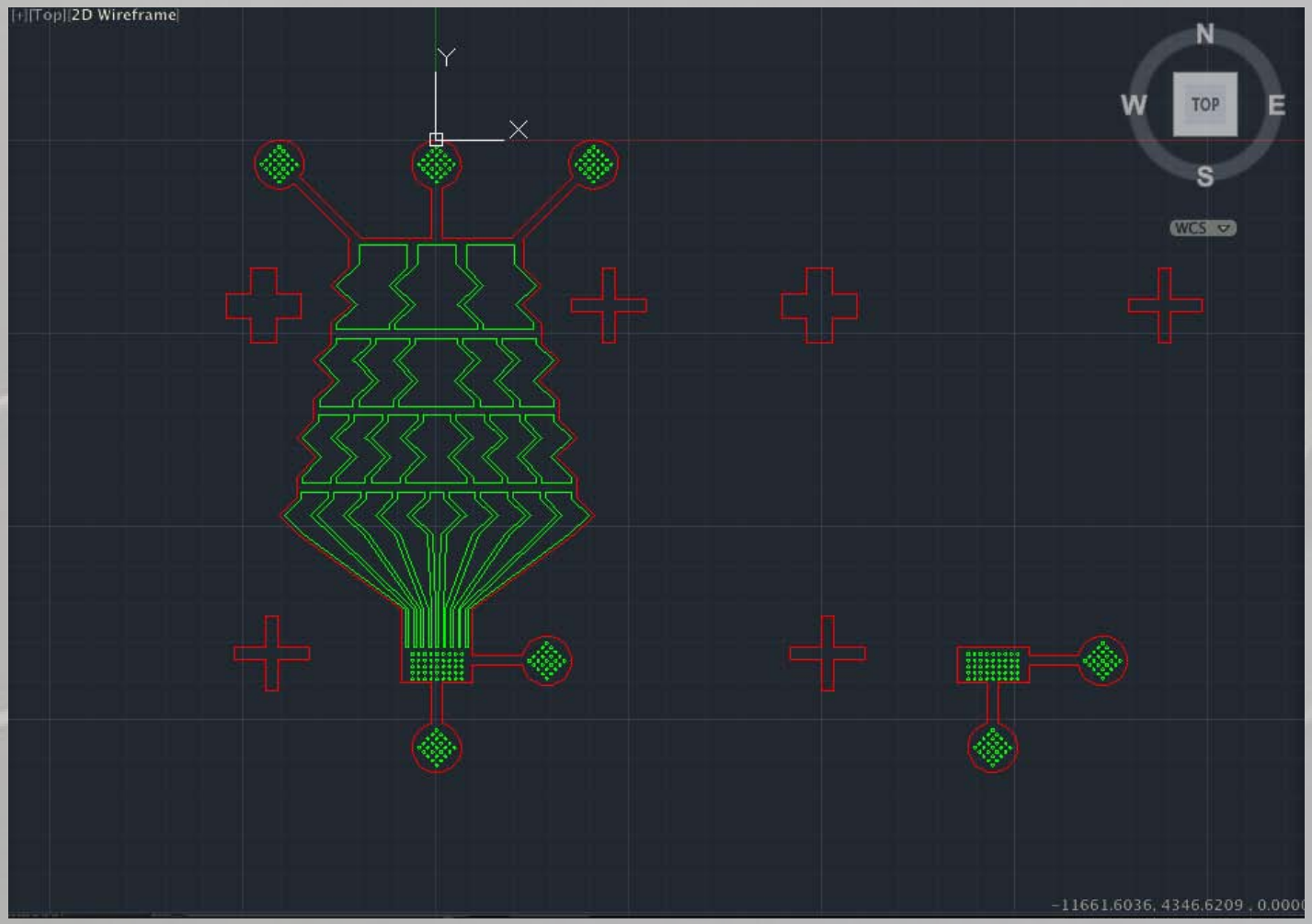
Example 2-Flow-Based

-Chemoeffectors generate a gradient and bacteria is presented after gradient created

Our Screening Device

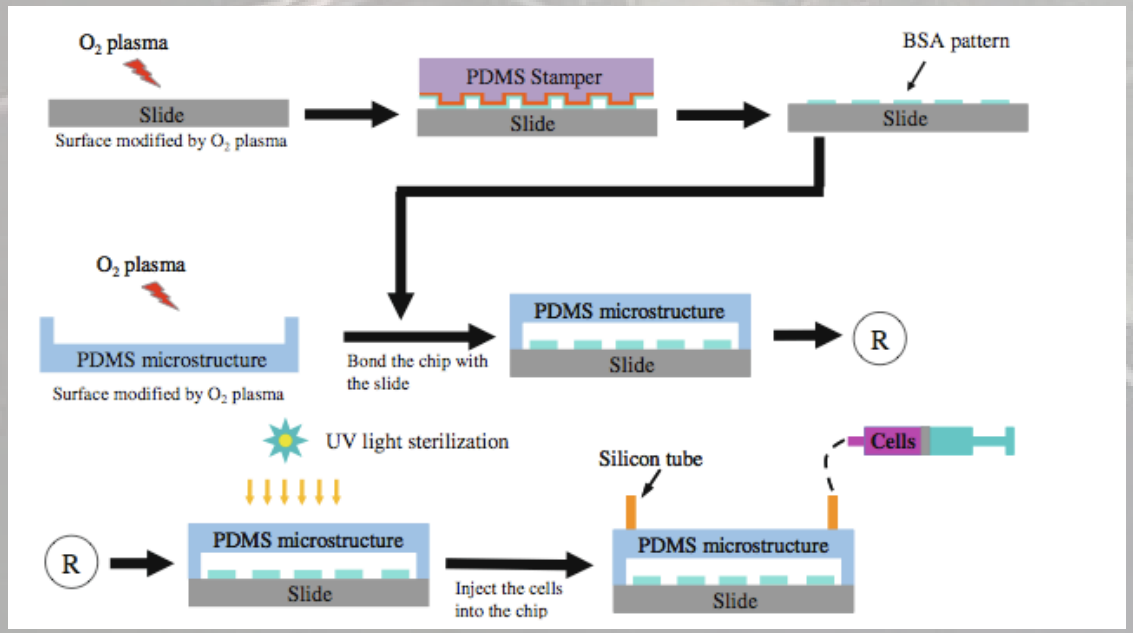
- What we will be doing in the experiment
  - Create a pathogen concentration gradient
  - Study the response of cells to varying pathogen levels
    - How much pathogen can cells endure?
  - Create a pathogen/antibiotic concentration gradient and view the response of the cells
- Improvements over other current designs
  - Three input wells opens up possibility of different experiments

Our Original Design

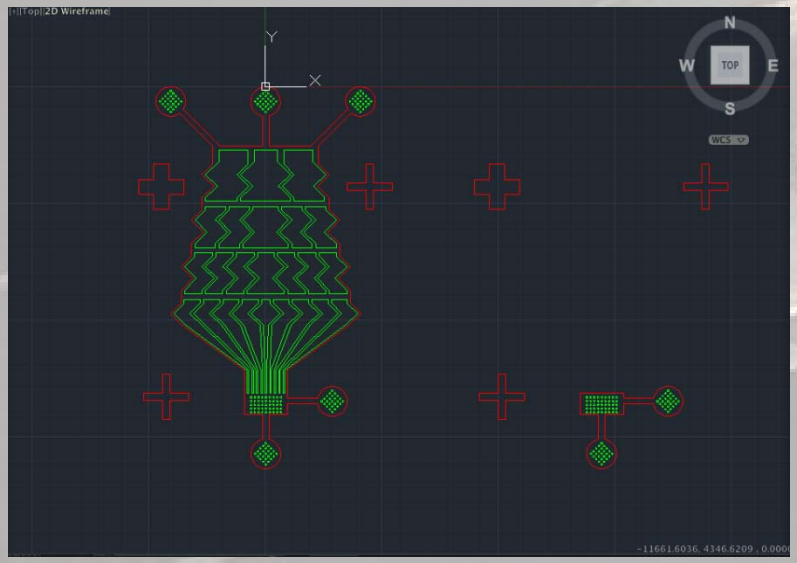


Device Design: Components

- Human Endothelial Cells
- Shiga Toxin-producing E. coli (STEC)

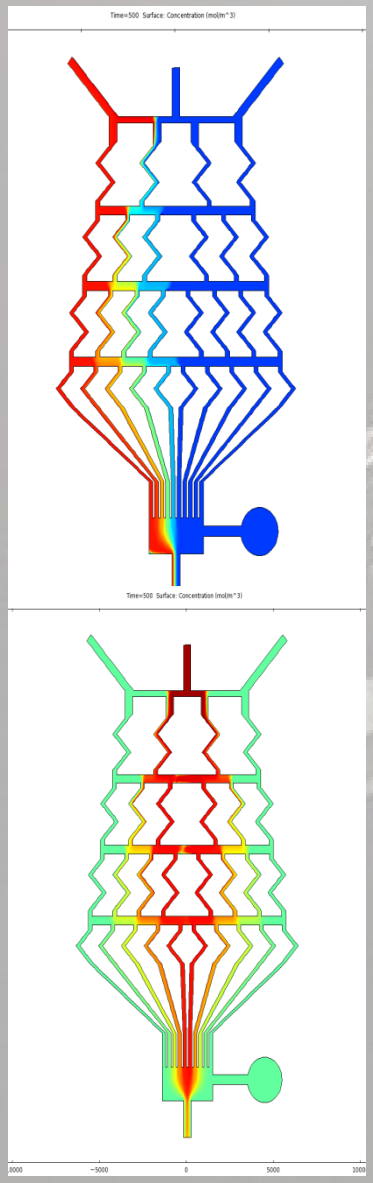


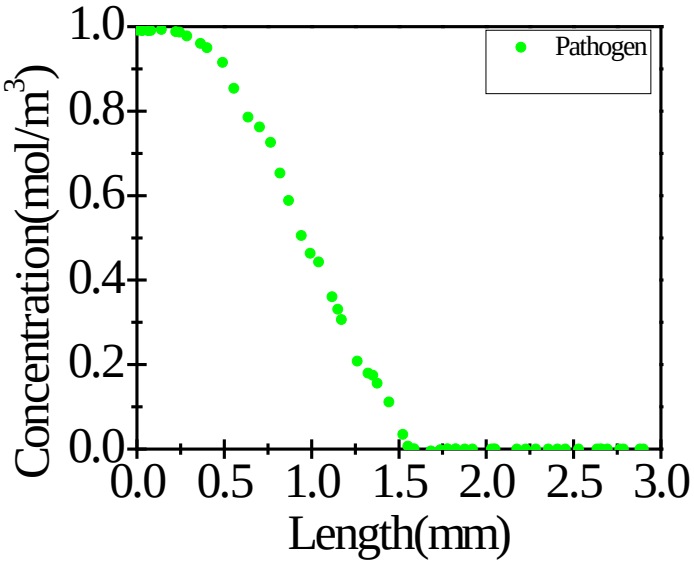
Device Design: Values

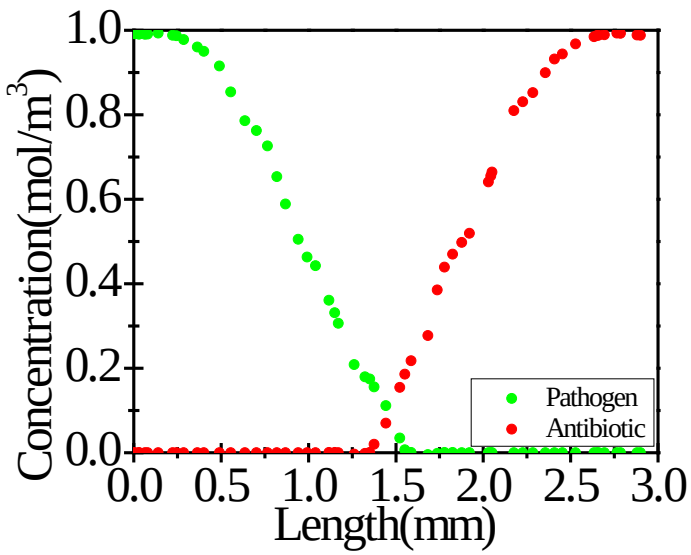


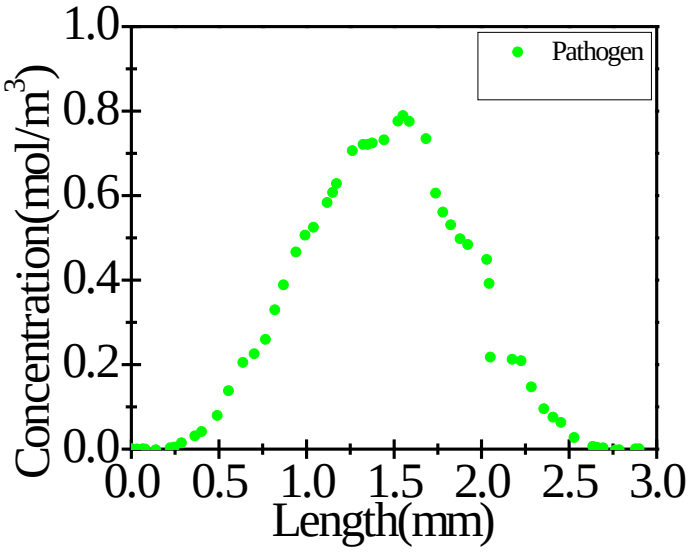
- Flow Rate to be used 20 ul/min
- Reynolds number 2.95

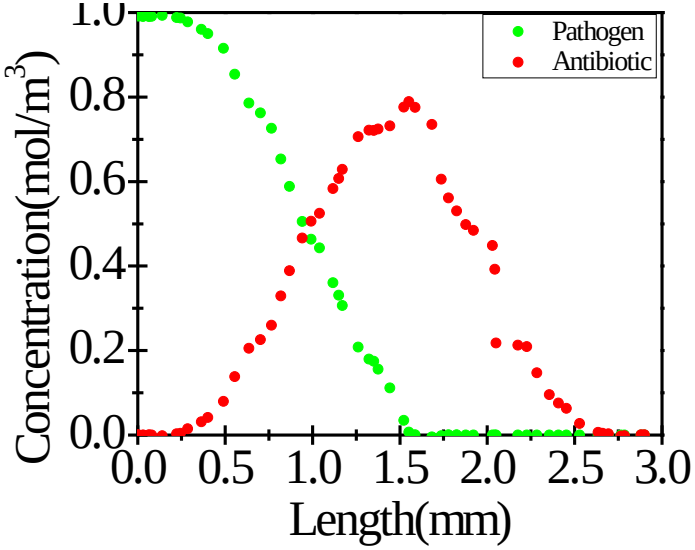
Device Design: Controlling Concentration





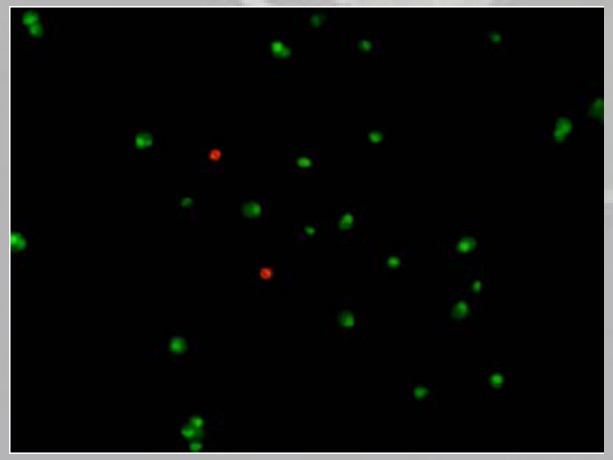






How to analyze results?

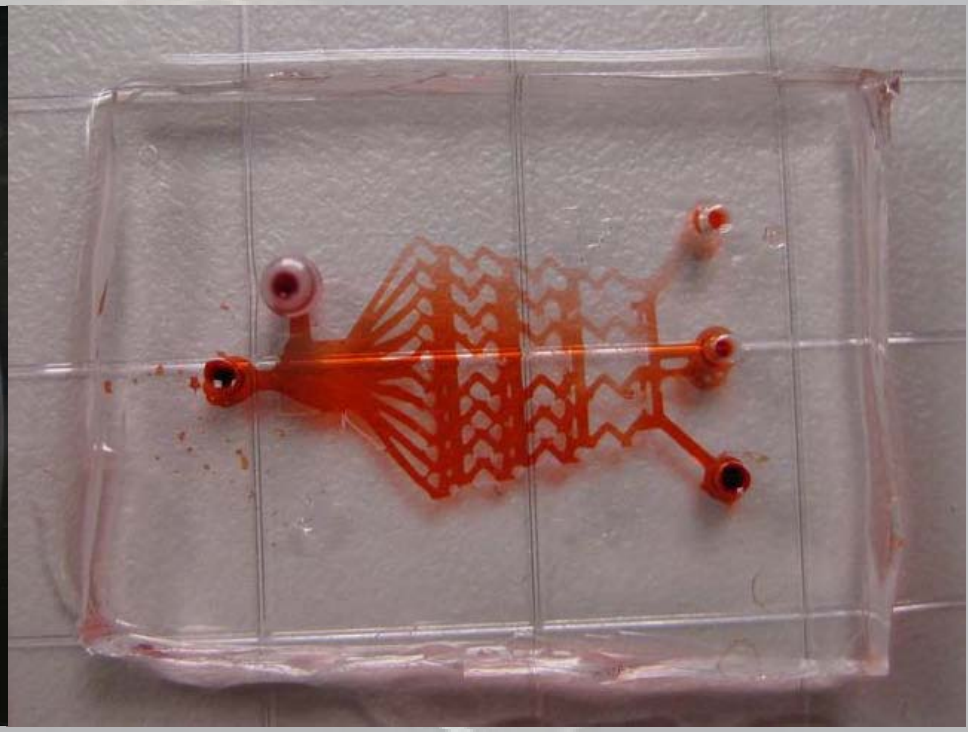
- Microscope
  - Capability to continuously view cell culture, to make sure cells are properly bound to substrate and stained
  - This is will allow to monitor cell growth and adjust cell concentration as needed
- Fluorescence
  - Live/dead staining
- Viewing Section



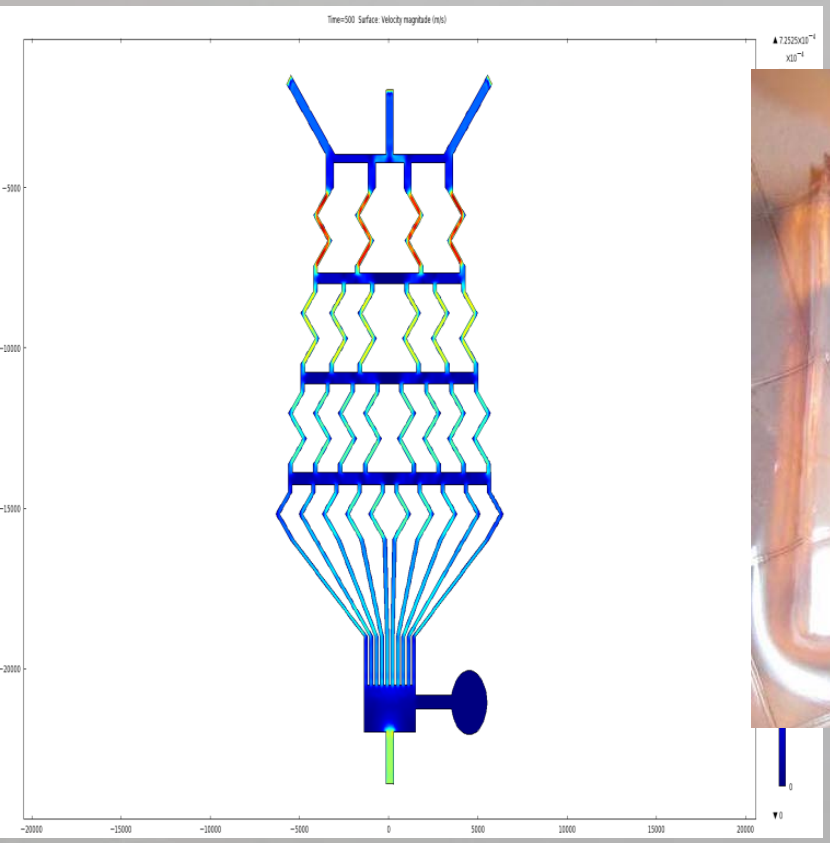
Complications

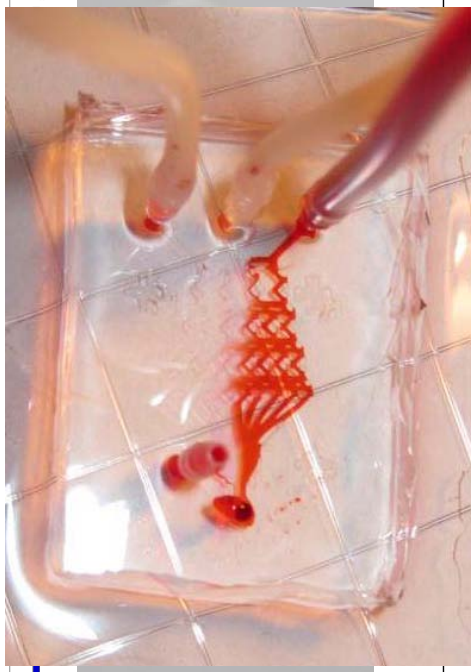
- Fabrication Issues
  - Silicon wafer moved during photolithography
    - Junctions and channels were compromised
  - Air bubbles trapped under wafer during soft lithography
  - Photolithography and soft lithography not performed in a clean room

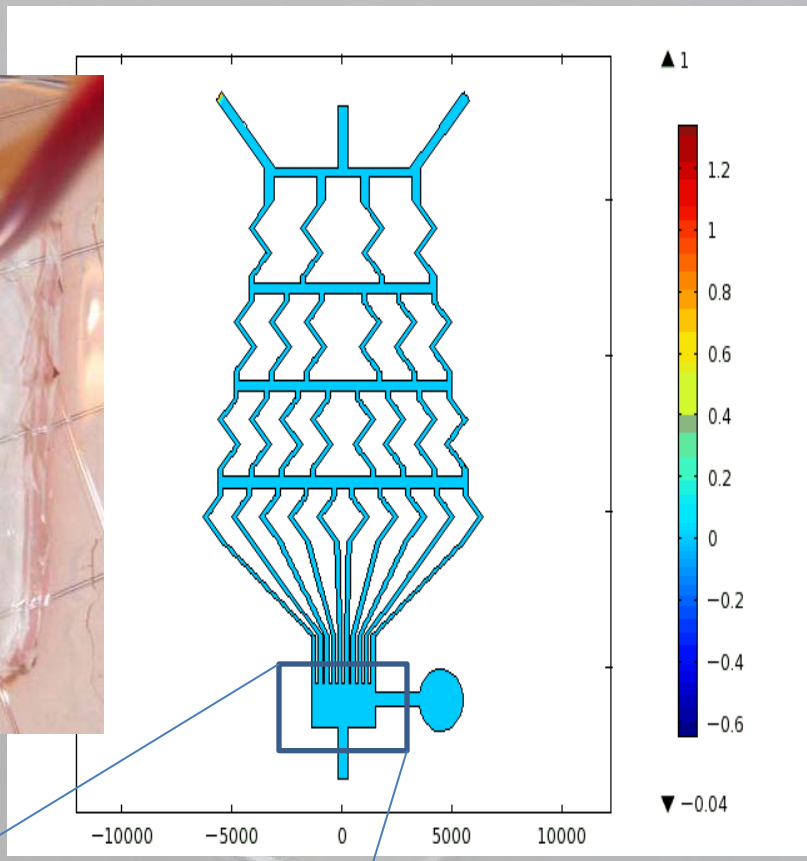
Device Design and Complications

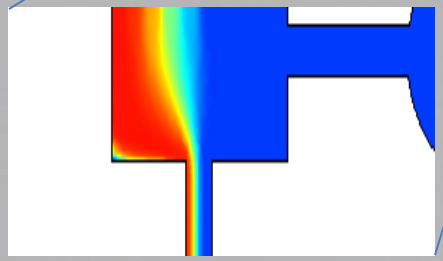


Running the Experiment

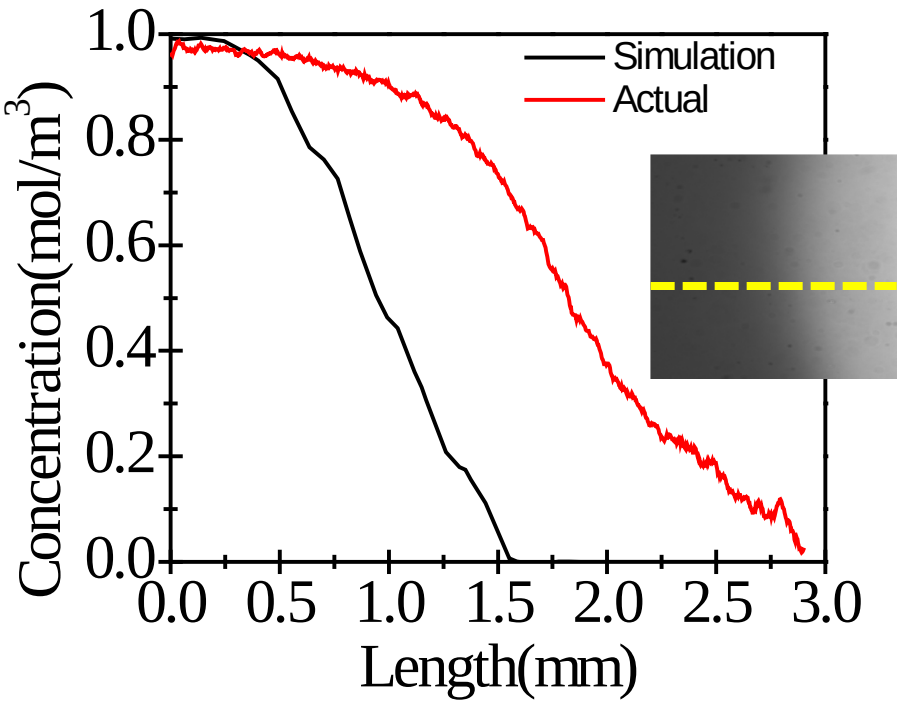


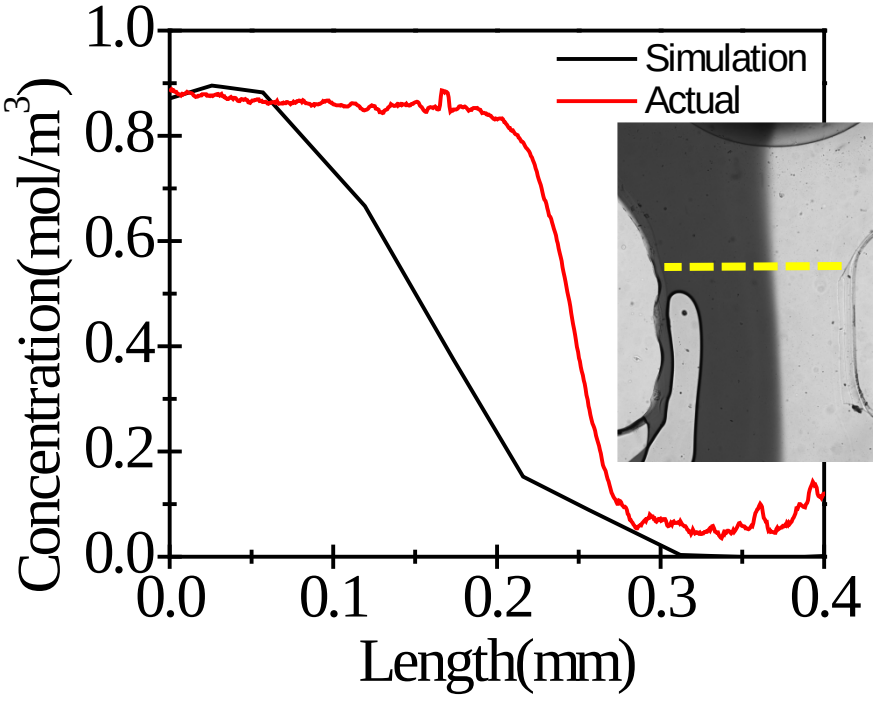




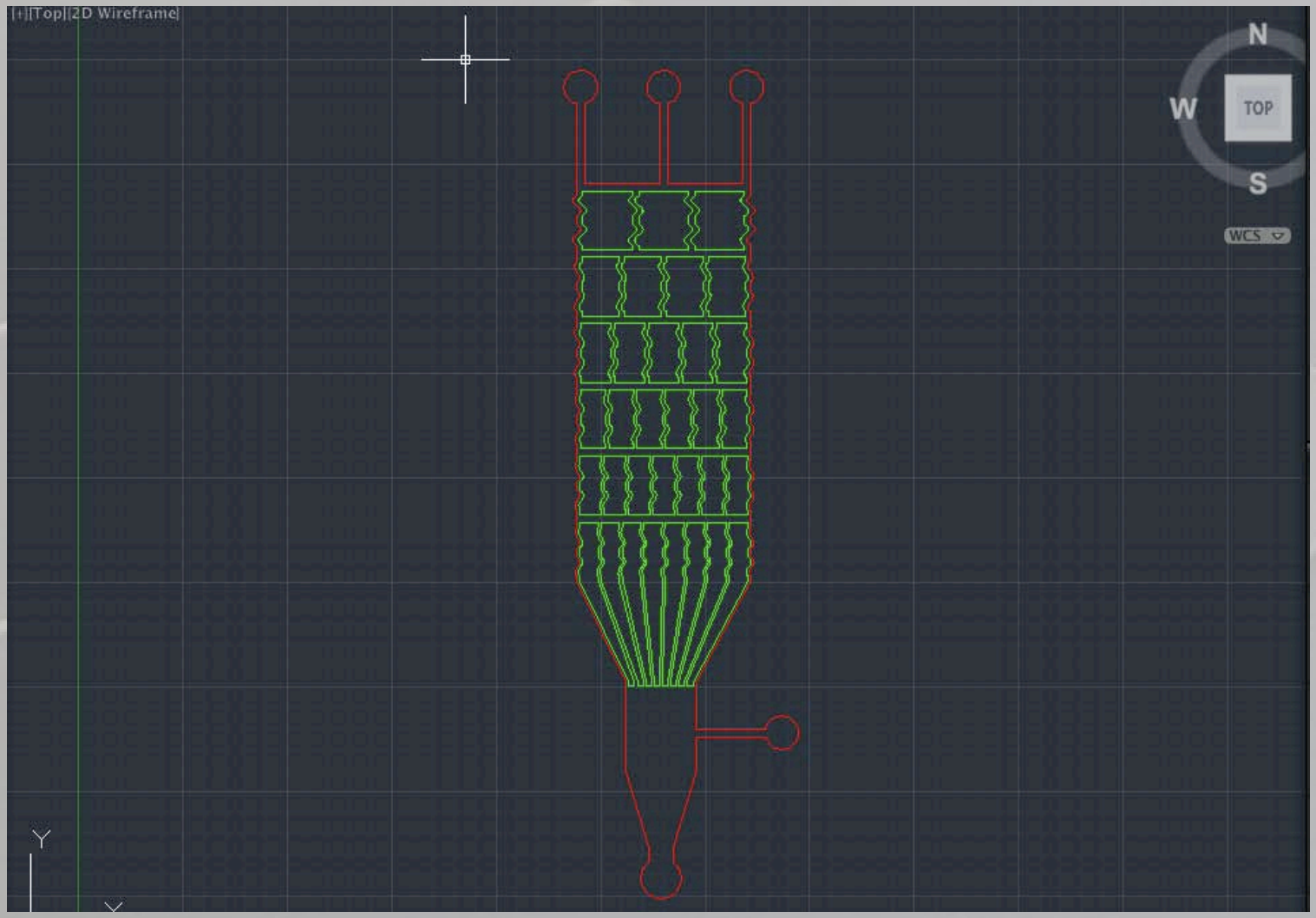


Analysis


Simulation
Actual
Concentration(mol/m3)
Length(mm)
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0

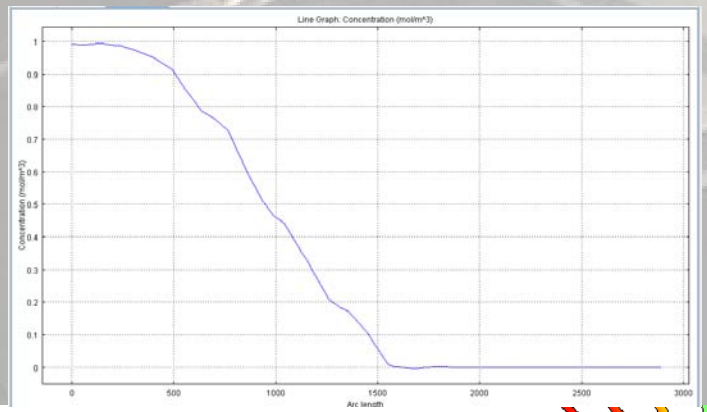

Simulation
Actual
Concentration(mol/m3)
Length(mm)
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.1
0.2
0.3
0.4

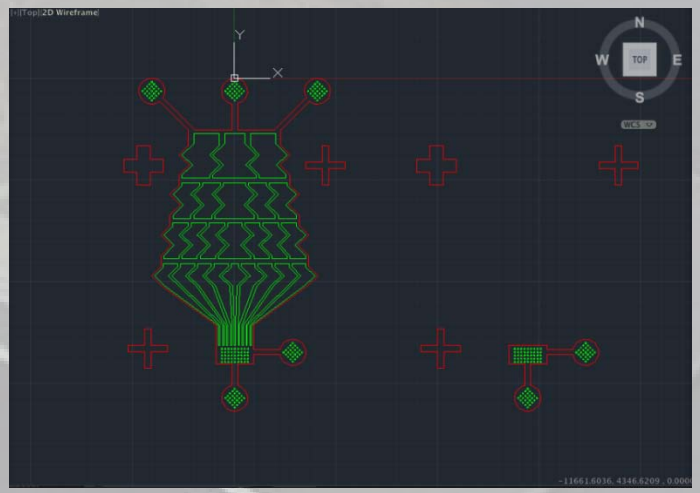
Our New Design

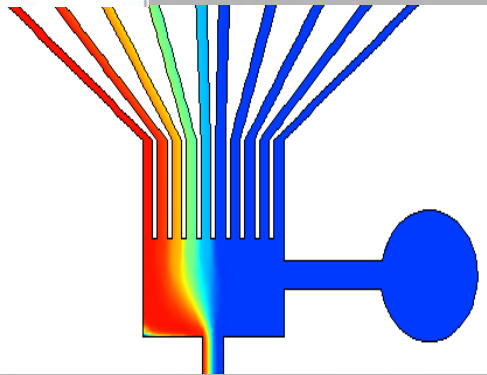


Complications/What We Do “Next Time”

- Spacing between channels
  - Uneven spacing in mixer develops a disrupted slope in gradient

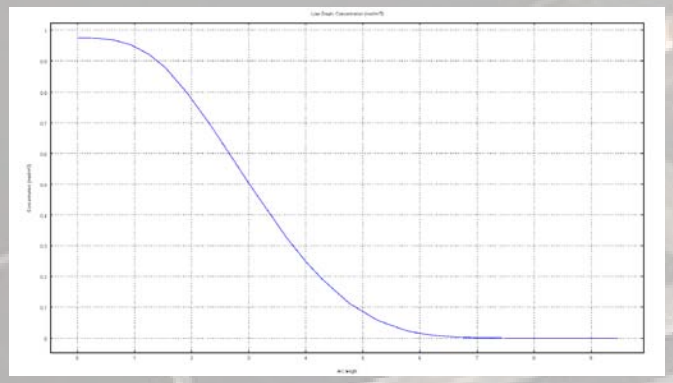


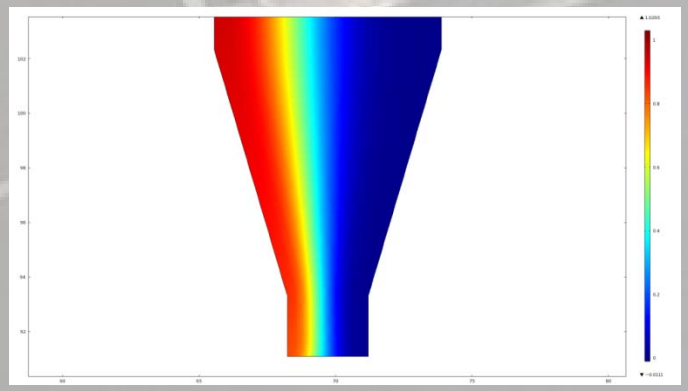


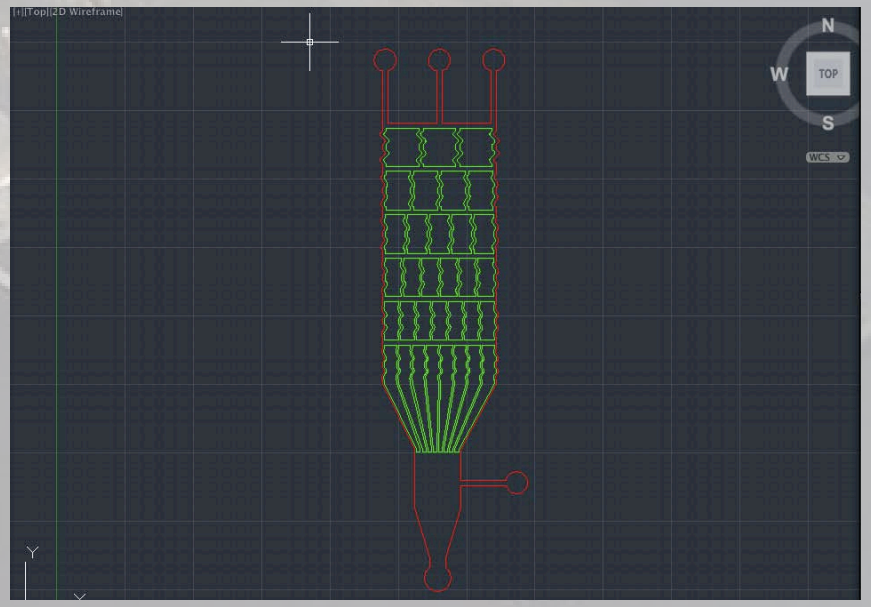


Complications/What We Do “Next Time”

- Spacing between channels

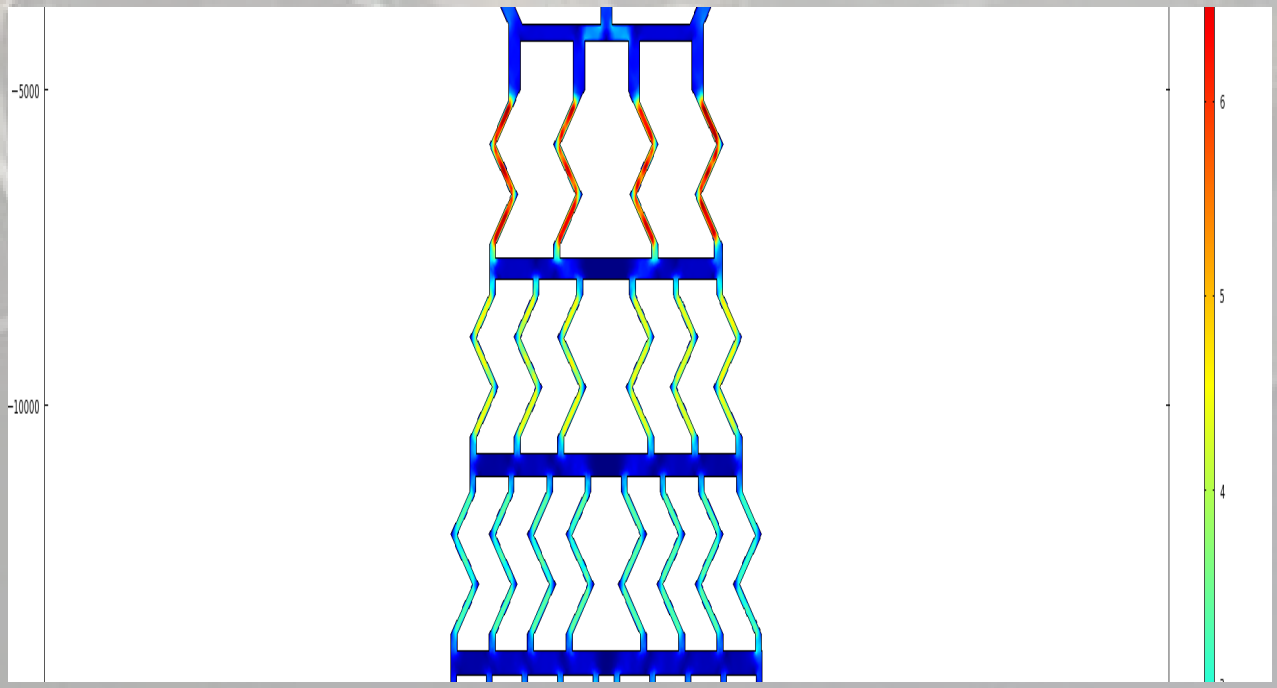






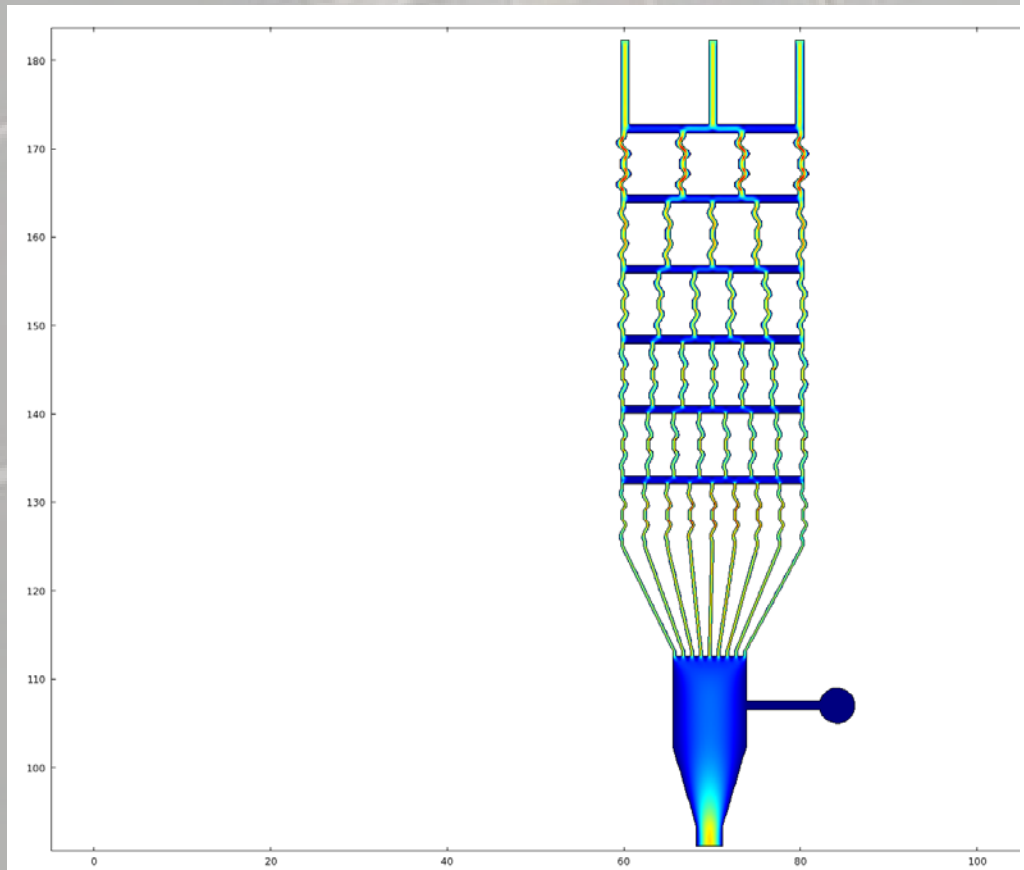
Complications/What We Do “Next Time”

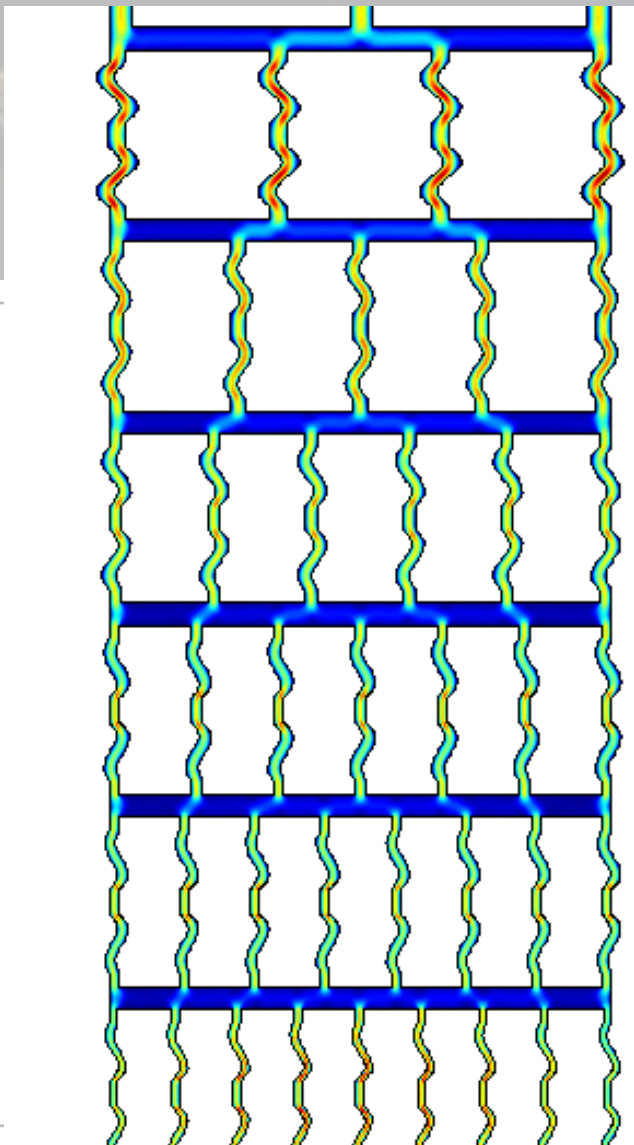
- Size and shape of channels
  - Different sizes leads to different and non-uniform velocities



Complications/What We Do “Next Time”

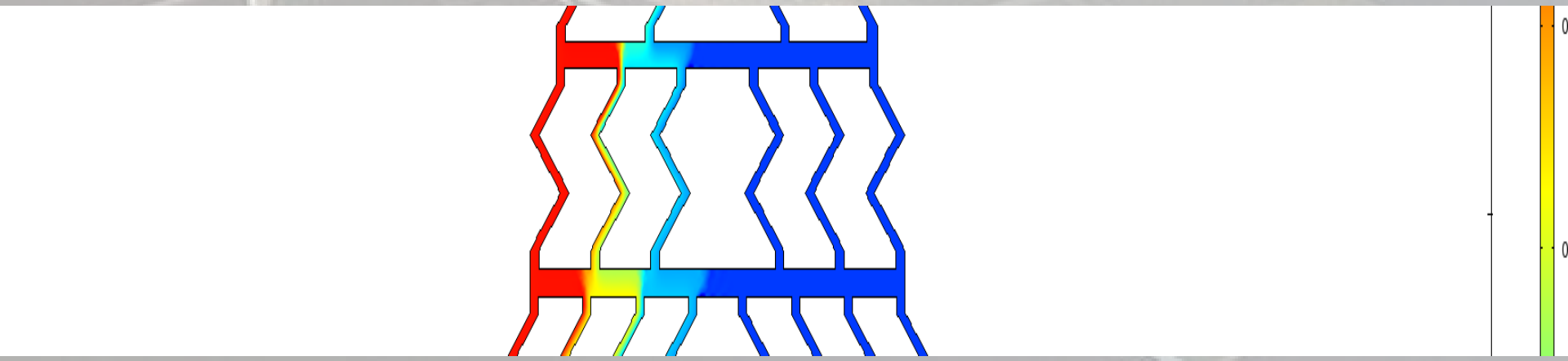
- Size and shape of channels
  - Better velocity profiles





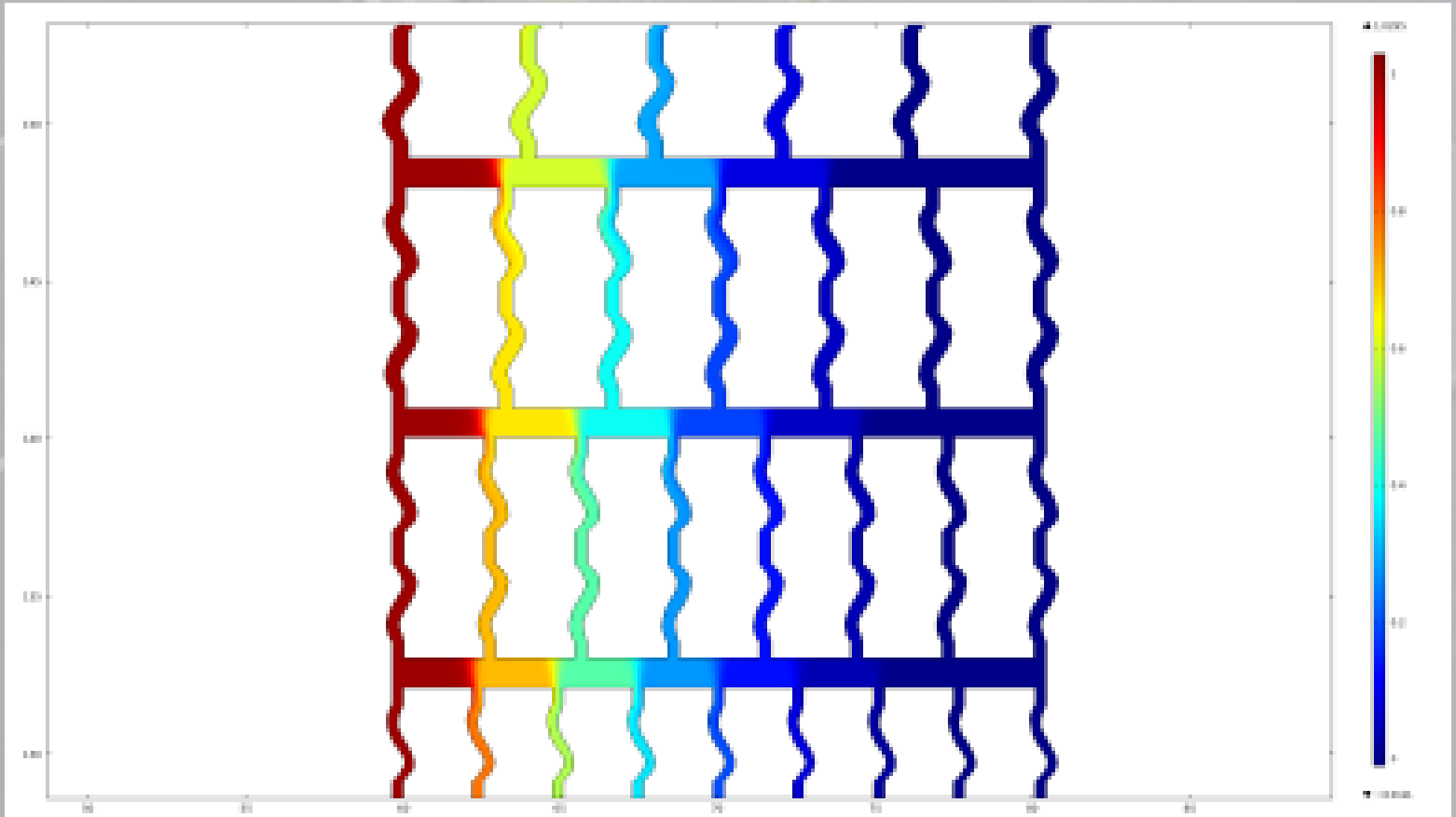
Complications/What We Do “Next Time”

- Size and shape of channels
  - Concentration not uniform



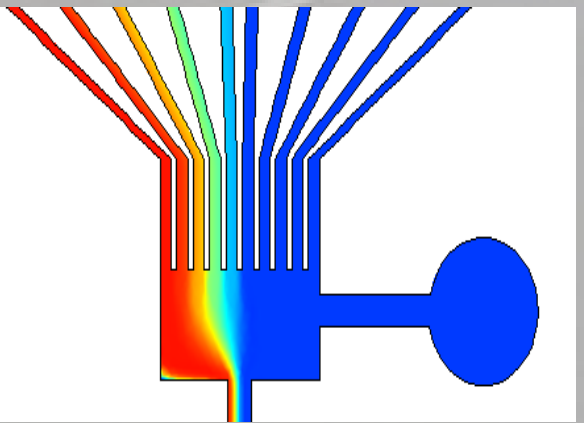
Complications/What We Do “Next Time”

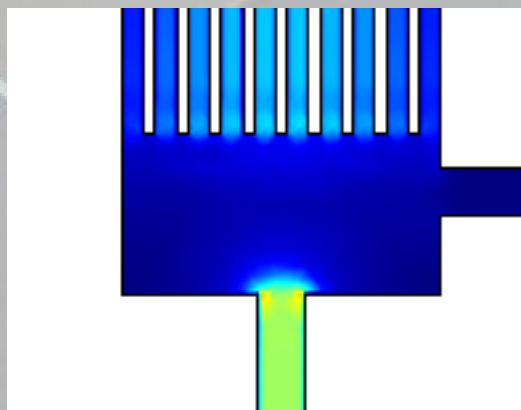
- Size and shape of channels
  - Concentrations uniform



Complications/What We Do “Next Time”

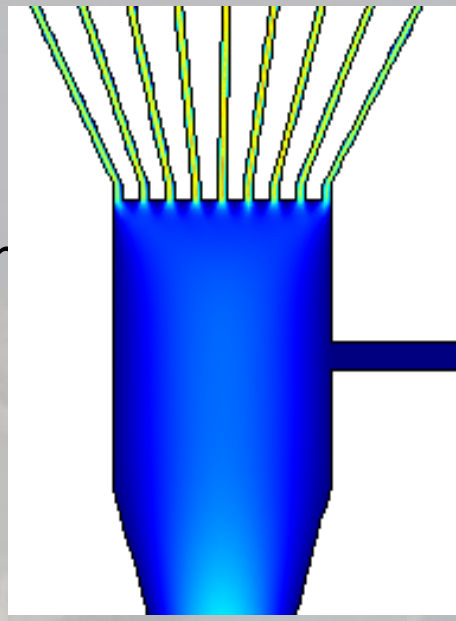
- Output channel may be too small and cause backup/turbulence in cell culture chambers
- SOLUTION: OUTPUT = INPUT SIZE

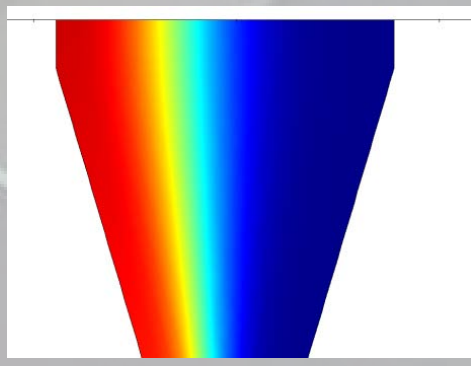




Complications/What We Do “Next Time”

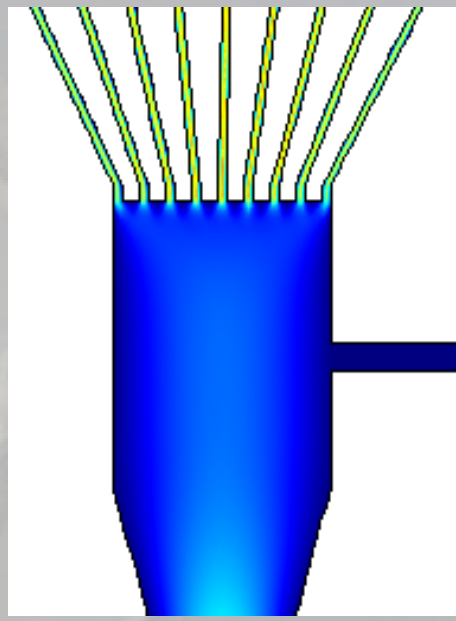
- Extend length of viewing panel
  - Allow gradient more space to reach equilibriu (so not step wise)
  - Avoid culturing and imaging cells where gradient is in flux
- Change outlet channel geometry to avoid sudden change in flow parameters and for diffusion to establish linear gradient
  - Thus do not need to worry about cell distribution – can compare cells at entrance and exit now





Complications/What We Do “Next Time”

- Cell distribution in chamber tough to uniform
- Step wise gradient of pathogen concentration
- SOLUTION – Chamber before cell chamber for diffusion to establish linear gradient thus do not need to worry about cell distribution – can compare cells at entrance and exit now



Complications/What We Do "Next Time"

- Cell culture chamber dropoff (increase in height from 50-75)
  - Leads to increase in flow rate (acceleration from gravity)
  - Dropoff from increased volume

  - SOLUTION-Can eliminate in photolithography to make a ramp and have cell height = dropoff

Or

Only use 1 layer and assume cell height is small

Questions?