2024 Buck Summer Scholar: Daniel Allred

My name is Daniel Allred. I am a student at the College of William & Mary, majoring in Biology and minoring in Data Science. At William & Mary, I work in Dr. Matthew Wawersik's lab which focuses on cellular and developmental genetics. Our current research investigates how the gene *Chigno* helps regulate stem cell development in fly testes. During the school year, I have been designing and conducting a transcriptomic analysis of *Chigno* in fly testes. At The Buck Institute, I had the opportunity to work in Dr. Chuankai Zhou's Lab, alongside postdoctoral researcher Dr. Meiying Wu. The Zhou lab focuses on proteostasis and its role in cellular aging, primarily using budding yeast as a model organism. One of the lab's main goals is to develop new methodologies that enable groundbreaking aging research.

My work with Dr. Meiying Wu centered on developing a microfluidic protocol to streamline the quantification of yeast replicative lifespan (RLS). RLS, defined by the number of replications a yeast cell undergoes, is a valuable measure of aging in budding yeast. Replication occurs when one yeast cell, called the mother, creates a new yeast cell, called the daughter. Quantifying RLS is a challenging and time-consuming task because scientists must count every replication of an individual yeast cell by observing that cell under a microscope. The biggest roadblock to quantifying RLS is dealing with the daughter yeast cells, which, after being produced, also start replicating. The average yeast RLS lasts 20 replications. With every replication of the mother and daughter cells the entire population of yeast doubles. Thus, over the RLS of a single yeast millions of cells are produced. This creates a crowded environment, making it difficult to identify individual replications. Previous methods required manual microdissection to remove new cells as they replicated. This prevented exponential growth and allowed for quantification but required significant time and labor. More recently, microfluidic approaches have been developed.

Microfluidics lie at the intersection of physics, engineering, and biology. They use small channels to move small amounts of liquid. Current microfluidic approaches for quantification of yeast RLS use mechanical traps. These traps take advantage of the size difference between mother and daughter yeast, allowing mother cells to be trapped while smaller daughter cells are washed away. Unfortunately, this approach has several limiting factors including restrictive requirements and relatively low sample size. The protocol we've been developing uses chemical adhesion to bind and image yeast. The protocol uses a relatively large channel and coats the surface with proteins. These proteins bind to the yeast and adhere them to the channel's surface. Any daughter cells formed by the first generation of mother cells will not be bound to the protein adhesive. Thus, as liquid flows through this channel providing nutrients and experimental conditions, it will wash away all newly formed daughter cells. This provides an efficient method to quantify yeast RLS. The process of troubleshooting and developing a reliable protocol is long and difficult. However, the finished protocol will provide a platform to conduct a range of new yeast RLS studies, with topics ranging from iron homeostasis to protein behavior.