

**Dielectrophoretic Separation of the Symbiotic Protists Present in Termite Hindguts.** Claire V. Crowther<sup>1</sup> Katalina D. Petersen<sup>2</sup> Mark A. Hayes<sup>1</sup> Gillian H. Gile<sup>2</sup> <sup>1</sup>School of Molecular Sciences, Arizona State University, Tempe, AZ, USA. <sup>2</sup>School of Life Sciences, Arizona State University, Tempe, AZ, USA.

**Introduction:** The ability for termites to survive on wood is due to their symbiotic relationship with protists that are present in their hindgut. Specifically, protists within the parabasalia phylum inhabit the hindguts of termites.<sup>1</sup> Current work with parasalid symbionts has focused on the composition of the hindgut community in a particular termite or on determining the phylogenetic affinity of particular parasalids.<sup>2,3</sup> The work detailed here seeks to understand the evolutionary dynamics of the symbiosis, specifically how parasalids have speciated faster than their hosts.

In order to further understand the morphological and molecular characteristics of the symbionts present in termite hindguts, the various species need to be isolated. One method is to culture a specific protist; however this is extremely difficult or currently impossible depending on the species. The other method is hand picking the cells but this is extremely time and labor intensive. A rapid and inexpensive separation system is needed, which has led to the development of a novel dielectrophoretic cell-separation device. Dielectrophoresis (DEP), a force achieved from non-uniform electric field, enables separations by exploiting subtle differences in the electrophysical properties of the analytes of interest. Insulators can be used to achieve the non-uniform electric fields needed for DEP, which is known as insulator-based dielectrophoresis (iDEP). This method has been used to manipulate several nano and micron size analytes, including polystyrene spheres<sup>4</sup>, bacteria<sup>5</sup>, and viruses<sup>6</sup>. This work aims to separate much larger protist cells that range in size from 10-250  $\mu\text{m}$ .<sup>7</sup>

To achieve these separations a larger iDEP device was developed, which has wider constrictions, a larger depth, and side channels to allow for easy removal of the analytes of interest. The design was modelled using a finite element modelling software (COMSOL Multi Physics). The model predicts the device is able to manipulate and separate the analytes of interest.

While modelling clearly indicates separations are achievable, several experimental factors are being addressed. Fabricating these preliminary larger devices requires different techniques than is presently utilized. Currently a CO<sub>2</sub> laser is being used to etch various plastics to create molds of the microdevices. Various challenges have been encountered in the fabrication process as the plastics easily warp and the accuracy of the laser is not consistent. Once these molds are made, a polymer is used to make a cast of the microdevice,

which can be bonded to a glass slide using oxygen plasma. This is currently limited by the ability to get two flat surfaces that can bond. Additionally, to ensure that protists can survive in a microdevice two common buffers used with protists, Ringers and Traegers, have been tested in smaller microdevices. Both buffers demonstrated that the microchannels can withstand the applied direct current potentials needed to achieve separations.

This work is designed to generate a new, low cost, rapid method of separation for analytes from 10 to at least 250  $\mu\text{m}$ . The probing of the morphological and molecular characteristics of the separated protist species will allow, for the first time, the ability to map the symbionts' transmission and speciation against the phylogeny of the termites. The isolated protist can also be targeted for transcriptome sequencing. The transcriptomes can then be used to determine cellulose and lignin-degrading enzyme genes, allowing for a better understanding of the origin and evolution of termite protists ability to digest wood. Furthermore, the development of a channel for this size of analytes is beneficial as it can be used for numerous other applications including the study of microbial community ecology and genomics.

**References:** [1] Nalepa C. A. (2015) *Ecol. Entomol.*, 40, 323-335. [2] Gerbod, D. et al. (2000) *Int. Microbiol.*, 3, 165-172. [3] Saldarriaga J. F. et al. (2011) *J. Eukaryot. Microbiol.*, 58, 487-496. [4] LaLonde et al. (2014) *J. Chromatogr. A.*, 1344, 99-108. [5] Jones P. V. et al. (2015) *Analyst*, 140, 5152-5161. [6] Ding, J. et al. (2016) *Analyst*, 141, 1997-2008. [7] Yamin M. (1979) *Sociobiol.*, 4, 3-117.