Utilization of Dielectrophoretic Microfluidic Channels in Protist Separation to Enable Symbiosis Research.
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Introduction: Rapid separation of species in a community is desired in microbiology. Dielectrophoresis, based on the creation on non-uniform electric fields can separate whole cells based on their physical differences. Research in termite hindgut communities brought about the need for simplified, low cost, and quick protist isolation. Take for example, *Heterotermes aureus*, the most common urban termite pest in Arizona; who has a characteristic hindgut community comprising of three protists ranging from 10-250 μm^2. In order to study *H. aureus* protists, they must be separated by hand using micropipette cell picking, a laborious process.

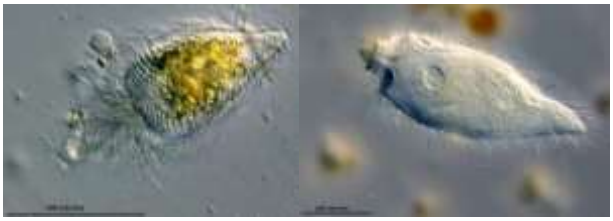

Figure 1: Holomastigotoides and Pseudotrichonympha from *H. aureus*

The scale and range of the protists created several challenges with current Dielectrophoretic separation techniques being deficient in channel depth and width. A novel Dielectrophoretic separation device is being fabricated that can handle the separation of larger scale cells.

Methods: The process used to create this device is intentionally designed to be inexpensive for ease of development and optimization of design.

Design. The initial design was created in AutoCAD, in order to develop a precise design with submicron accuracy. At each constriction point cells with different electrophysical properties are trapped. The samples will run from left to right based on the application of voltage with electrodes placed in the inlet and outlets (Figure 2).

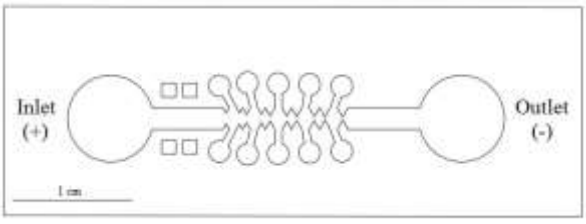

Figure 2: Current AutoCAD Design

Figure 2 displays the most recent design based on the current fabrication limits.

Modeling. Each design was modeled, using a finite element mesh software (COMSOL Multi-physics) to visualize the forces present in the device. Figure 3 illustrates the area that traps analytes flowing through the smallest gate (constriction point) in the channel, 200 μm at the narrowest point.

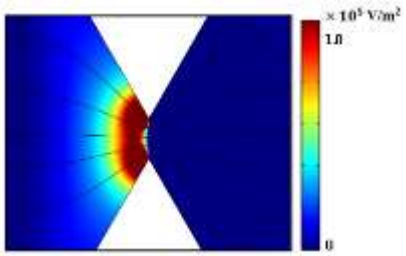

Figure 3: Trapping Ratio

Fabrication. A CO_2 laser printer was used to etch the design into a variety of plastics to create a mold. The microchannels are made from polydimethylsiloxane (PDMS) which is treated with O_2 plasma and bonded to a glass slide.

Testing. Trager and Ringers buffer were tested on smaller scale microchannels to ensure stability with applied DC potentials of 100-1200 V.

Impact on Field: Dielectrophoretic channels have previously been used to separate >10 μm biomolecules[1], but cannot currently separate larger protist cells. Our process is inexpensive, quick, and allows for the separation of larger analytes. The successful development of a microfluidic channel on this scale can have innumerable uses in microbial community ecology, microbial genomics, and symbiosis research.

References: [1] Jones P. V., Staton S. J. R., and Hayes M. A. (2011) *Anal Bioanal Chem*, *401*:2103. [2] Yamin M. (1979) *Sociobiology*. *4*:3-117.