MICROBIOLOGY

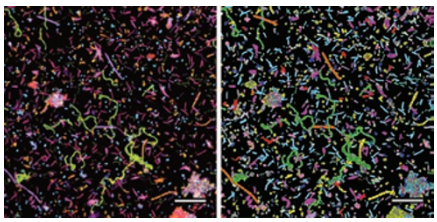
Identifying order in a plaque

An image-analysis technique deconvolutes combinations of labels, revealing which bacteria are where in microbial communities.

Everywhere from the human mouth to abandoned mine shafts, microbes live in diverse, interacting communities. Whereas sequencing techniques are getting better at identifying what species are present, no comprehensive techniques exist to determine how these microbes are spatially arranged. Such information could be crucial to understanding, for example, how microbe-microbe interactions establish a biofilm, how biofilms turn virulent as well as how biofilms can be engineered or controlled. The major difficulty has been that the number of bacteria types in a microbial community far exceeds the number of fluorophores researchers can use to label them, severely limiting the number of microbial taxa that can be observed.

Recently, researchers led by Gary Borisy at the Marine Biological Laboratory in Woods Hole described an approach to labeling and image-processing that can identify many types of microbes in a single image. It is based on an established technique called fluorescence *in situ* hybridization, or FISH, in which DNA molecules attached to fluorophores hybridize to complementary DNA or RNA sequences inside cells. The research team first created a suite of FISH probes to label ribosomal RNA so that each taxon would be labeled by distinct sets of fluorophores. The raw images obtained were difficult to interpret because of overlapping spectra from the different fluorophores, so the researchers used commercial spectral unmixing software to determine which probes were bound to which cells. They also wrote software to statistically evaluate their results and to assign unique colors to each of these combinations, making each observed taxa a different color. As the researchers had used combinatorial labeling and spectral imaging with FISH, they dubbed the technique CLASI-FISH.

After testing the technique on simple, cultured microbes, they applied it to a natural microbial community, the plaque on laboratory members' teeth. The research team showed that combinations of six fluorophores could identify 15 taxa in the same image and demonstrated (as expected) that the plaque was primarily made up of early colonizers such as *Streptococcus*, *Prevotella*, *Actinomyces* and *Veillonella* species. Further analysis showed that most of the interspecies associations involved *Prevotella* and *Actinomyces*, suggesting that these genera are particularly important in establishing and maintaining biofilms.



Combining a few fluorophores can identify many microbial species. Merged raw spectral image (left) and taxon-assigned segmented image (right). Reprinted from the *Proceedings of the National Academy of Sciences USA*.

Although previous imaging studies were limited to only a handful of species, CLASI-FISH could potentially be used to explore hundreds. In this work, the researchers showed that eight fluorophores could identify 28 unique combinations of two fluorophores each, but the team is developing new algorithms to expand the number of fluorophores distinguishable in a single image. Labeling each taxon with, say, three of a possible 15 fluorophores could provide 455 combinations.

In addition, the Borisy lab members are working with collaborators to apply CLASI-FISH to study fully intact biofilms as well as to study microbial communities introduced into the digestive tracts of mice. "Now we can see how microbes relate in space," says Borisy. This could allow researchers to develop new hypotheses about how the structure of microbial communities affects human disease and antibiotic resistance.

And the technique is not restricted to studying microbes, says Borisy. With appropriate probes, CLASI-FISH could be used to monitor the spatiotemporal arrangement of cellular organelles in a cell or the arrangement of cell types in a tissue.

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RESEARCH PAPERS

Valm, A. M. *et al.* Systems-level analysis of microbial community organization through combinatorial labeling and spectral imaging. *Proc. Natl. Acad. Sci. USA* **108**, 4152–4157 (2011).