JOHN BARRY, and WENDY TRZYNA, Department of Biological Sciences, Marshall University, Huntington, WV, 25755**. Second harmonic generation microscopy imaging of cellulose transportation during the encystment process of Acanthamoeba castellanii**.

Acanthamoeba are single-celled protists able to survive in many different environments, but are most commonly found in soil and freshwater sources. Due to their free-living nature, Acanthamoeba often face a variety of precarious, environmentally stressful conditions, which for many microbes means death. Acanthamoeba are able to cope with extreme conditions by means of a biphasic life-cycle resulting in a round, resistant double-walled dormant cyst state when conditions are adverse, and a dividing, metabolically active free-living trophozoite state when conditions are favorable. The purpose of this study is to observe the transformation from a trophozoite to a mature cyst stage, specifically analyzing the change in morphology associated with the process of encystation. In order to directly observe the production of cellulose, the main component of the interior cell wall that gives an Acanthamoeba cyst its durability, cells were subjected to a non-nutrient medium with added salt concentrations, which has been shown in preliminary trials to be an encystation-inducing stressor. During the process of encystment, trophozoites were imaged frequently using second harmonic generation microscopy to identify cellulose forming in the cell walls of the cysts. Through this method, possible locations of intracellular cellulose while it is being transported to the cell wall can be observed, as well as the dynamic morphology of the cell caused by the build-up of cellulose, resulting in round, mature cysts. Calcofluor white, a cellulose-specific stain commonly used for identifying cell walls in plants and algae, was also implemented to achieve the same goals.