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Journal

Journal of Oral Microbiology, 18(1)

ISSN

2000-2297

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Publication Date

2026-12-31

DOI

10.1080/20002297.2026.2640344

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Title Page

Interrogation of Imaging-based Interspecies Dynamics in the Oral Microbiome

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Abstract

The oral cavity presents a highly dynamic environment where inter-microbial communications play a pivotal role. Understanding the spatial organization of microbial ecosystems has been highlighted on the microbiome and polymicrobial infection. Furthermore, cross-feeding and modulation by metabolites from the oral microbiota and host cells, such as lactate and reactive oxidative species, impact the stability and functionality of microbial communities. Traditional research focusing solely on the compositional aspects of these communities is insufficient to understand the sophisticated interactions. Advancements in imaging technologies, bolstered by multi-omics analyses and artificial intelligence (AI)-driven insights, provide a more integrated understanding of the dynamics and function of the oral microbiome. Real time imaging and resolution-enhancing methods at the single-cell level have unraveled the ecology and dynamics of microbial communities, indicating unique three-dimensional architectures and biogeographical patterns associated with disease status in polymicrobial interplays. Emerging computational techniques can account for the spatial features of oral microbiome by creating image-like representations that capture the complex relationships between host tissues and microbial communities. Spatial multi-omics, help address the limitations of single-cell sequencing, deciphering molecular mechanisms between species in these biogeographical patterns. To process the massive volume of imaging-based data, AI-assisted analysis enables complex dataset integration, predictive capacity, and personalized treatment, bringing a whole new level of understanding of the oral microbiome and its relationships with the host. In this review, we highlight recent imaging-based technologies used to study the spatial biogeography of interspecies and interkingdom relationships within oral microbial communities, focusing on how these interactions and functional/metabolic alterations associated with health and disease. We further outline limitations of AI-generated predictions and imaging-based observational data. Finally, we elaborate on potential biomarkers for early diagnosis and new effective therapeutic strategies to reshape microbial dynamics.

Keywords: Spatial organization, Oral microbiome, Interspecies interaction, Spatial omics, Imaging, Artificial intelligence, Computational modeling

Introduction

The oral environment is exceptionally complex, hosting nearly 1,000 species of bacteria, viruses, fungi, archaea, and protozoa (Li et al. 2022). Within this dynamic milieu, interspecies interactions influence both the structure and function of microbial communities. Networks of synergistic and antagonistic relationships within oral microbiome give rise to emergent properties such as persistence, stability, and large-scale spatial organization that mediate the shift from a state of health to diseases (Chetty and Blekhman 2024).

Heterogeneous and non-random spatial organization is repeatedly observed in natural microbial ecosystems, and the oral cavity is no exception (Martinez-Garcia et al. 2022). Distinct microbial habitats with unique spatial patterns exist throughout the mouth, forming an ecological gradient from front to back, regardless of the underlying tissue type (teeth, alveolar mucosa, keratinized gingiva, or buccal mucosa) (Proctor et al. 2018). Microorganisms live side by side, stack on top of each other, and intertwined to form a complex interacting community (Mark Welch and Borisy 2025). Meanwhile, dense and highly structured microbial consortia on the human tongue exhibit taxon clustering in domains suggestive of clonal expansion (Wilbert et al. 2020).

Emerging research increasingly highlights the crucial role of spatial structures and interspecies connections, both physical and chemical, in shaping microbial ecosystems (Lin et al. 2024). Physical proximity is vital for microbial interactions; direct cell-to-cell contacts govern large-scale biofilm architecture and create the spatial context for chemical signaling and metabolic cooperation (Diaz and Valm 2020). These spatially organized interactions drive synergistic relationships that enhance colonization, persistence, and pathogenicity. Specific physical associations among diverse species may facilitate direct interspecies nutrient exchange, while localized, metabolite-mediated chemical signaling enables non-contact communication and coordinated functional behaviors over longer distances (Marcelino et al. 2023). Notably, alterations in these spatial organizations have been linked to oral diseases, with distinct three-dimensional architectures and biogeographical patterns observed under dysbiotic

conditions. The shift from health to disease is further reinforced by a feedforward loop involving microbiota, inflammation, and host factors (Marcelino et al. 2023).

With advances in sequencing technology, the site-specific hypothesis has gained strong support. For example, a recent study analyzing short-read metagenomic data for the abundant oral genus *Streptococcus* found that even closely related species predominantly inhabit different regions of the mouth (McLean et al. 2022). However, most current sequencing-based approaches, while providing robust compositional data, inherently disrupt spatial context and fail to capture the interactive dynamics among microbes and between microbes and the host (Ntekas and De Vlaminck 2024).

Imaging techniques capable of resolving microbial dynamics across experimental scales and environmental conditions remain indispensable for dissecting spatial ecology in complex ecosystems (Espinoza Miranda et al. 2025). Electron microscopy requires thin sectioning and lacks inherent taxonomic labeling, limiting its utility in community-scale spatial ecology studies. In contrast, optical microscopy, when combined with labeling techniques, offers unparalleled spatial resolution, real-time visualization, and the ability to capture molecular and cellular interactions within their native microenvironments, making them indispensable tools for studying spatial dynamics (Figure 1). In this review, we synthesize advances that bridge spatial organization and metabolic interactions in the oral microbiome. We highlight imaging-based technologies, complemented by multi-omics integration and AI-driven analytics, to advance the understanding of spatial organization and metabolic interactions within the oral microbiome.

1. Novel fluorescence imaging techniques reveal highly structured oral microbiota

Building on the site-specific colonization, imaging techniques have dramatically enhanced our ability to understand dynamic microbial organization and quantify interactions *in situ*, providing critical spatial and temporal context that is often missing in bulk analyses (Baker et al. 2024). Recent breakthroughs in fluorescence microscopy have been particularly transformative for the spatial mapping (biogeography) of oral

microbiota. However, despite significant progress, fluorescence-based approaches have historically been limited by the number of taxa detectable in a single experiment (Barbosa et al. 2023). Recent innovations incorporate highly multiplexed strategies, enabling the parallel interrogation of dozens to hundreds of microbial taxa or functional markers within a single sample (Figure 2).

When coupled with advanced optical systems, such as super-resolution microscopy or confocal laser scanning, fluorescence imaging reveals how different microbial taxa arrange themselves relative to one another and to host surfaces (Mark Welch et al. 2020). The microbiotas of dental plaque, the tongue dorsum, and the keratinized gingiva are the most distinctive from one another (Eren et al. 2014) (Figure 2 upper panel). Species-level imaging using CLASI-FISH (combinatorial labeling and spectral imaging–fluorescence in situ hybridization) has revealed the dominance of certain species on the tongue dorsum, supporting sequencing-based evidence that most oral microbes are site specialists (Wilbert et al. 2020). CLASI-FISH also facilitated the study examining plaque biofilm and suggests that *Corynebacterium matruchotii* may play an important role as a physical bridge between the base of the biofilm and its outer layers (Mark Welch et al. 2016). Interestingly, multiplex immunohistochemistry showed that fungi and bacteria mostly occupy separate dentinal tubules within carious dentin and rarely colocalize, which may partly reflect their limited likelihood of direct physical interaction (Sulyanto et al. 2024).

Beyond site-specific localization, mesoscale analysis further reveals that microbial architecture is governed by extracellular cues and biophysical properties, including chemical gradients (nutrients, oxygen, and antimicrobials), which both determine and are shaped by intercellular interactions and the interplay between cells and their environment. Such methods highlight microbial “hot spots”, where local interactions (e.g., metabolite exchange or competitive inhibition) may play a crucial role in shaping community assembly and function (Wilbert et al. 2020). Across longitudinal samples, researchers observed recurrent, distinctive microarchitectures, such as clusters of *Lautropia* cells, indicating that oral microbiome spatial structure can be stable over time (Shi et al. 2020). In dental plaque, *Corynebacterium* cells create habitat for other

bacteria like a tree, some binding to the tips of filaments, for example, *Streptococcus* spp., where they may have direct access to the oral environment, such as saliva, forming a characteristic "corncob" structure; others might be embedded deeper in the mass of biofilm, where they find a more sheltered, low oxygen environment that they need (Mark Welch et al. 2016) (Figure 2 middle panel). This spatial organization allows each microbial participant to interact with multiple, though limited, potential partners and is thought to promote the long-term stability of the community (Morillo-Lopez et al. 2022). Another study of multispecies biofilms showed that *Streptococcus mutans* densely clustered in a "rotund" extracellular scaffold, creating a localized low-pH zones that lead to enamel demineralization which possible links spatial structure to the onset of caries (Kim et al. 2020) (Figure 2 middle panel). High phylogenetic resolution fluorescence *in situ* hybridization (HiPR-FISH) further uncovered colocalization of *Rothia* and *Phocaeicola* cells in oral plaque samples, suggesting a potential metabolic synergy (Hahm 2024). Spatial analysis in dental implant biofilms similarly revealed disease-associated architectures characterized by ecosystem instability: bacterial blooms form large patches, creating open niches that allow colonization by new or opportunistic existing community members due to the absence of key functional roles. This supports a model of peri-implant dysbiosis that hypothesizes altered biofilm spatial architecture facilitates the pathogenic colonization (Grodner et al. 2024). By integrating confocal live-cell imaging and computational image analysis, Cho et al. (2023) demonstrated that *S. sputigena* cells co-localized within *S. mutans*-derived EPS α-glucans, indicating *S. mutans* may facilitate the motile bacterium *Selenomona* colonization and biofilm formation. Moreover, using dynamic tracking via flow-cell microfluidic to capture real-time imaging, researchers observed native cross-kingdom assemblages of *Candida albicans* and *S. mutans* with highly structured arrangement in saliva of children with severe tooth decay, exhibiting coordinated "leaping-like" and "walking-like" motions while continuously growing, a unique form of migratory spatial mobility that facilitates rapid biofilm expansion and potentially exacerbates tooth decay (Ren et al. 2022) (Figure 2 middle panel). Quantitative mesoscale image analysis can be extended to evaluate the immunogenic potential of discrete microbial consortia within the periodontal pocket. Such integrative approaches enable spatial mapping of dysbiotic clusters relative to tissue interfaces and local inflammatory microenvironments (Van et

al. 2020). By linking biofilm architecture with host immune gradients and functional readouts, mesoscale imaging frameworks may therefore provide prognostic insight into disease progression and therapeutic responsiveness while refining mechanistic models of host-mediated microbial selection in periodontitis (Chew et al. 2025).

At an even finer scale, recent advances have enabled imaging at single-cell resolution (Figure 2 lower panel). Super-resolution confocal imaging with Airyscan detected α-glucans on bacterial and fungal surfaces within interkingdom assemblages in saliva from caries-active patients, highlighting a complex bacteria-fungi-EPS biostructure associated with early childhood caries (Ren et al. 2022). However, in most cases, densely packed biofilms hinder the analysis of individual cells and their interactions. Heterotropic expansion microscopy addresses this challenge by coupling the microbial community to a swellable polyelectrolyte gel while leaving the cells themselves unswollen. Isotropic gel expansion decrowds the cells, enhancing spatial resolution with conventional microscopes and providing an unprecedented view of microbial heterogeneity (Dong et al. 2024). This improved spatial detail has revealed distinct species-level interactions; for example, *Fusobacterium nucleatum* shows stronger adhesion to *Streptococcus sanguinis* than to *S. mutans*, suggesting specific preferences that may influence community assembly. Although significant progress has been made, microbiome imaging remains fundamentally constrained by complex microbial communities and the limited number of spectrally distinguishable fluorophores. Sequential FISH methods can markedly expand multiplexing, and error-correction strategies may further improve target-identification accuracy, enabling finer-resolution spatial mapping of oral microbial communities (Cao et al. 2023). In addition, there is lack of research leveraging these highly multiplexed spatial imaging platforms to elucidate how microbial dysbiosis in one oral niche affects ecological balance in other niches. Using periodontal dysbiosis and tongue as an example, by simultaneously resolving taxonomic identity, spatial proximity, and functional markers across multiple oral habitats, these methods can reveal whether dysbiotic consortia or periodontal pathogens directly colonize on the tongue or reshape its microbial ecology through metabolite diffusion, inflammatory signaling, or host-derived factors. In turn, spatial analysis can further identify niche-specific microbial reservoirs on the tongue that may

sustain periodontal pathogens or facilitate recolonization of the subgingival environment. Importantly, mapping microbial co-colonization and metabolic gradients across niches enables the identification of transmission pathways and inter-niche microbial networks that are obscure in the bulk sequencing approaches. Such insights are critical for redefining diseases not solely as a site-confined disease but as a spatial interconnected oral ecosystem disorder, thereby informing targeted interventions that disrupt pathogenic cross-niche interactions.

Admittedly, beyond physical interactions, the biochemical mechanisms underlying microbial spatial organizations require further validation. Key questions remain as to how metabolic exchanges are coordinated in the presence of oral pathogens or commensals, and whether polymicrobial interactions influence the growth dynamics of the spatial structure. Future studies using *ex vivo* and *in vivo* polymicrobial models are needed to investigate spatiotemporal dynamics within complex communities (Table 1). In addition to traditional *in vitro* studies, *in situ* approaches such as imaging-based spatial omics offer powerful tools to uncover these mechanisms.

2. Imaging-based spatial omics decodes the spatial organization and interspecies interactions

Metabolites act as nutritional currencies and communication signals within oral biofilms, enabling cross-feeding and collective resilience (Zhang et al. 2024). They also modulate host responses by triggering inflammation through lipopolysaccharides and peptidoglycan or promoting immune tolerance via tryptophan derivatives and short-chain fatty acids from *Porphyromonas* and *Fusobacterium*, which are commonly found in periodontal pockets (Park and Kim 2021). However, traditional approaches for studying metabolites in complex communities have relied largely on bulk extraction and analysis, which destroy the sample and eliminate spatial context. These methods average signals across diverse cell populations and microenvironments, obscuring critical metabolic heterogeneity and interactions that are spatially organized within biofilms. In contrast, emerging imaging-based spatial omics tools allow direct visualization and quantification of metabolites distributions within intact microbial communities.

To understand how metabolites mediate interspecies interactions within the oral cavity, it is critical to resolve their spatial distribution and cell-specific activity within intact communities. Raman Spectroscopy (RS), a vibrational spectroscopic technique that leverages the Raman effect, can detect and map metabolites at single-cell resolution. Raman spectra obtained from biofilms of different cariogenic *streptococci* revealed that each species produced a distinct spectral profile, especially in regions corresponding to lipids, amide proteins, and carbohydrates (Gieroba et al. 2020). Notably, recent advances like stimulated Raman scattering (SRS) microscopy have achieved sub-micrometer resolution and video-rate imaging speeds in live cells and tissues (Cheng and Xie 2015). *Pseudomonas aeruginosa* biofilms, where stratified metabolic activity across biofilm depth was visualized with high spatial resolution using SRS imaging of carbon-deuterium bonds, revealed phenazine-dependent biosynthetic hotspots in hypoxic subregions (Schiessl et al. 2019). Furthermore, Raman spectral markers can serve as indicators of biofilm response to treatments and detect specific metabolite-driven interactions, as illustrated by the reduced Raman peak observed in *S. mutans*-rich dental biofilms treated with quaternary ammonium silane, enabling real-time monitoring of biofilm breakdown during antimicrobial treatment (Daood et al. 2020). Using *in situ* Raman microprobe, peroxynitrite was observed to be produced by the *Porphyromonas gingivalis* on an antibacterial bioceramic surface, providing insight into location-specific oxidative stress chemistry within the biofilm (Pezzotti et al. 2016). Such observations highlight localized chemical activity but do not define causality in the absence of genetic or biochemical manipulation.

Surface-enhanced Raman scattering or spectroscopy (SERS) is a powerful technique combining nanotechnology and biomedicine, using plasmonic metallic nanostructures to detect molecular fingerprints with ultra-high sensitivity-even at the single-molecule level. Nanolaminated plasmonic crystals (NLPCs) with dense Au-SiO_2-Au nanogap hotspots enabled highly sensitive, real-time monitoring of living *Pseudomonas syringae* biofilms under phage infection. The platform captured dynamic biochemical changes, revealing virus dose-dependent biofilm disruption, cell lysis, and host metabolic shift during viral replication (Garg et al. 2023). Similarly, SERS-based nanoplasmonic platforms could be applied to monitor the spatiotemporal dynamics of oral

Streptococcus-Veillonella interactions, enabling real-time visualization of metabolic exchanges (e.g., lactate consumption, short-chain fatty acid production) and biofilm development under therapeutic or ecological perturbations.

Interspecies and intraspecies interactions in the oral environment often involve diverse, untargeted metabolites or signaling molecules, requiring broad, discovery-driven mapping of the metabolic landscape that exceeds the molecular coverage achievable by RS. Matrix-assisted laser desorption/ionization imaging mass spectrometry (MALDI-IMS) meets this need by simultaneously detecting and mapping hundreds to thousands of molecules in a single scan, directly from biological surfaces without labeling or targeting. By raster-scanning across a sample, MALDI-IMS generates a mass spectrum at each pixel, which can be computationally reconstructed into ion images showing the intensity of specific peaks across the sample surface. Unlike traditional assays that lose spatial context, MALDI-IMS preserves the molecule layout intact, providing label-free, spatially resolved metabolomic profiles. In oral cancer, MALDI-IMS identified localized biomarkers such as LRP 6 in oral squamous cell carcinoma (Yuan et al. 2017), demonstrating its spatial power but not functional causality.

Building on the experience in cancer diagnosis, MALDI-IMS has also been explored to understand spatial chemistry in biofilms. Researchers monitored *N*-acyl-homoserine lactones (AHLs) production during *Pseudomonas putida* biofilm development, demonstrating uniform AHL signals distribution at early stage. As the biofilm matured, AHLs shifted to the peripheral accumulation of metabolites such as quinolones and pyochelin, correlating with swarming motility at the expanding edges (Pitchapa et al. 2022). Moreover, advanced instrumentation such as high-resolution MALDI or post-ionization techniques enabled mapping of interspecies metabolites exchanged between competing adjacent bacterial colonies. A pulse infrared (IR)-MALDI-IMS revealed that unique quorum-sensing molecules were strongly upregulated and concentrated at the immediate contact zone in co-cultures of *Staphylococcus aureus* and *P. aeruginosa*, where molecules produced by one colony diffused into the territory of the other (Brockmann et al. 2021). These spatially resolved insights into microbial metabolism are directly relevant to oral biofilms, which also exhibit microscale zones of antagonism

and cooperation, such as acidogenic *Streptococcus* species adjacent to acid-sensitive commensals, or bacteria-fungal interfaces in candidal infections. Thus, MALDI-IMS could be applied to dental plaque or mucosal biofilm samples to discover metabolites that distinguish pathogenic microenvironments from those associated with health, providing hypotheses that require downstream functional testing.

Building on this capability, emerging multimodal platforms further enhance spatial resolution and molecular identification. Advanced technologies, such as a spatial metabolomics pipeline (metaFISH) (Bourceau et al. 2023), have been a pioneering application combined MALDI-IMS with fluorescent microscopy to link chemistry with microbial identity. By overlaying MALDI maps with FISH on the same tissue section, metaFISH enables simultaneous visualization of spatial distribution of metabolites and microbial taxa at micrometer to single-cell resolution (Stüwe et al. 2025). Using this approach, the spatial metabolome of a deep-sea mussel and its intracellular symbiotic bacteria were resolved at the scale of individual epithelial cells, demonstrating metabolite exchange patterns that define symbiotic micro-niches (Geier et al. 2020; Stüwe et al. 2025). Applied to oral systems, metaFISH could map metabolite gradients and microbial localization within dental plaque or mucosal biofilms. This capability is particularly relevant for dissecting species-specific interactions and bacterial coaggregation patterns by directly linking physical microbial associations with localized metabolic activities. Notably, coaggregation involving probiotic strains within oral biofilms has been recognized as an important factor of dysbiosis and biofilm immunogenicity (Yang et al. 2025), underscoring a critical need in our understanding of how spatial organization and local metabolic microenvironments mediate probiotic function *in situ*. By simultaneously visualizing bacteria strains, their coaggregating partners, and associated metabolite signatures, metaFISH could address this gap by elucidating how probiotic incorporation into oral biofilms modulates community metabolism, host-relevant metabolites, and inflammatory potential. More broadly, this approach could uncover species-specific metabolic interactions, identify metabolite-based biomarkers, and monitor the effects of antimicrobial or probiotic interventions on oral metabolic homeostasis. Together, this integrative imaging framework holds strong potential for dissecting microbe-host and microbe-microbe interactions in oral biology.

RS, MALDI-IMS, and metaFISH are complementary tools that allow unprecedented visualization of metabolites *in situ* (Figure 3). Together, these spatial omics approaches enable researchers to visualize the chemical architecture of oral microbial communities; in fact, we can detect where acids are produced, showing where signaling molecules concentrate, and how all these chemical factors align with the microbial organization; however, these data remain correlational and cannot reveal mechanistic interactions without complementary functional validation (Table 1). These findings will allow us to develop precision tools to modulate the microbiome and nudge the ecology of the mouth toward a healthy state.

3. Artificial intelligence and computational techniques revolutionize the spatial structure of microbiome research

Imaging-based assays now routinely yield terabytes of multi-channel, three-dimensional data, so extracting biological meaning increasingly depends on purpose-built analytic pipelines rather than manual inspection. Machine-learning frameworks are proving especially powerful. Figure 4 presents how AI and machine learning methodologies have been widely implemented in imaging data science and illustrates how the computational framework integrates into the overall analytical workflow across imaging platforms. Borowa et al. (2023) explored bacterial species classification from polyculture microscopic images using multi-instance learning (a machine learning approach that handles grouped unlabeled data), bypassing the need for time-consuming monoculture preparation. This method enables faster and more interpretable diagnosis in complex microbial communities. Hahm et al. (2024) employed spatially resolved imaging (HiPR-FISH) to visualize the ecological structure of oral biofilms, demonstrating how pathogenic consortia in peri-implantitis form tightly clustered niches.

Imaging-based spatial omics is revolutionizing microbiome research by providing direct insight into the interactions between microbial communities and their host environments. Spatial host–microbiome sequencing (SHM-seq) is an all-sequencing-based approach closely integrated with deep learning. It captures tissue, polyadenylated

RNAs, and bacterial 16S rRNA directly from tissue by modifying a spatially barcoded glass surface, enabling simultaneous capture of both bacterial 16S rRNA and host transcripts (Lötstedt et al. 2024). Our ability to model these complex spatial structures has significantly improved with the emergence of artificial intelligence (AI)-assisted tools and machine learning techniques. For example, PM-CNN (Phylogenetic Multi-path Convolutional Neural Network) (Wang et al. 2024) models phylogenetic relationships using multi-layer hierarchical clustering of microbes and therefore provides more accurate microbiome-based disease prediction. Density-based unsupervised machine learning techniques can focus on spatial clustering. Different multi-omics approaches that integrate multiple data streams generate massive and complex data sources. The newly developed AI-powered, biology-inspired, multi-scale modeling framework may pave the way for a new level of understanding of spatial structure (Wu and Xie 2025).

Moreover, the unique oral ecological environment, characterized by diverse niches such as saliva flow, mucosal surfaces, and tooth structures, gives rise to highly complex imaging datasets. These complexities underscore the need for advanced AI applications to integrate and interpret data from such heterogeneous sources. Pais et al. (2025) developed predictive models using automated machine learning techniques to integrate datasets from saliva and subgingival biofilms. Their approach identified combinations of clustered microbial species to predict peri-implantitis. Similarly, Zhao et al. (2024) used a deep learning model, PSPNet (a model for image segmentation), to capture complex microbial patterns beyond individual taxa. This method can process complex datasets, capture detailed context, and provide a comprehensive assessment of the oral microbiome for disease diagnosis.

Despite the promise of AI in oral microbiome research, the utility of these models depends heavily on data quality, diversity, and interpretability. Many studies report high predictive accuracy (Pais et al. 2025; Zhao et al. 2025; Ding et al. 2022), yet sample size limitations, cohort diversity, and lack of experimental validation constrain their translational relevance. As noted in several prior studies (Pais et al. 2025; Zhao et al. 2025; Ding et al. 2022), different metrics have been used to evaluate model performance.

For example, Ding et al. (2022) used Pearson's correlation coefficient to assess the agreement between predicted and observed results. Zhao et al. (2025) reported low mean absolute error to demonstrate prediction accuracy alongside computational efficiency. Importantly, Pais et al. (2025) reported high sensitivity, reaching up to 95% in saliva samples, but relatively lower specificity in classification performance. To ensure that AI-generated predictions reflect true biological phenomena, complementary *in vitro* experiments and *in vivo* studies (e.g., animal models or clinical cohorts) are essential. Integration of host responses and spatial microbial organization further underscores the need for experimental confirmation (Hahm 2024; Eriksson et al. 2022).

4. Potential biomarkers for early diagnosis and therapeutic strategies to reshape microbial dynamics

Therapeutic modulation of oral microbiome will depend on an accurate understanding of how microbial communities dynamically-spatially change over time and which interspecies interactions are required for maintaining healthy microbiome. To achieve this, incorporating biogeographical data will be key to informing and refining studies that examine the metabolic underpinnings of oral microbial ecology (Baker et al. 2024).

Several metabolites have been identified as potential biomarkers for dysbiosis and disease susceptibility. For instance, galactose utilization, resulting from streptococcal sugar metabolism, may alter the physiology of *P. gingivalis*, impairing its ability to form biofilms and interact with host tissues (Chen et al. 2023). Para-aminobenzoic acid (PABA), a metabolic product of *S. gordonii*, modulates *P. gingivalis* virulence by altering exopolysaccharide composition and fimbrial expression (Stocke and Lamont 2024). Butyrate, a short-chain fatty acid (SCFA) produced by *P. gingivalis*, *Tannerella forsythia*, and *F. nucleatum*, plays a significant role in immune suppression by reducing neutrophil phagocytosis and downregulating intercellular adhesion molecules (ICAM-1), thereby promoting chronic inflammation in periodontal disease (Du et al. 2024). The spatial distribution of these metabolites provides critical insights into disease progression and identified opportunities for targeted intervention.

Given the critical role of metabolites in microbial dynamics and host immune responses, targeting key metabolic pathways has emerged as a promising therapeutic strategy in oral health. Probiotic approaches, such as *Lactobacillus* and *Bifidobacteria* species, can modulate lactate metabolism to counteract acidification and reduce caries risk (Luo et al. 2024). In periodontal disease, *Limosilactobacillus reuteri* has also been studied as an adjunctive therapy, with emerging evidence indicating that its probiotic benefits extend beyond direct antagonism of individual pathogens such as *Fusobacterium nucleatum*, instead reshaping biofilm composition and immunogenicity more broadly (Yang et al. 2025). Beyond lactate metabolism, oral nitrate-reducing bacteria on the tongue dorsum and within subgingival plaque contribute to the nitrate-nitrite-nitric oxide pathway, with potential implications for nitric oxide bioavailability and local redox and inflammatory balance (Rosier et al. 2024). In this context, spatially resolved metabolomic imaging approaches such RS, MALDI-IMS, and metaFISH could directly evaluate the probiotic potential by linking niche-specific colonization with localized metabolic gradients and host-microbe signaling *in situ*.

Together, these findings highlight a conceptual shift toward precision modulation of microbial metabolism as a means to restore oral homeostasis. Nanomaterials, specifically those generating or scavenging reactive oxygen species (ROS), are being explored for their dual ability to disrupt pathogenic biofilms while mitigating oxidative stress. Protein therapies targeting specific metabolic enzymes, such as lactate oxidase or catalase (Cao et al. 2025), are also under investigation for their potential to restore redox balance and inhibit pathogen growth. In particular, enzyme-functionalized nanoparticles stand out as a highlight treatment (Cao et al. 2024), combining the precision of enzymatic activity with the stability and delivery advantages of nanotechnology. These systems can degrade harmful metabolites, such as butyrate or lactate, while simultaneously modulating microbial interactions and immune responses, offering a multifaceted approach to restoring oral microbial balance. This convergence of metabolic and nanotechnological strategies represents a transformative step toward precision-targeted therapies that not only address oral diseases but also have the potential to improve systemic health outcomes.

Importantly, the efficacy of these metabolism-targeted strategies is intrinsically linked to the spatial organization of microbial communities. Metabolite gradients, microenvironments, and host-microbe signaling are not uniformly distributed but instead localized microbial interactions within spatial structures. As such, therapeutic outcomes cannot be fully captured by compositional analysis alone, underscoring the need for evaluation frameworks that reflect both function and spatial context. While current oral microbial dysbiosis indices, such as periodontal indice, primarily rely on compositional shifts in relative abundance, emerging spatial imaging techniques offer an opportunity to refine these metrics by incorporating architectural and proximity-based parameters (Chen et al. 2022). High-plex fluorescence imaging and analysis can resolve contact-dependent interactions between homeostatic and dysbiotic taxa, biofilm depth gradients, and epithelial interface engagement that are not captured by sequencing alone. Such spatially informed descriptors integrating interspecies proximity, vertical stratification, and tissue invasion, may enhance the diagnostic and prognostic sensitivity of existing microbial indices (Bostanci et al. 2025). A similar framework may be extended to oncologic settings, where integrative spatial omics analyses of tumor and adjacent normal tissues have begun to reveal structured microbial alterations associated with oral cavity squamous cell carcinoma. By coupling taxonomic indices with spatial and host-interface features, future studies may better understand whether specific microbial consortia function as passive biomarkers or active contributors to carcinogenic microenvironments (Cai et al. 2024).

Conclusions and Perspectives

Investigating the link between the native spatial architecture of the microbiota and its function requires sampling strategies that maintain microbial populations within their original topology. Such approaches enable simultaneous visualization of microorganisms and host structures across scales. Conventional thin-section imaging, however, fails to fully capture the spatial complexity of microbial communities. Alternative low-perturbation tools, such as ridged plastic tongue scrapers, can recover biofilm fragments that retain spatial organization, although compression during collection remains a limitation (Wilbert et al. 2020; Borisy and Valm 2021). Recent advances include tissue-clearing techniques that maintain the spatial integrity of

mucosal microbiota, host tissues, and the fragile mucus layer (Mondragón-Palomino et al. 2022). Despite this progress, the method has yet to be applied to oral biofilms or integrated into clinical patient sample workflows, which needs further validation.

In vitro models replicating the in-situ microenvironment have emerged to study spatial interactions within the oral microbiome. Although short-range factors such as direct adhesion and steep micron-scale gradients yield a powerful influence on microbial niches, longer-range factors likewise shape oral biofilm composition in complex ways. Interactions might occur between taxa separated by centimeters across the mouth through small molecules diffusing in air or transported via salivary flow (Borisy and Valm 2021). Spatiotemporal tracking of microbial populations similarly offers further insights into how these interspecies interactions evolve and drive broader shifts in microbial architecture over time (Molina-Santiago et al. 2022). Moreover, organs-on-a-chip approach combined with microfluidics enables real-time characterization of biofilm morphology, colonization density, and spatial arrangement with *in vitro* models mimicking human oral conditions including salivary flow and different niches (Huang et al. 2023). By reconstructing high-resolution three-dimensional or time-lapse maps, these approaches reveal how local niches shape microbial ecology at the microscale, ultimately influencing transitions from health- to disease-associated communities (Mark Welch et al. 2019).

However, imaging-based findings have inherent limitations. Despite technical advances, microscopy remains fundamentally descriptive, signals are influenced by labeling strategies, photophysics, sampling/fixation artifacts, image processing, and downstream analytic pipelines, making results vulnerable to confounding and bias. Accordingly, targeted genetic or chemical perturbations, controlled *in vitro* assays, and *in vivo* or clinical studies are required to validate observations and establish causality.

The spatial and functional specificity of chemical interactions such as metabolites within the oral cavity underscores their potential as both diagnostic biomarkers and therapeutic targets. Advances in spatial metabolomics have revealed location-specific metabolites, highlighting the importance of micro-environmental factors in shaping

microbial interactions. The future of oral health management lies in precision microbiome modulation, where spatially and functionally relevant metabolites serve as both early disease indicators and therapeutic targets, promoting a balanced oral microbiome. Integrating AI-driven analyses with advanced imaging and multi-omics approaches can create predictive models that simulate the effects of microbial interaction, metabolite production and host response on community stability and health outcomes, which can lead to more accurate predictions of disease progression and personalized therapeutics (Figure 4).

Author Contributions

Y. Liu, Y. Wang, and J. Wen, contributed to conception, design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript; Z. Xiang, Z. Wang, and N. Ghasemi, contributed design, data acquisition, analysis, and interpretation, drafted and critically revised the manuscript. All authors gave final approval and agreed to be accountable for all aspects of the work.

Declaration of Conflicting Interests

The authors declare the following competing interests: J. Wen has a financial interest in Vivibaba, and the regents have licensed intellectual property invented by J. Wen to Vivibaba. No funding was provided by this company to support this work. All other authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Fundings

The authors disclosed receipt of the following support for the research, authorship, and/or publication of this article: This work was supported by the National Institutes of Health, National Institute of Dental and Craniofacial Research grants K23DE032419 (to Y. L.), National Institute of Neurological Disorders and Stroke grant R01NS137919 (to J. W.), National Institutes of Health, National Institute of Dental and Craniofacial Research grants K01DE032775 and R03DE033631 (to Y. W.).

Data Availability Statement

Making data available does not apply to this review paper.

References

Baker JL, Mark Welch JL, Kauffman KM, McLean JS, He X. 2024. The oral microbiome: diversity, biogeography and human health. *Nat Rev Microbiol*. 22:89–104.

Barbosa A, Miranda S, Azevedo NF, Cerqueira L, Azevedo AS. 2023. Imaging biofilms using fluorescence in situ hybridization: seeing is believing. *Front Cell Infect Microbiol*. 13.

Borisy GG, Valm AM. 2021. Spatial scale in analysis of the dental plaque microbiome. *Periodontol 2000*. 86:97–112.

Borowa A, Rymarczyk D, Ochonska D, Sroka-Oleksiak A, Brzychczy-Wloch M, Zielinski B. 2023. Identifying bacteria species on microscopic polyculture images using deep learning. *IEEE J Biomed Health Inform*. 27(1):121–130.

Bostanci N, Manoil D, Van Holm W, Belibasakis GN, Teughels W. 2025. Microbial markers for diagnosis and risk assessment for periodontal diseases: a systematic literature search and narrative synthesis. *J Clin Periodontol*. 52(Suppl 29):125–154.

Bourceau P, Geier B, Suerdieck V, Bien T, Soltwisch J, Dreisewerd K, Liebeke M. 2023. Visualization of metabolites and microbes at high spatial resolution using MALDI mass spectrometry imaging and in situ fluorescence labeling. *Nat Protoc*. 18(10):3050–3079.

Brockmann EU, Potthoff A, Tortorella S, Soltwisch J, Dreisewerd K. 2021. Infrared MALDI Mass Spectrometry with Laser-Induced postionization for imaging of bacterial colonies. *J Am Soc Mass Spectrom*. 32(4):1053–1064.

Cai L, Zhu H, Mou Q, Wong PY, Lan L, Ng CWK, Lei P, Cheung MK, Wang D, Wong EWY, Lau EHL, Yeung ZWC, Lai R, Meehan K, Fung S, Chan KCA, Lui VWY, Cheng ASL, Yu J, Chan PKS, Chan JYK, Chen Z. 2024. Integrative analysis reveals associations between oral microbiota dysbiosis and host genetic and epigenetic aberrations in oral cavity squamous cell carcinoma. *NPJ Biofilms Microbiomes*. 10(1):39.

Cao Z, Liu C, Wen J, Lu Y. 2024. Innovative formulation platform: paving the way for superior protein therapeutics with enhanced efficacy and broadened applications. *Adv Mater.* 36:2470326.

Cao Z, Ren J, Yang A, Wang Z, Love M, Chen W, Yuan X, Guo X, Chen I, Lu Y, et al. 2025. A multi-enzyme nanocascade to target disease-relevant metabolites. *Small.* 21:e2408481.

Cao Z, Zuo W, Wang L, Chen J, Qu Z, Jin F, Dai L. 2023. Spatial profiling of microbial communities by sequential FISH with error-robust encoding. *Nat Commun.* 14(1):1477.

Chen T, Marsh PD, Al-Hebshi NN. 2022. SMDI: an index for measuring subgingival microbial dysbiosis. *J Dent Res*. 101(3):331–338.

Chen WA, Dou Y, Fletcher HM, Boskovic DS. 2023. Local and systemic effects of Porphyromonas gingivalis infection. *Microorganisms.* 11:470.

Cheng JX, Xie XS. 2015. Vibrational spectroscopic imaging of living systems: An emerging platform for biology and medicine. *Science*. 350(6264):aaa8870.

Chetty A, Blekhman R. 2024. Multi-omic approaches for host-microbiome data integration. *Gut Microbes.* 16:2297860.

Chew RJJ, Goh CE, Lin XYS, Oh FJB, Sim RP, Preshaw PM, Tan KS. 2025. Prognostic Impact of Microbiome Dysbiosis: A Prospective Study. *J Clin Periodontol*. 52(2):215-224.

Cho H, Ren Z, Divaris K, Roach J, Lin BM, Liu C, Azcarate-Peril MA, Simancas-Pallares MA, Shrestha P, Orlenko A, Ginnis J, North KE, Zandona AGF, Ribeiro AA, Wu D, Koo H. 2023. *Selenomonas sputigena* acts as a pathobiont mediating spatial structure and biofilm virulence in early childhood caries. *Nat Commun*. 14(1):2919.

Daood U, Burrow MF, Yiu CKY. 2020. Effect of a novel quaternary ammonium silane cavity disinfectant on cariogenic biofilm formation. *Clin Oral Investig*. 24(2):649–661.

Diaz PI, Valm AM. 2020. Microbial interactions in oral communities mediate emergent biofilm properties. *J Dent Res.* 99:18–25.

Ding H, Yang Y, Li X, Cheung GS-P, Matinlinna JP, Burrow M, Tsoi JK-H. 2022. A simple AI-enabled method for quantifying bacterial adhesion on dental materials. *Biomater Investig Dent*. 9(1):75-83.

Dong P-T, Shi W, He X, Borisy GG. 2024. Adhesive interactions within microbial consortia can be differentiated at the single-cell level through expansion microscopy. *Proc Natl Acad Sci U S A.* 121:e2411617121.

Du Y, He C, An Y, Huang Y, Zhang H, Fu W, Wang M, Shan Z, Xie J, Yang Y, et al. 2024. The role of short chain fatty acids in inflammation and body health. *Int J Mol Sci.* 25:7379.

Eren AM, Borisy GG, Huse SM, Mark Welch JL. 2014. Oligotyping analysis of the human oral microbiome. *Proc Natl Acad Sci U S A*. 111(28):E2875–E2884.

Eriksson K, Lundmark A, Delgado LF, Hu YO, Fei G, Lee L, et al. 2022. Salivary microbiota and host-inflammatory responses in periodontitis-affected individuals with and without rheumatoid arthritis. *Front Cell Infect Microbiol*. 12:841139.

Espinoza Miranda SS, Abbaszade G, Hess WR, Drescher K, Saliba A-E, Zaburdaev V, Chai L, Dreisewerd K, Grünberger A, Westendorf C, et al. 2025. Resolving spatiotemporal dynamics in bacterial multicellular populations: approaches and challenges. *Microbiol Mol Biol Rev*. 0:e00138-24.

Garg A, Nam W, Wang W, Vikesland P, Zhou W. 2023. In situ spatiotemporal SERS measurements and multivariate analysis of virally infected bacterial biofilms using nanolaminated plasmonic crystals. *ACS Sens*. 8(3):1132–1142.

Geier B, Sogin EM, Michellod D, Janda M, Kompauer M, Spengler B, Dubilier N, Liebeke M. 2020. Spatial metabolomics of in situ host-microbe interactions at the micrometre scale. *Nat Microbiol*. 5(3):498–510.

Gieroba B, Krysa M, Wojtowicz K, Wiater A, Pleszczyńska M, Tomczyk M, Sroka-Bartnicka A. 2020. The FT-IR and Raman spectroscopies as tools for biofilm characterization created by cariogenic streptococci. *Int J Mol Sci*. 21(11):3811.

Grodner B, Wu DT, Hahm S, Takayasu L, Wen N, Kim DM, Chen CY, De Vlaminck I. 2024. Microscale spatial dysbiosis in oral biofilms associated with disease. *bioRxiv*. 2024.07.24.604873.

Hahm S. 2024. Highly spatial mapping of oral microbial communities in peri-implant health and disease. *Harvard University Repository.*

Huang C, Sanaei F, Verdurmen WPR, Yang F, Ji W, Walboomers XF. 2023. The application of organs-on-a-chip in dental, oral, and craniofacial research. *J Dent Res.* 102:364–375.

Kim D, Barraza JP, Arthur RA, Hara A, Lewis K, Liu Y, Scisci EL, Hajishengallis E, Whiteley M, Koo H. 2020. Spatial mapping of polymicrobial communities reveals a precise biogeography associated with human dental caries. *Proc Natl Acad Sci U S A*. 117:12375–12384.

Kim D, Koo H. 2020. Spatial design of polymicrobial oral biofilm in its native disease state. *J Dent Res*. 99(6):597–603.

Li X, Liu Y, Yang X, Li C, Song Z. 2022. The oral microbiota: community composition, influencing factors, pathogenesis, and interventions. *Front Microbiol.* 13.

Lin Y, Liang X, Li Z, Gong T, Ren B, Li Y, Peng X. 2024. Omics for deciphering oral microecology. *Int J Oral Sci.* 16:1–11.

673 Lötstedt B, Stražar M, Xavier R, Regev A, Vickovic S. 2024. Spatial host-
674 microbiome sequencing reveals niches in the mouse gut. *Nat Biotechnol*. 42(9):1394–
675 1403.

676 Luo SC, Wei SM, Luo XT, Yang QQ, Wong KH, Cheung PCC, Zhang BB. 2024.
677 How probiotics, prebiotics, synbiotics, and postbiotics prevent dental caries: an oral
678 microbiota perspective. *NPJ Biofilms Microbiomes*. 10(1):14.

679 Marcelino VR, Welsh C, Diener C, Gulliver EL, Rutten EL, Young RB, Giles EM,
680 Gibbons SM, Greening C, Forster SC. 2023. Disease-specific loss of microbial cross-
681 feeding interactions in the human gut. *Nat Commun.* 14:6546.

682 Mark Welch JL, Borisy GG. 2025. Dental plaque: Who is in the neighborhood and
683 why it matters. *JADA Found Sci*. 4:100051.

684 Mark Welch JL, Dewhirst FE, Borisy GG. 2019. Biogeography of the oral
685 microbiome: the site-specialist hypothesis. *Annu Rev Microbiol.* 73:335–358.

686 Mark Welch JL, Ramírez-Puebla ST, Borisy GG. 2020. Oral microbiome geography:
687 micron-scale habitat and niche. *Cell Host Microbe*. 28(2):160–168.

688 Mark Welch JL, Rossetti BJ, Rieken CW, Dewhirst FE, Borisy GG. 2016.
689 Biogeography of a human oral microbiome at the micron scale. *Proc Natl Acad Sci U*
690 *S A*. 113(6):E791–E800.

691 Martinez-Garcia R, Tarnita CE, Bonachela JA. 2022. Spatial patterns in ecological
692 systems: from microbial colonies to landscapes. *Emerg Top Life Sci.* 6:245–258.

693 McLean AR, Torres-Morales J, Dewhirst FE, Borisy GG, Mark Welch JL. 2022. Site-
694 tropism of streptococci in the oral microbiome. *Mol Oral Microbiol.* 37:229–243.

695 Molina-Santiago C, Pearson JR, Berlanga-Clavero MV, Pérez-Lorente AI, de Vicente
696 A, Romero D. 2022. A noninvasive method for time-lapse imaging of microbial
697 interactions and colony dynamics. *Microbiol Spectr.* 10:e00939-22.

698 Morillo-Lopez V, Sjaarda A, Islam I, Borisy GG, Mark Welch JL. 2022. Corncob
699 structures in dental plaque reveal microhabitat taxon specificity. *Microbiome*.
700 10(1):145.

701 Mondragón-Palomino O, Poceviciute R, Lignell A, Griffiths JA, Takko H, Ismagilov
702 RF. 2022. Three-dimensional imaging for the quantification of spatial patterns in
703 microbiota of the intestinal mucosa. *Proc Natl Acad Sci U S A.* 119:e2118483119.

704 Ntekas I, De Vlaminck I. 2024. Spatial methods for microbiome–host interactions.
705 *Nat Biotechnol*. 42:1359–1360.

706 Pais RJ, Botelho J, Machado V, Alcoforado G, Mendes JJ, Alves R, Bessa LJ. 2025.
707 Exploring AI-driven machine learning approaches for optimal classification of peri-

implantitis based on oral microbiome data: A feasibility study. *Diagnostics (Basel, Switzerland)*. 15(4):425.

Park J, Kim CH. 2021. Regulation of common neurological disorders by gut microbial metabolites. *Exp Mol Med*. 53(12):1821–1833.

Pezzotti G, Bock RM, McEntire BJ, Jones E, Boffelli M, Zhu W, Baggio G, Boschetto F, Puppulin L, Adachi T, Yamamoto T, Kanamura N, Marunaka Y, Bal BS. 2016. Silicon nitride bioceramics induce chemically driven lysis in *Porphyromonas gingivalis*. *Langmuir*. 32(12):3024–3035.

Pitchapa R, Dissook S, Putri SP, Fukusaki E, Shimma S. 2022. MALDI mass spectrometry imaging reveals the existence of an N-acyl-homoserine lactone quorum sensing system in *Pseudomonas putida* biofilms. *Metabolites*. 12(11):1148.

Proctor DM, Fukuyama JA, Loomer PM, Armitage GC, Lee SA, Davis NM, Ryder MI, Holmes SP, Relman DA. 2018. A spatial gradient of bacterial diversity in the human oral cavity shaped by salivary flow. *Nat Commun.* 9:681.

Ren Z, Jeckel H, Simon-Soro A, Xiang Z, Liu Y, Cavalcanti IM, Xiao J, Tin N-N, Hara A, Drescher K, Koo H. 2022. Interkingdom assemblages in human saliva display group-level surface mobility and disease-promoting emergent functions. *Proc Natl Acad Sci U S A.* 119:e2209699119.

Rosier BT, Johnston W, Carda-Diéguez M, Simpson A, Cabello-Yeves E, Piela K, Reilly R, Artacho A, Easton C, Burleigh M, Culshaw S, Mira A. 2024. Nitrate reduction capacity of the oral microbiota is impaired in periodontitis: potential implications for systemic nitric oxide availability. *Int J Oral Sci*. 16(1):1.

Shi H, Shi Q, Grodner B, Lenz JS, Zipfel WR, Brito IL, De Vlaminck I. 2020. Highly multiplexed spatial mapping of microbial communities. *Nature*. 588(7839):676-681.

Schiessl KT, Hu F, Jo J, Nazia SZ, Wang B, Price-Whelan A, Min W, Dietrich LEP. 2019. Phenazine production promotes antibiotic tolerance and metabolic heterogeneity in *Pseudomonas aeruginosa* biofilms. *Nat Commun*. 10(1):762.

Stüwe M, Petersen LE, Liebeke M. 2025. From microbes to molecules: unveiling host-microbe interactions with spatial metabolomics. *Mol Syst Biol*. 21:947-951.

Sulyanto RM, Beall CJ, Ha K, Montesano J, Juang J, Dickson JR, Hashmi SB, Bradbury S, Leys EJ, Edgerton M, et al. 2024. Fungi and bacteria occupy distinct spatial niches within carious dentin. *PLoS Pathog.* 20:e1011865.

Van Dyke TE, Bartold PM, Reynolds EC. 2020. The Nexus Between Periodontal Inflammation and Dysbiosis. *Front Immunol*.11:511.

Wu Y, Xie L. 2025. AI-driven multi-omics integration for multi-scale predictive modeling of genotype-environment-phenotype relationships. *Comput Struct Biotechnol J*. 27:265–277.

Wang Q, Fan X, Wu S, Su X. 2024. PM-CNN: microbiome status recognition and disease detection model based on phylogeny and multi-path neural network. *Bioinform Adv*. 4(1):vbae013.

Wang XW, Wang T, Liu YY. 2024. Artificial intelligence for microbiology and microbiome research. *arXiv Preprint*. arXiv:2411.01098.

Wilbert SA, Mark Welch JL, Borisy GG. 2020. Spatial ecology of the human tongue dorsum microbiome. *Cell Rep.* 30:4003–4015.e3.

Yang L, Sriram G, Chew RJJ, Tan KS. 2025. *Limosilactobacillus reuteri-Fusobacterium nucleatum* interactions modulate biofilm composition and immunogenicity. *J Periodontal Res.* 60(10):1006-1017.

Yuan Y, Xie X, Jiang Y, Wei Z, Wang P, Chen F, Li X, Sun C, Zhao H, Zeng X, Jiang L, Zhou Y, Dan H, Feng M, Liu R, Wang Z, Chen Q. 2017. LRP6 is identified as a potential prognostic marker for oral squamous cell carcinoma via MALDI-IMS. *Cell Death Dis*. 8(9):e3035.

Zhang Y, Cai Y, Zhang B, Zhang YPJ. 2024. Spatially structured exchange of metabolites enhances bacterial survival and resilience in biofilms. *Nat Commun*. 15(1):7575.

Zhao Z, Liu X, Li M, Liu J, Wang Z. 2025. Oral microbe community and pyramid scene parsing network-based periodontitis risk prediction. *Int Dent J*. 75(2):700–706.

Figure Caption

Figure 1. The need of imaging-based approaches in studying dynamic interspecies and microbe-host interactions within oral microbial communities. Created with BioRender.com.

Figure 2. Biogeography of the oral microbiome at different scales. The right panel of the figure is adapted with permission from ref. (Morillo-Lopez et al. 2022; Kim and Koo 2020; Ren et al. 2022; Wilbert et al. 2020; Mark Welch et al. 2016; Kim et al. 2020). Created with BioRender.com.

Figure 3. Overview of complementary spatial imaging techniques for mapping microbial organization and metabolic features in the oral microbiome. Raman, MALDI mass spectrometry imaging, and FISH fluorescence-based approaches provide distinct but complementary spatial information on chemical composition, metabolite distributions, and microbial localization. Scale bar, 150 μm. The imaging results are adapted with permission from ref. (Geier et al. 2020 and Wang et al. 2014). Created with BioRender.com.

Figure 4. Advances in multimodal imaging, AI-driven analytics, and multi-omics integration in oral microbiome research. Created with BioRender.com.