

## Biological and Technological Tools to Probe Systems Level Structure of Human Oral Biofilms

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Just as the function of metazoan tissues and organs is mediated by the patterned assembly of phenotypically distinct cells during development, the functions of oral microbes in promoting health and disease arise from the emergent properties of their patterned structure: the dental plaque biofilm. In our earlier work, we expanded the number of different microbes that could be simultaneously identified in a single image through combinatorial labeling and spectral imaging. We mapped the structural interaction network of 15 different genera of oral microbes [1]. We and others further identified abundant and conspicuous, filamentous organisms in as central features in dental plaque biofilm communities. These organisms are species of *Corynebacterium*, *Leptotrichia*, *Capnocytophaga* and *Fusobacterium*, yet the individual roles of these geometrically eccentric cells in structuring oral biofilms remains unknown [2]. There exists a critical need to understand the role filamentous organisms play in biofilm assembly because it is well understood that changes in taxonomic abundance, ecological succession and biofilm structure mediate the transition from health to inflammatory periodontal diseases in the mouth. To address this knowledge gap, we have developed two tools to probe dental plaque structure at the systems level: one biological and one technological.

Our biological tool is an *in vitro* dental plaque model that we have adapted for multispectral imaging. We collected dental plaque from 20 healthy donors and used this mixed species inoculum to seed cultures in saliva-coated microfluidic devices. We verified that our *in vitro* communities are species-rich and recapitulate the oral communities from their respective donors through 16S RNA sequencing with 16 or more genera present at greater than 1% abundance. We further mapped the spatial heterogeneity of *in vitro* biofilms with probes for 8 genera and multispectral confocal microscopy. This biological tool allows us to perform controlled hypothesis testing to identify cellular and molecular components of long-range spatial organization in complex communities.

Our technological tool is an algorithm that greatly improves spectral unmixing of fluorescently labeled microbes in mixed communities. Our algorithm uses a Poisson regression approach and further utilizes *a priori* knowledge about the labeled specimen and the resulting image, e.g., sparseness, to impose novel constraints on the unmixing solution. We demonstrate with model images an improvement of 20% or higher in the accuracy of spectral unmixing with this approach compared to non-negative least squares.

In future, we will assimilate our two tools to probe the cellular and molecular basis of multi-species microbial community structure and function. Our work demonstrates the dynamic interplay between biological insight and technological advances and will allow a greater understanding of spatial structure in the human microbiome and specific role of biogeography in maintaining health and allowing disease in the oral cavity.

References:

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- [2] JL Mark Welch et al., Proc. Nat. Acad. Sci USA **113** (2016), p. E791-800. doi: 10.1073/pnas.1522149113