20.345: Biological Instrumentation II
Department of Biological Engineering
Massachusetts Institute of Technology
Cambridge, MA 02139

Integrated technology in microfluidic chemotaxis assay for high through-put scientific discovery

20.345 Final Project Proposal

PRINCIPAL INVESTIGATOR: Emmanuel A. Quiroz
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Emmanuel A. Quiroz, Bioinstrumentation II Laboratory
Department of Biological Engineering, Massachusetts Institute of Technology

I. Summary

Roman Stocker's lab has come up with a microfluidic device that quantifies chemotaxis assays (Seymour *et al*, 2009). This stopped- flow unsteady gradient approach demonstrates the ability of microfluidics to model essential features of the chemical landscape of natural, marine microbial environments. Stocker's approach has the potential of providing microbial research with a device that determines population-scale transport parameters directly from single-cell trajectories yet is simple in its assembly. However, the simplicity of the system limits the speed at which novel research can be complete.

The objective of this project is to further develop Stocker's microfluidic system that will allow researchers to run multiple chemotaxis assays in parallel for high though-put assays. This proposal includes my methods and materials to develop such a device as well as a scheduled list of goals to achieve my objective.

I. Background and Motivation

Microfluidics has provided biological research with an unprecedented way to study the motile behavior of cells by providing a degree of control of chemical and physical environments of cells. By reducing experimental analysis to the microscale level, microfluidics avoids turbulent nature of fluid flow which allows the generation of smoother chemical gradients essential in chemotaxis assays. There have been multiple approaches to coupling microfluidics with chemotaxis assays and each is designed to address a defined scientific question.

The earliest bacterial chemotaxis assays relied on flow to generate chemoeffector gradients as well as model environmentally realistic flow (Ahmed, Shimizu, & Stocker, 2010). The general method was to flow bacteria in parallel with a chemoeffector gradient and observe the bacterial distribution at the end of the channel. An example of this approach is shown in Mao *et al*'s three-inlet parallel-flow device (Fig. 1) (Mao, Cremer, & Manson, 2003).

The disadvantage of this approach is that the Hysdrodynamic shear forces may reorient cells and impair chemotaxis analysis. This limitation can be mitigated by diffusion-based gradient generators, which produce flow-free and shear-free environments for bacterial chemotaxis assays.

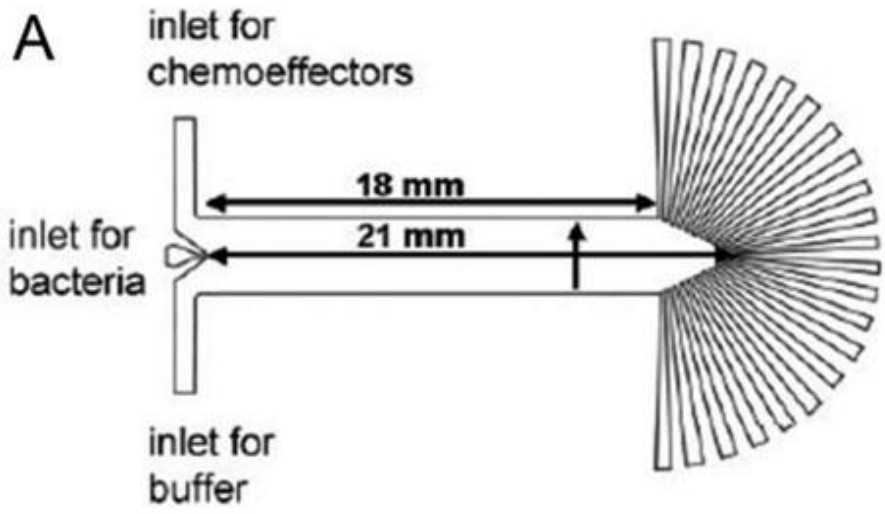

Figure 1: The three-inlet parallel device of Mao *et al*. Outer channels contain chemoeffector or buffer to create gradient across microchannel. Bacteria are injected in middle inlet at steepest part of chemoeffector gradient. Bacterial distribution quantified by counting cells in outlet ports.

Flow-free, or 'stopped-flow', chemotaxis assays allow for the analysis of individual bacterial movement in the absence of shear forces and relies on natural diffusion to generate a gradient. In this approach, flow is only used to set up an initial gradient, but is stopped, allowing the gradient to evolve by diffusion alone. These devices produce unsteady gradients that are advantageous in representing environmental conditions of nutrients especially in marine bacteria and in characterizing bacterial responses to wide range of concentration and gradients within a single experiement. The bacterial distribution is measured by recording the kinetic paths of individual bacteria using videomicroscopy. An example of this approach is Seymour *et al*'s nutrient pulse generator (Fig 2) (Seymour *et al*, 2009).

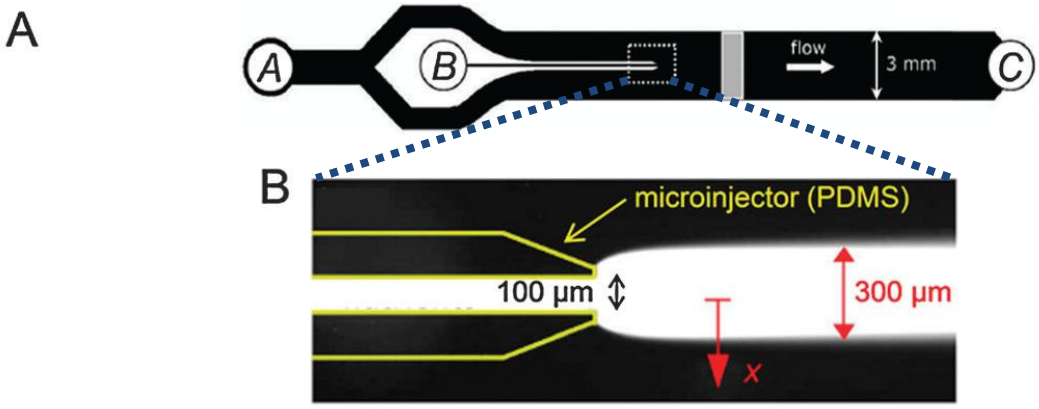

Figure 2: The nutrient pulse generator of Seymour *et al*. (A) The device has two inlets, one for a chemoeffector (inlet B) and one for a bacterial suspension (inlet A). Bacteria and chemoeffector band created by microinjector are flowed to setup initial gradient. (B) The band width of the chemoeffector band can be adjusted by changing the flow rates in inlets A & B.

The disadvantage of this device is that there are constraints on observation time. The stopped-flow approach is useful for quantifying chemotaxis when the timescale of gradient relaxation is much greater than the characteristic times of bacterial response and behavior (Ahmed *et al*, 2010). The solution currently in motion is to design devices that generate steady gradients for studies with longer time scales.

Microfluidics has created a 'toolbox' of a number of bacterial chemotaxis assays that will continue to increase as more scientific questions emerge. Coupled with videomicroscopy, some of these devices enable the observation of both population and individual bacterial response with high spatial and temporal resolution. Even so, the systems of these devices can be improved to provide more high through-put analysis that will advance chemotaxis research.

For my project I will develop an automated microfluidic system using Seymour *et al*'s microinjector approach to provide a high through-put method of acquiring bacterial chemotaxis parameters of varying populations simultaneously. The microinjector device was chosen for its simple design, diverse application to both fluid-flow and flow-free approaches, and for its ability to measure individual cell kinetics. The development of a faster and systematic device is especially useful in the research of marine microbes' response to settling dissolved organic matter.

Currently, more and more scientific questions emerge; therefore multiple microfluidic devices have been designed to address each question specifically. However, there is a lack of integrating technology to these biological devices. These devices need to go beyond being engineering innovations to become an enabling technology of new scientific discovery (Ahmed *et al*, 2010). The objective of this project seeks to accomplish this endeavor.

IV. EXPERIMENTAL GOAL (what accomplish and techniques planning to use)

The main goal of this project is to develop a microfluidic chemotaxis assay system that integrates computer automated pneumatics and step-driven videomicroscopy to Seymour *et al*'s microinjector device. The system will also enable the researcher to run parallel microinjector devices increasing efficiency of scientific discovery and opens a realm of experiments that require simultaneous analysis of cell populations

V. DEVELOPMENT PLAN

The system consists of four major sections, with the first being the detection through videomicroscopy. The microscope will be a brightfield and fluorescent microscope as shown in Fig 3. CCD will acquire images at a given rate and the kinetic paths will be tracked using particle tracking algorithms. The stage of the microscope will be driven by a step-motor and will use algorithms developed in the Bioinstrumentations II class.

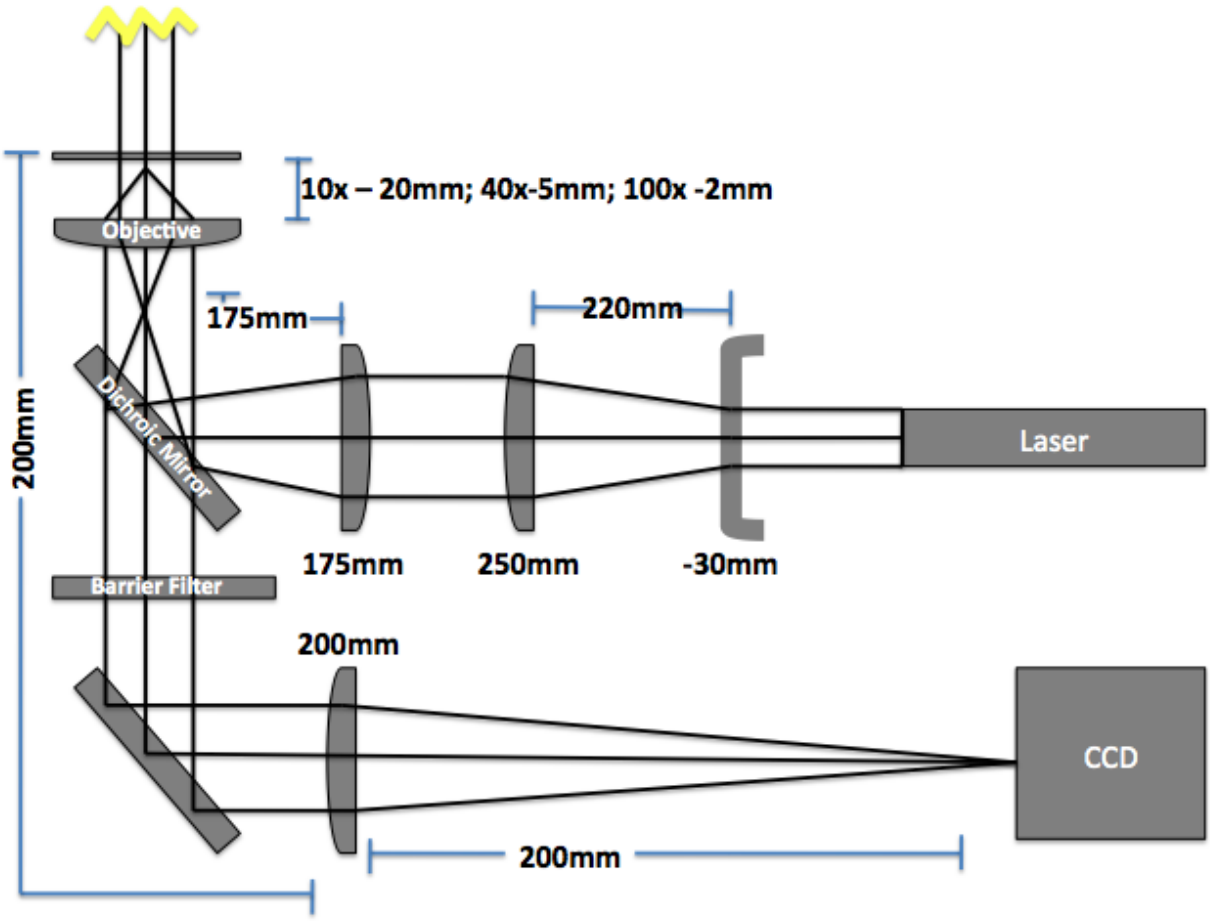

Figure 3: Bright field and Fluorescent Microscope Block Diagram

The second section of the system is the pneumatic controls that will allow computer regulated fluid flows (Fig 4). This consists of computer controlled pressure regulators and valves to adjust microinjector bandwidth and to easily switch to a buffer wash procedure. This pneumatics approach is similar to that used by the Manalis Lab for their Suspended Microchannel Resonator system. The third section of the system involves the manufacturing a microfluidic device that has 3 – 4 microinjectors in parallel. The device is made by PDMS molds and should be fairly easy to alter to suit the systems needs. The fourth section of the system involves the programs involved in controlling the step motor stage, pneumatic regulation, and cellular kinetic path tracking.

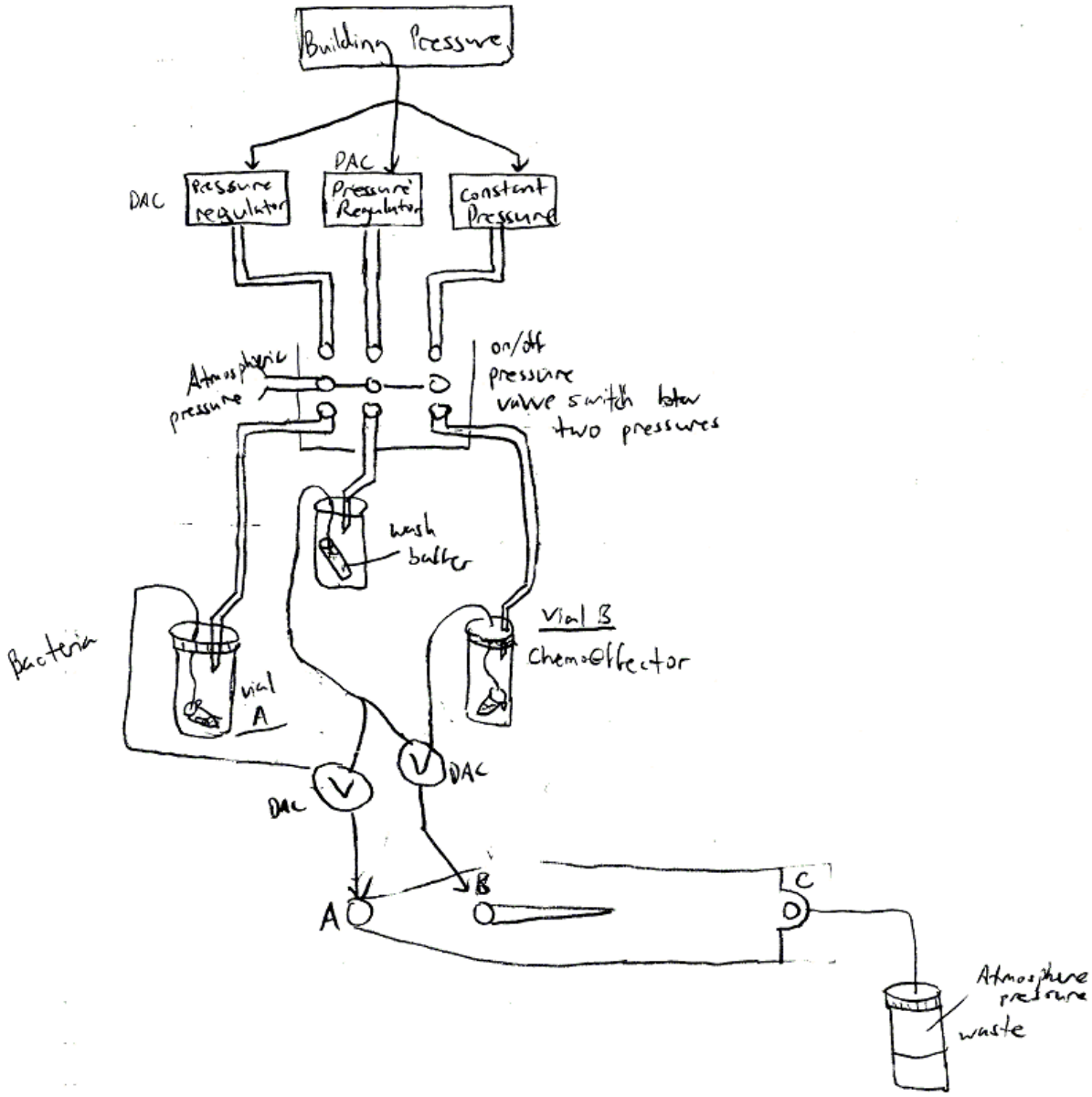

Figure 4: Pneumatic Control Diagram

1. Planned Experiments

a.) General Microinjector Experiment Set up. The general procedure of the microinjector approach by Stocker *et* al is as follows:

- A chemoeffector band with uniform concentration is injected into the inlet of the center stream using a microinjector while simultaneously injecting bacteria into a separate inlet that flows along both sides of the center stream.
- The width of microinjector stream (chemoeffector band) is controlled by the relative flow rate of the two inlets.

- Chemotaxis analysis begins once the flow has been stopped, allowing the chemoeffector band to naturally diffuse in the lateral direction with a nearly Gaussian shape.
- Bacteria and chemoeffector are visualized with fluorescent markers and tracing dyes, respectively. The kinetic paths of the cells are recorded using fluorescent videomicroscopy and particle/cellular tracking computer programs.

b.) Equal E. coli populations analyzed in parallel: this experiment will validate that the parallel microinjector devices are functioning to give equal results.

c.) Novel experiment using parallel microinjector system: To be determined

2. Tentative Schedule and Goals

Week (Friday)	Goal
March 4	- Order Materials
March 11	- Build Brightfield/Fluor Microscopy system
March 18	- Manufacture parallel microinjector devices
March 25	…SPRING BREAK…
April 1	- Assemble automated pneumatics system
April 8	- Exp 2: Run parallel experiments w/equal & known results
April 15	- Troubleshot device/system
April 22	- Exp 3: Run parallel experiments to discover novel results
April 29	- Analyze results and address system limitations/improve.
May 6	- First draft paper on automated system techniques and novel chemotaxis assay results
May 13	- Final draft paper …

VI. ITEMIZED REQUIRED MATERIAL

1. Video Microscopy

- CCD camera
- 175mm lens, 250mm lens, -30mm lens, 200mm lens
- Dichroic mirror
- Objective lens (10x, 40x, or 100x)
- 3 mirrors

2. Pneumatics

- DAC

VII. MATERIALS OUTSIDE OF LAB

1. Video Microscopy

- step motor specimen stage (and circuitry)

2. Pneumatics

- Straight tubing
- Capillary tubing (PEEK tubing or Teflon lined tubing)
- 2 DAC controlled valves and pressure regulators
- Mechanical pressure regulator
- Glass vials with rubber top
- Channel splitters

3. Microfluidics

- Microinjector device with 3-4 microchannels in parallel

4. Chemotaxis Experiments

- Chemoeffector
- E. coli
- Buffer

VI. References

Ahmed, T., Shimizu, T. S., & Stocker, R. (2010). Microfluidics for bacterial chemotaxis. *Integrative Biology* , 604 - 629.

Mao, H., Cremer, P. S., & Manson, M. D. (2003). A sensitive, versatile microfluidic assay for bacterial chemotaxis. *PNAS* , 5449 - 5454.

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