In Situ Analysis of Microbial Communities Using Expansion Microscopy

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***Short Abstract* — We demonstrate the utility of expansion microscopy (ExM) in visualizing and analyzing spatial distributions of microbial species in gut microbiota of several organisms. First, we developed a new super-resolution imaging method to improve the photostability and brightness in expansion microscopy. Second, by tuning the enzymatic digestion of microbial cell walls, we use the expansion ratios in ExM as an in situ measure of bacterial physiology. This method enables multiplex imaging of complex bacterial communities while simultaneously providing spatial and cell physiological information.**

***Keywords* — Expansion microscopy, Microbial community, Bacterial physiology**

I. Background

THERE is a global race in microbiome research to map spatial organization of microbial species in densely packed communities. This information is critical for understanding the physiological and molecular interactions among species. The rapid progress in adapting fluorescence microscopy [1,2] and super-resolution imaging techniques [3] have greatly advanced the characterization of spatial structures in microbial communities and bacterial physiology in individual cells at the molecular level. However, progress is limited in linking these two scales due to lack of methods to characterize the diversity in physiology of microbial species within a population in situ.

II. Results

Here we describe a new super-resolution imaging method, based on expansion microscopy (ExM) [4], to achieve super-resolution imaging of dense bacterial populations in two organisms, planarian flatworms and mice, and simultaneous measurement of bacterial mechano-physiology.

A. Locked expansion microscopy

Expansion microscopy is an optical imaging technique based on physical expansion of tissues anchored to a hydrogel. The current method relies on a polyelectrolyte hydrogel that is expanded by electrostatic repulsion; as a result, the hydrogel shrinks in ionic buffers. This technical limitation precludes the possibility of using most anti-photobleaching systems, which typically require buffering. This restricts the application of ExM in thick tissues with weak fluorescence signals, as these applications typically demand long imaging times and suffer from photobleaching. Here, we developed a novel method, locked ExM, in which the first expanded polyelectrolyte hydrogel is embedded in a second interpenetrating hydrogel mesh that expands through entropic forces. The second interpenetrating mesh retains the size of expanded tissues even in buffers with extreme ionic strengths. This method has allowed us to use anti-photobleaching systems in ExM to achieve sub-diffraction-limit resolution with high photostability in order to capture features that are otherwise impossible to image.

B. Differentiation of bacterial cell wall properties by expansion ratios in situ

In locked ExM, the mechanical properties of a specimen are critical to the extent of expansion. In particular, bacteria rely on their cell walls to bear mechanical stress and maintain cell shape. The mechanical property of the cell wall also reflects the physiological states of bacteria. We found that the expansion ratios of microbial species depend on their specific cell wall structures, which is characteristic to species and cell physiology. By using this information, our method allows multiplex imaging to identify different bacterial species, characterize their physiological states, and measure their spatial organization in complex communities in vivo. We use this method to study the gut microbiota in two organisms: the planarian flatworm, as a model for regeneration, and mice.

References

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