## Quantitative Imaging of Gut Microbiota Spatial Organization

**Awardee:** Kerwyn Casey Huang
**Award:** New Innovator
**Awardee Institution:** Stanford University
**Co-authors:** Kristen Earle, Gabriel Billings, Justin Sonnenburg
**Co-authors' institution:** Stanford University

I will discuss a pipeline for the assessment of intestinal microbiota localization within immunofluorescence images of fixed gut cross-sections. The pipeline includes a flexible software package, BacSpace, for high-throughput quantification of microbial organization, including proximity to host mucus and epithelium. Using gnotobiotic and humanized (colonized with human microbiota) mice, we demonstrate that elimination of fiber from the diet, which is known to increase microbiota utilization of host mucosal glycans, results in thinner mucus in the distal colon, increased proximity of microbes to epithelium, and heightened expression of the inflammatory marker REG3β. We demonstrate that hypotheses of how microbe-microbe metabolic interactions impact spatial organization can be tested using this quantitative method, including the role of mucus processing in the colocalization of a promiscuous polysaccharide utilizer with the pathogen *Salmonella typhimurium*. Furthermore, we quantify the invasion of Helicobacter pylori into the glands of the mouse stomach, illustrating the generalization of this approach. This broadly applicable framework will accelerate the elucidation of the roles of microbiota localization in health and disease.