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REVIEW

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The Transformative Role of Biophotonics in Rapid, Real-Time, and In Situ Studies of Microbial Structure, Cellular Processes, and Interactions

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Abstract: Photonics and optical technologies provide powerful, noninvasive means to study microorganisms, including those that remain unculturable, by detecting light signals arising from specific structural and biochemical features. These include intrinsic chromophores such as aromatic amino acids, nicotinamide adenine dinucleotide hydride, and flavins; spectral signatures of functional groups in proteins, lipids, and nucleic acids; refractive index contrasts from cell walls and organelles; and scattering patterns shaped by microbial morphology. This review synthesizes current advances in applying optical and photonic methods to microbial research, with the explicit aim of showing how these tools enable in situ, real-time characterization of microbial structure, metabolic state, behavior, and interactions. We overview high-resolution imaging modalities (confocal, multiphoton, and super-resolution microscopy) for visualizing morphology and dynamics, spectroscopic techniques (Raman, surface-enhanced Raman scattering, Fourier transform infrared spectroscopy, fluorescence) for label-free molecular fingerprinting, and biosensors (fiber-optic, plasmonic, lab-on-chip) for rapid detection. We also examine light-based manipulation strategies (optogenetics, optical tweezers, laser ablation) that allow precise control of microbial processes and discuss emerging integrations with artificial intelligence analytics, quantum sensing, and space-adapted photonics. By linking the physical principles of signal generation to microbial targets, we highlight how photonics is transforming microbial ecology, diagnostics, and biotechnology and identify the technical challenges driving the development of next-generation optical tools.

Keywords: biophotonics, microbial ecology, super-resolution microscopy, optical biosensors, label-free imaging, plasmonic sensors, Raman spectroscopy

1. Introduction

Microorganisms—including bacteria, archaea, fungi, protists, and viruses—are fundamental to Earth's biosphere, driving carbon, nitrogen, phosphorus, and sulfur cycling through processes such as nitrogen fixation, chemosynthesis, and decomposition [1, 2]. Their metabolic versatility sustains ecosystem productivity, even in extreme environments, by exploiting inorganic energy sources (e.g., hydrogen, nitrite, ammonia, sulfur, iron). Microbial communities interact via mutualism (e.g., mycorrhizal nutrient exchange), commensalism, amensalism (e.g., *Lactobacillus* inhibiting *Pseudomonas*), and parasitism (e.g., pathogenic fungi), shaping nutrient flow, resilience, and applications such as bioremediation [3].

Microbial ecology studies the diversity, distribution, interactions, and functional roles of microorganisms in natural and engineered systems, connecting taxonomy with function through tools like metagenomics, transcriptomics, metabolomics, microscopy, and cultivation [3–5]. Community structure is influenced by abiotic factors (temperature, pH, nutrient availability, habitat) and biotic interactions (competition, cooperation, symbiosis, predation) [4, 5]. Insights inform climate change mitigation, wastewater treatment, agriculture, infectious disease control, and biotechnology.

Increasingly, high-resolution imaging, ecological modeling, and systems biology predict microbial responses to environmental change and enable their sustainable use.

Research faces the "Great Plate Count Anomaly" [4–6]: most microbes are unculturable with current methods, limiting functional studies in native contexts. Sequencing reveals genetic potential but rarely captures dynamic behavior, spatial organization, or real-time activity [6–9]. As microorganisms are invisible to the naked eye, biophotonics—light-based technologies for biological analysis—has become central to microbial detection [5, 6]. By exploiting signals from endogenous chromophores (nicotinamide adenine dinucleotide hydride (NADH), flavins, aromatic amino acids), structural domains (cell walls, membranes), and

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morphological optical scattering, photonics enables noninvasive, label-free, in situ monitoring while preserving natural interactions [7]. Optical contrast arises when photons interact with molecular bonds (FTIR vibrational absorption), polarizability (Raman scattering), endogenous fluorophores, or refractive index gradients (phase-contrast, label-free), sampled at each pixel to render biostructures and their dynamics [10, 11].

Biophotonics offers speed, sensitivity, selectivity, and resolution for visualizing microbes at cellular and subcellular scales, probing morphology, community structure, gene expression, metabolic state, and interactions directly in environmental matrices [7–11]. In situ, real-time observation captures transient events and dynamic processes missed by static or artificial approaches [12–14].

In this review, we link optical principles to the detection of specific microbial structures and functions, covering advanced imaging, spectroscopy, biosensing, and light-based manipulation. We highlight applications in microbial ecology and biotechnology and emerging trends such as artificial intelligence (AI), quantum sensing, and space-adapted photonics and identify technical challenges and opportunities for next-generation platforms in environmental monitoring, medical diagnostics, and industry.

2. Advanced Imaging Techniques for Microbial Visualization

Advanced imaging overcomes the limitations of conventional microscopy by enabling visualization of structures, dynamics, and interactions from subcellular details to complex microbial communities.

2.1. Confocal microscopy

Confocal microscopy uses a pinhole aperture to block out-of-focus light, restricting illumination and detection to a single focal plane [15,16]. A focused laser scans the sample point by point, and fluorescence or reflected light from the focal volume is detected to produce high-resolution, high-contrast optical sections. These 2D sections can be computationally reconstructed into detailed 3D structures within the specimen [15,16]. Confocal microscopy detects biostructures by collecting fluorescence (or reflected light) emitted from labeled targets, while a pinhole rejects out-of-focus photons, yielding optically sectioned images. This enables quantitative mapping of protein localizations, membranes, nucleic acids, and 3D biofilm architecture with submicron precision [15, 16]. This technique is a critical tool in microbiology, especially for studying biofilms and complex microbial communities [17]. It provides high-resolution, 3D imaging of these structures, offering insights into their morphology, behavior, and interactions [18]. A key advantage is its ability to image live cells without causing significant damage, allowing for real-time observation of microbial dynamics [19]. Confocal microscopy also enables quantitative analysis of microbial structures, such as size and distribution, using advanced image analysis software. A challenge with this technique, however, is overlapping emission spectra during multicolor imaging, which often requires a process called spectral unmixing to resolve [19].

2.2. Multiphoton microscopy

Multiphoton microscopy is a fluorescence imaging technique in which two or more near-infrared (NIR) photons are absorbed simultaneously to excite a fluorophore, a nonlinear process that confines excitation to the focal volume [20]. This intrinsic optical sectioning reduces out-of-focus photodamage, enables deeper tissue penetration, and improves imaging of thick or living specimens [21]. Multiphoton microscopy detects biostructures via nonlinear excitation (two- or three-photon absorption) that confines fluorescence to the focal volume, improving depth penetration and reducing photodamage. It also provides label-free contrasts such as second-harmonic generation (SHG) from ordered matrices and coherent Raman signals (coherent anti-Stokes Raman scattering (CARS)/Stimulated Raman Scattering (SRS)) from specific bonds, revealing endogenous metabolism (NADH/flavins), lipids, and extracellular polymeric substances (EPS) features in thick biofilms and tissues [20, 21]. Unlike confocal microscopy, this technique uses a nonlinear process called two-photon excitation that only occurs at the focal point [20]. It typically uses pulsed IR lasers, which allow the light to penetrate deeper into biological structures—up to 1 mm—by significantly reducing light scattering and absorption [20]. Because excitation is confined to the focal plane, all fluorescence is inherently confocal, eliminating the need for a pinhole and reducing phototoxic damage to out-of-focus areas [21]. This makes multiphoton microscopy particularly useful for studying microbial communities in thick biological matrices like biofilms or soil [22]. Its applications include intravital imaging of organs to study their physiology and label-free imaging of microbes within complex root, mineral, and soil matrices [22]. The technique can also simultaneously capture two-photon excitation fluorescence, CARS, SHG, and sum-frequency mixing, providing both contrast and chemical information [22]. However, challenges include issues with probe access, tissue optics, and photon scarcity at high imaging rates, which can reduce image contrast.

2.3. Super-resolution microscopy (SRM)

SRM comprises optical methods that surpass the diffraction limit of conventional light microscopy (~200 nm lateral, ~500 nm axial) by exploiting physical and photochemical control of fluorophores to achieve nanometer-precision localization [20, 21]. Techniques modulate excitation/emission in space or time or use stochastic activation of single molecules and then apply computational reconstruction to recover high spatial frequencies. As a result, SRM breaks the Abbe limit and resolves 10–50 nm subcellular architecture in fixed or live specimens [20, 21].

SRM resolves microbial structures by surpassing diffraction limits and converting specific light–matter interactions into measurable contrast. In stimulated emission depletion microscopy (STED), a primary excitation beam is paired with a donut-shaped depletion beam that drives peripheral fluorophores to the ground state via stimulated emission, leaving only the focal core emissive; this shrinks the fluorescent spot to 30–80 nm and enables point-scanned, sub-diffraction imaging [23, 24]. Reversible Saturable Optical Fluorescence Transitions (RESOLFT) uses lower-power depletion to switch fluorophores off, reducing phototoxicity [25]. STED works with conventional fluorescent proteins, and commercial systems enable real-time, multicolor imaging of cytoskeletal and division machinery (e.g., discontinuous FtsZ helices in *Bacillus subtilis*; neighboring MreB filaments in live *Escherichia coli*) [25, 26]. Limitations include high depletion intensities that induce photobleaching/phototoxicity and point-scanning speeds that can limit temporal resolution [26]. Single-molecule localization microscopy techniques—such as photoactivated localization microscopy (PALM) and stochastic optical reconstruction microscopy (STORM)—activate or exploit stochastic blinking of sparse emitters and fit their Point Spread Functions (PSFs) with nanometer precision across thousands of frames (PALM with photoactivatable proteins; STORM

with photoswitchable dyes) [27, 28]. Typical precisions are ~10–30 nm (PALM) and ~10–20 nm (STORM), enabling quantitative measurements of dimensions, molecular densities, and multicolor interactions and revealing nanoscale organization (e.g., OmpR, membrane clusters, chemosensory arrays) [27, 29]. Advantages are top camera-based precision and molecular counting; drawbacks include slow temporal resolution, live-cell windows often <15 min due to photodamage, strict probe requirements and intracellular dye permeability limits, and potential fixation artifacts [26–28]. Minimal photon fluxes (MINFLUX) technique localizes a single emitter by positioning a donut-shaped excitation minimum with feedback to minimize fluorescence, achieving ~1–5 nm precision with few photons (e.g., YscL of the T3SS in *Yersinia enterocolitica*), but it requires exceptionally stable, specialized instrumentation and currently offers limited multiplexing [30, 31]. Structured illumination microscopy (SIM) shifts high spatial frequencies into the passband using rotating/translating sinusoidal grids; moiré patterns are deconvolved to double resolution (~100 nm) at gentle light doses, enabling live imaging of pathogens (e.g., *Brucella abortus* in host cells) and proteins mediating interbacterial interactions (e.g., the T6SS component TssB) [30, 31]. Super-resolution optical fluctuation imaging (SOFI) analyzes higher-order temporal cumulants of fluorescence fluctuations to narrow the effective PSF and separate overlapping emitters without single-molecule fitting, aiding studies in dense fields (e.g., iron acquisition receptors such as Pfe in *Pseudomonas aeruginosa*) [32, 33]. In microbiology, these methods are complementary: STED/RESOLFT provide direct, real-time imaging with conventional labels [25, 26]; PALM/STORM deliver molecular precision and counting [27–29]; SIM offers gentle live-cell dynamics; and SOFI adds computational robustness in crowded scenes. Technique choice balances resolution versus speed, phototoxicity/bleaching, labeling and permeability constraints, and computational demands to match the biological question.

2.4. Digital holographic microscopy (DHM)

DHM is a label-free, quantitative phase imaging technique that records interference between a coherent reference beam and light scattered by a specimen, producing a digital hologram containing both amplitude and phase information [34]. Numerical wave-propagation reconstruction yields high-contrast phase images that reveal morphology, refractive index, and dynamic changes in live samples. By compressing 3D information into a single image, DHM enables nanometer-resolution measurement of phase shifts caused by a sample's optical thickness and refractive index [34]. DHM detects biostructures label-free by measuring quantitative phase—optical path length set by refractive index and thickness—reconstructed from interference with a reference beam. It reports cell area, thickness, dry mass, growth/viability, and 3D motility without staining. DHM provides real-time, label-free 3D observations for applications in cell biology, diagnostics, and the food industry [35]. It enables cell counting, tracking growth, viability, and death with accuracy comparable to manual methods, and can monitor 3D bacterial motility, such as *E. coli* movement during biofilm formation [36], as well as lipid accumulation in microalgae for nutraceutical and biodiesel production [34–36]. DHM is affordable, fast, and simple, yielding parameters like cell area, thickness, and volume from a single hologram [35]. A key limitation is the difficulty in tracking very small bacteria with refractive indices like the surrounding medium [34, 35].

2.5. Light sheet fluorescence microscopy (LSFM)

LSFM uses a thin laser sheet to illuminate a single plane of a fluorescently labeled specimen, collecting emission orthogonally to minimize photobleaching and phototoxicity [37]. This setup enables optical sectioning without depth scanning and rapid, high-signal-to-noise 3D imaging of large, living samples, including whole communities, host tissues, and biofilms [37, 38]. LSFM is perfect for long-term studies of microbial behavior and for imaging entire organisms or thick transparent tissues. Its low phototoxicity makes it ideal for observing dynamic biological processes over long periods without causing damage or altering function [38]. In microbial ecology, LSFM is used to monitor artificial closed ecosystems, study bacterial biofilms, and observe morphological changes in algae colonies [39]. It has also been used to investigate the population dynamics of gut microbial communities in model organisms, revealing complex behaviors like varying growth rates and competitive exclusion [39]. LSFM's advantages include its high resolution, speed, and ability to image tissues thicker than 1 cm in a nondestructive way, which makes it great for 3D reconstruction [40]. While a lack of commercial instruments and inconsistent terminology were once issues, current technical challenges include aligning dual-sided light sheets to reduce shadows, managing the Gaussian beam profile for large specimens, and issues with objective immersion for high-resolution imaging [39, 40]. Additionally, specimens must be transparent, and image processing for 3D reconstruction can be time-consuming [38–40].

Figure 1 [16, 26, 27, 30, 31, 33, 34, 39] highlights applications of microscopic techniques in microbial research, and these techniques are scored in Figure 2 by spatial resolution, acquisition speed, and field of view (the area of the specimen captured in a single image at one time).

3. Spectroscopic Techniques for Microbial Characterization

Spectroscopy enables label-free chemical analysis of microbial cells, revealing molecular composition, metabolism, and community dynamics.

3.1. Raman spectroscopy and surface-enhanced Raman spectroscopy (SERS)

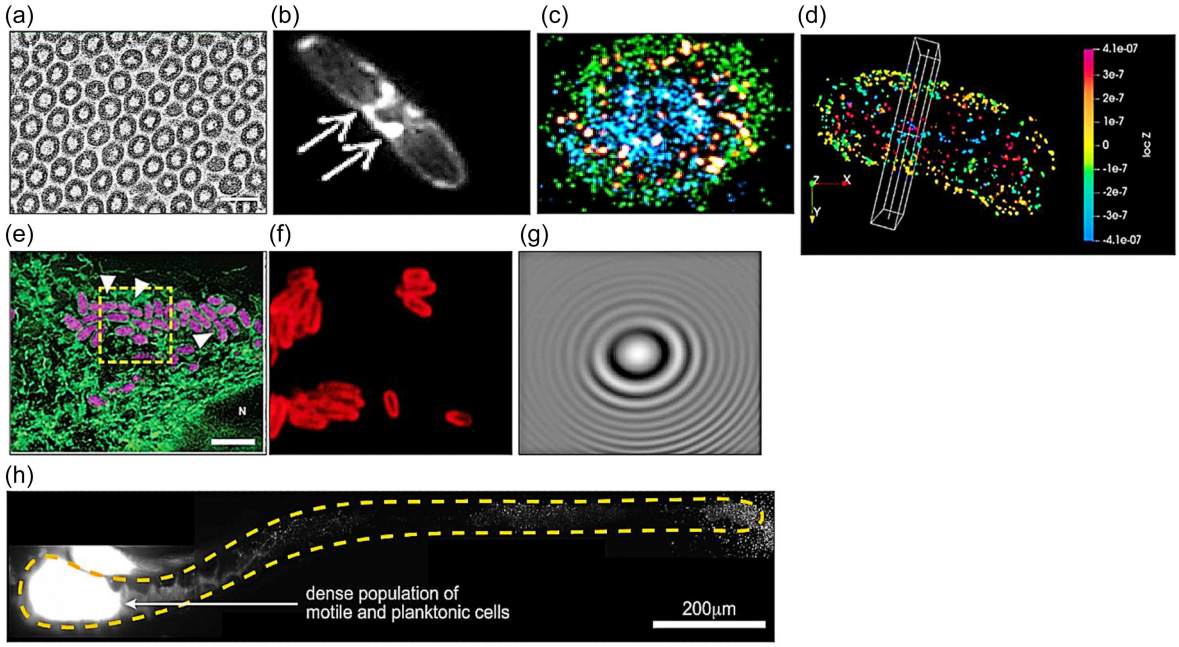
1) Raman spectroscopy

Raman spectroscopy is a vibrational spectroscopic technique based on the inelastic scattering of monochromatic light (usually from a laser) by molecular vibrations, phonons, or other excitations in a sample [41]. This Raman scattering shifts the energy (and wavelength) of the scattered photons according to the vibrational energy levels of the molecules, providing a molecular "fingerprint" that enables identification and characterization of chemical composition, molecular structure, and intermolecular interactions [41]. Only vibrational modes that change a molecule's polarizability are Raman-active, which distinguishes Raman selection rules from IR absorption.

Raman spectroscopy detects biostructures via inelastic scattering from vibrational modes that change molecular polarizability (Raman-active), producing label-free chemical fingerprints. It identifies cell-wall components, nucleic acids, proteins (e.g., phenylalanine ~1004 cm^{-1}), lipids, pigments, and stress metabolites at single-cell resolution [41, 42]. This technique is widely applied in biology and medicine for quantifying biomolecules, hyperspectral molecular imaging of cells and tissues, and medical diagnosis. A significant advantage of Raman spectroscopy in microbiology is its nondestructive and label-free nature, requiring minimal to no sample preparation [42]. This allows for *in situ* and near real-time chemical information from microorganisms without compromising their integrity [41, 42]. Unlike infrared (IR)

Figure 1

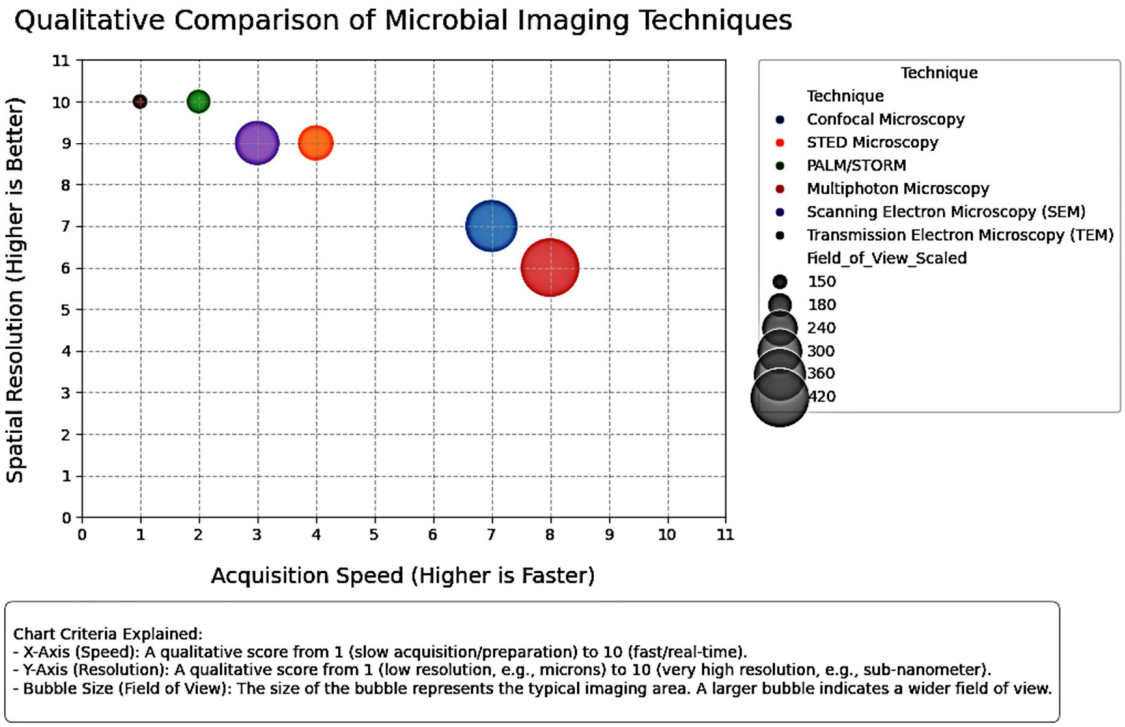
Applications of imaging techniques in microbial research. (a) Confocal images of *Cellulophaga lytica* biofilms, (b) STED image of protein components of the MreB-associated cytoskeleton in *E. coli*, (c) PALM and STORM showing presence of OmpR (red hot), DNA (cyan) and membrane (green) in cross section of *E. coli*, (d) distribution of YscL of the T3SS in *Y. enterocolitica* shown by MINFLUX, (e) *B. abortus* (magenta) infecting HeLa cells (green) shown by SIM, (f) Pfe siderophore expressing *P. aeruginosa* by SOFI, (g) DHM holograms of freely swimming *E. coli*, and (h) LSFM showing the spatial organization of *Vibrio cholerae* in zebrafish



Note: Panels (a), (b), (d), (e), and (g) are adapted under a CC BY license. Panels (c) and (f) are adapted with permission from the American Chemical Society and Oxford University Press, respectively. Sources are cited in relevant sections.

Figure 2

An overview of various microscopic techniques



Note: These techniques were scored based on published values of spatial resolution/acquisition speed ranges and field of view from the cited literature after experts' opinions.

spectroscopy, the quality of the Raman signal is barely affected by the presence of water, making it reliable for aqueous biological samples and *in situ* monitoring of processes like fermentation. While powerful, a major limitation is the inherently weak scattering signal from most samples, often leading to long acquisition times, especially for hyperspectral imaging [41, 42]. This can be a significant obstacle for high-throughput applications, as acquiring a large image can take days. Another challenge lies in developing robust and trustworthy quantitative methods for data analysis [41, 42].

2) SERS

SERS is a highly sensitive variant of Raman spectroscopy that enhances weak Raman scattering signals by several orders of magnitude using nanostructured metal surfaces (typically silver, gold, or copper) [43]. The enhancement arises primarily from localized surface plasmon resonance, which amplifies the electromagnetic field near the metal nanostructures, and secondarily from chemical enhancement due to charge-transfer interactions between the analyte and the metal surface. This enables molecular detection down to single-molecule sensitivity [44].

SERS detects biostructures by plasmonically enhancing Raman signals at metal nanostructure "hot spots," boosting weak vibrational fingerprints. It sensitively reads extracellular metabolites, envelope signatures, or antibody-tagged epitopes for strain-specific detection [43]. SERS enables label-free chemical fingerprinting of microbial cells and metabolites, with applications in medical diagnostics, food safety, and biodefense [44]. It can directly detect single *E. coli* cells without culture, achieving detection limits down to 1 CFU/mL in milk [44]. SERS-tags—gold nanoparticles coated with Raman reporters and specific antibodies—offer selective, highly sensitive single-cell detection, with high specificity against nontarget strains [43–45]. Limitations include limited molecular interpretability in label-free approaches, variability from nanoparticle preparation, and the small sensing area and shallow penetration depth of some sensors, reducing effectiveness for complex biofilms [43–45].

3.2. Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy is an analytical technique that measures how a sample absorbs IR light as a function of wavelength, revealing its molecular vibrational modes [46]. A broadband IR source is directed into an interferometer (typically a Michelson interferometer) to generate an interference pattern (interferogram), which is recorded after passing through the sample [46]. A Fourier transform then converts the interferogram into an IR absorption spectrum, providing a molecular "fingerprint" for qualitative and quantitative analysis of specific biomolecules like lipids, proteins, and carbohydrates within cells [46, 47]. In contrast to Raman, FTIR detects vibrational modes that involve a change in the dipole moment (IR-active), yielding complementary information to polarizability-dependent modes.

FTIR spectroscopy detects biostructures by absorption from vibrational modes that change dipole moment (IR-active), resolving amide, lipid, carbohydrate, and nucleic-acid bands. It profiles biofilm EPS, cell-wall chemistry, growth-phase shifts, and antibiotic responses through band positions and intensities [46, 47]. FTIR is widely used in microbial cell biology and environmental microbiology to identify species and strains via spectral fingerprints, monitor growth phases, and characterize cell walls, distinguishing Gram-positive from Gram-negative bacteria [46, 47]. It can track antibiotic penetration, such as rifampicin in *E. coli*, by observing changes in cell wall and protein peaks [48], and monitor biofilms from bacteria, fungi, and algae. ATR-FTIR enables in situ, nondestructive, real-time observation of biofilm development and EPS functional groups critical for adhesion [47]. FTIR also profiles microbial communities, assesses stress and viability, studies biosorption of metals, and tracks pollutant biodegradation via band changes [46–48]. Advantages include noninvasiveness and small sample requirements, but complex biological spectra often require extensive reference data and advanced analysis [46–48].

3.3. Fluorescence spectroscopy

Fluorescence spectroscopy measures light re-emitted by fluorophores after they absorb excitation light and briefly enter an excited state [48]. Analysis of the emitted intensity and wavelength reveals molecular identity, environment, concentration, and dynamics [49], with broad applications in environmental monitoring, industry, medical diagnostics, and biotechnology [47, 49].

Fluorescence spectroscopy detects biostructures by emission from intrinsic fluorophores (NADH, flavins, aromatic amino acids) or dyes, with spectra and lifetimes reflecting identity and environment. It discerns species via autofluorescence patterns and reports metabolic state and viability [49, 50]. In microbial characterization, fluorescence spectroscopy is crucial for bacterial identification and pathogen detection. It can leverage the intrinsic autofluorescence of cellular molecules (e.g., aromatic amino acids like tryptophan, tyrosine, and phenylalanine, or NADH and flavins) to provide label-free identification and insights into metabolic states [51]. For instance, autofluorescence spectra have been used to distinguish between different bacterial species like *E. coli, Enterococcus faecalis*, and *Staphylococcus aureus* with high sensitivity and specificity in less than 10 minutes, often aided by principal component analysis [48–50]. It has also been applied to detect the presence of pathogenic microorganisms in drinking water by spectrally studying UV-induced autofluorescence from single organisms in a water flow, serving as an early warning system [47, 49]. Environmental applications include characterizing and monitoring wastewater by identifying peaks related to living and dead cellular material and detecting human and chicken waste in rivers [50]. Advantages of fluorescence spectroscopy include its high sensitivity, strong selectivity, small sample volume requirement, and ease of use [51]. It provides more physical parameters and is linear to sample concentration over a broad range [49, 50]. However, its application range can be insufficient, and it is sensitive to environmental factors, which can introduce interference [50]. Background fluorescence from the sample matrix can sometimes mask the signal from bacteria, though imaging techniques can help distinguish between them [50].

3.4. Flow cytometry

Flow cytometry is a high-throughput, laser-based analytical technique used to measure the physical and chemical characteristics of individual cells or particles as they flow in a fluid stream past an optical interrogation point [51]. Cells are hydrodynamically focused into a single file, illuminated by one or more lasers, and analyzed based on light scattering (size, granularity) and fluorescence emission from labeled markers [51]. Flow cytometry enables high-throughput, single-cell analysis, making it ideal for studying microbes in bacteriology and virology [52].

It supports mixed-population characterization, microbial kinetics (growth, reproduction, metabolic needs), and detection of particles below the resolution limit of visible light via fluorescence [52]. Cell sorting allows isolation of single cells or rare events for downstream analysis and can be combined with imaging to study growth in complex 3D environments such as biofilms [51,53]. Limitations include high assay costs (e.g., antibiotic resistance testing) [54], complex spectral compensation with multiple fluorophores [51], bottlenecks in analysis time despite >20,000 particle/s sorter speeds [53,55], biosafety risks from aerosol generation [55], and slower performance than automated image processing for very high-throughput screening [53,55].

4. Optical Sensing and Biosensors

Optical biosensors enable real-time, sensitive, and portable microbial detection across diverse environments.

4.1. Fiber-optic biosensors (FOBs)

FOBs combine a biological recognition element (e.g., enzyme, antibody, nucleic acid) with an optical fiber transducer to detect specific analytes [55]. Interaction with the biorecognition layer alters light properties—intensity, wavelength, phase, or polarization—within the fiber, which are measured via fluorescence, absorbance, interferometry, or surface plasmon resonance and converted into quantitative signals [55]. FOBs detect biostructures by changes in guided light (intensity, wavelength, phase, polarization) within the fiber's evanescent field when captured bacteria, toxins, or nucleic acids bind to surface receptors. Fluorescence, absorbance, interferometric, or plasmonic readouts convert binding into quantifiable signals [55, 56]. FOBs offer high sensitivity, Electromagnetic Interference (EMI) immunity, and remote, real-time monitoring, with small, durable designs suited for in situ use in water, soil, and industrial environments [56,57]. Applications include pathogen detection and biomedical diagnostics, such as antibody-based fluorescent waveguide sensors and portable systems like RAPTOR™, which detect agents within 1–3 minutes [58]. Mach–Zehnder interferometers enable label-free bacterial detection at limits as low as 7 CFU/mL for *E. coli* [58,59], while nanomaterial integration enhances sensitivity [56,57]. Limitations include the fragility of some fiber Bragg grating sensors, limited high-frequency response, wavelength drift, cross-sensitivity to temperature or strain, and fabrication challenges. Plastic optical fibers are cheaper but less sensitive and have higher signal loss than glass [57–59].

4.2. Plasmonic sensors

Plasmonic sensors are label-free optical devices that detect analytes via surface plasmon resonance (SPR)—coherent oscillations of conduction electrons at a metal–dielectric interface (typically gold, silver, or copper) excited by light [60,61]. In SPR, p-polarized laser light is directed onto a thin metal film through a prism (Kretschmann configuration), and biomolecule binding alters the local refractive index within the ~200 nm evanescent field, shifting the resonance [60,61]. Localized SPR (LSPR) occurs in metal nanoparticles or nanostructures, yielding wavelength-dependent absorption and scattering peaks with a shorter decay length (~5–60 nm), offering greater sensitivity to small, surface-proximal biomolecules [60,61].

SPR/LSPR systems detect changes in refractive index caused by microbial cells, subcellular fragments, or biomolecules such as cell-wall components (lipopolysaccharides, peptidoglycan), secreted proteins and toxins, EPS from biofilms, nucleic acids, or specific antigenic epitopes [62]. Recognition elements, including antibodies, aptamers, lectins, or phage-derived peptides, immobilized on sensor surfaces enable targeted detection by inducing measurable spectral or angular shifts [62]. SPR instrumentation typically includes a collimated laser diode (red–NIR range) or broadband light source, coupling optics (prism or grating), a sensor chip with a ~50 nm metal film on glass and functionalized biorecognition layer, and a detector (CCD array or photodiode) [60]. LSPR platforms use nanostructured substrates (gold/silver nanoparticles, nano-islands, nanorods) fabricated by lithography or colloidal synthesis, with peak shifts measured by spectrometry under white light [61, 62]. Performance enhancements can be achieved using 2D materials (e.g., graphene, MoS_2) for improved adsorption and field strength, and microfluidic integration enables low-volume, real-time analysis [62]. Plasmonic sensors enable rapid (<30 min) pathogen detection, including *Salmonella enterica* and *E. coli* in food and clinical samples [63], identification of mycotoxins and bacterial toxins, real-time biofilm monitoring via EPS detection, viral sensing using LSPR with spike protein antibodies, and assessment of cell lysis or metabolic inhibition through refractive index shifts after antibiotic exposure [62]. They provide high-sensitivity, label-free real-time analysis, with SPR optimal for larger assemblies or whole cells and LSPR for small biomolecules and conformational changes [62,64]. Limitations include nonspecific binding, bulk refractive index effects, instability or degradation of biorecognition layers, and—in SERS-enabled formats—variability from nanoparticle fabrication and interference from complex samples [62,64].

4.3. Lab-on-a-chip (LOC) and microfluidics

LOC devices integrate multiple laboratory functions onto a single miniaturized platform, typically a few square centimeters in size [65]. These devices are designed for automation and high-throughput screening, using microfluidics to precisely manipulate very small fluid volumes (picoliters or less) within microchannels. While photolithography is a common fabrication method, newer techniques like 3D printing and soft lithography are emerging to enable faster prototyping and reduce costs [65]. LOC devices offer portable, rapid, and efficient microbial detection and characterization, making them ideal for point-of-care testing in resource-limited settings [66,67]. By integrating multiple laboratory functions onto a miniaturized platform, LOC enables fast diagnosis and management of bacterial or viral infections. In antimicrobial susceptibility testing, they deliver results far faster than conventional methods—for *S. aureus*, within 10 minutes to 7 hours versus 24–48 hours traditionally [68]. Miniaturization reduces reagent use, waste, and costs and, in some cases, allows single-cell testing without pre-incubation [69]. Integrated sensors, electrodes, and optical elements can measure parameters such as pH, temperature, and fluorescence, providing real-time data on microbial responses [66]. LOC devices face challenges despite their promise. Micro-manufacturing is often complex and costly, requiring specialized expertise, though 3D printing offers partial relief [65,67]. Precise fluid control is difficult due to intricate networks and multiple pumps [67], and many LOCs remain at the proof-of-concept stage, needing further validation [65–67]. At microliter volumes, surface effects like capillary forces hinder replication of conventional lab processes [65–68], and miniaturized detection can suffer from low signal-to-noise ratios [67]. AI integration also

struggles with limited generalizability and insufficient real-world testing [68].

4.4. Laser-induced breakdown spectroscopy (LIBS)

LIBS is an elemental analysis technique that uses a focused, high-energy laser pulse to ablate a minute amount of material from a sample, creating a hot microplasma. As the plasma cools, excited atoms and ions emit light at characteristic wavelengths, which is spectrally analyzed to determine the sample's elemental composition [69]. LIBS is a robust and versatile tool for elemental analysis and direct microbial identification, particularly where traditional methods are insufficient. Its advantages include its non-destructive nature, as it only ablates a small amount of material, and its rapid analysis time, which is measured in seconds to milliseconds [70]. LIBS provides wide elemental coverage, from lithium to uranium, and has high sensitivity for trace elements [71]. It requires minimal to no sample preparation, is capable of real-time and even remote measurements, and can perform simultaneous multi-element analysis [71]. In microbiology, LIBS is used to detect bacteria, molds, yeasts, spores, and even viruses [72]. It enables rapid genus-level discrimination of microbes—such as *Acinetobacter baylyi, B. subtilis, and E. coli*—by measuring elemental signatures (Na, Mg, P, K, Ca, Fe) [72] and can distinguish Gram-positive spores from Gram-negative bacteria using elemental ratios (Ca/Na, K/Na) [73]. LIBS is applied in food safety to identify pathogens in milk or chicken broth [74], assess membrane composition changes in Gram-negative bacteria, and classify pathogens in mixed cultures under stress [73]. Limitations include possible sample melting or recrystallization [69, 70], lower accuracy and precision in field conditions compared to lab-based atomic emission spectroscopy [64, 70], high setup costs, limited expertise [70], environmental sensitivity, and spectral distortion from plasma self-absorption [69, 71]. In biomedical contexts, LIBS may also have reduced accuracy and detection limits versus other methods [69, 71].

5. Light-Based Manipulations

Photonics enables precise microbial manipulation, advancing synthetic biology and applied microbiology.

5.1. Optogenetics

Optogenetics combines genetic engineering with photonics to achieve noninvasive, precise spatiotemporal control of cellular processes using light [74]. It employs photoresponsive proteins (e.g., opsins, photoreceptors, engineered light-sensitive domains) that change conformation upon absorbing specific wavelengths, thereby modulating downstream pathways [75]. Light-based activation is tunable, reversible, and orthogonal to native biochemistry, reducing side effects and crosstalk, and unlike chemical inducers, optical stimulation avoids issues with diffusion, degradation, and clearance [75]. In microbial systems, the optical signal in optogenetics typically originates from intrinsic chromophores bound to photoreceptor proteins, such as flavin mononucleotide in Light, Oxygen, or Voltage (LOV) domains, p-coumaric acid in photoactive yellow protein, or retinal in channelrhodopsins [76]. Upon excitation at specific wavelengths—blue light (~450–490 nm) for LOV domains or red/far-red light for phytochromes—these cofactors undergo spectral absorption changes that induce conformational and allosteric rearrangements in engineered fusion proteins [77]. This structural transition can regulate gene transcription, protein localization, or enzymatic activity [77]. To assess optogenetic activity in microbes, researchers often measure output proteins such as fluorescent reporters, enzymes, or structural proteins, enabling the monitoring of pathway activation, gene expression dynamics, or phenotype changes [75, 76].

Microbial optogenetics instrumentation uses LEDs or laser diodes matched to the photoreceptor's action spectrum (blue 450–470 nm, green 520–540 nm, red/far-red 630–740 nm) [77]. Light delivery methods include LED arrays for microtiter plates, fiber-coupled/collimated beams for microfluidics, and laser scanning or DMD projection for single-cell studies [75]. A programmable control unit adjusts intensity, duration, and spatial patterns, while fluorescence microscopy, confocal imaging, or photodetectors/spectrometers quantify responses [76, 78]. Microbial control circuits can be one-component systems integrating photoreception and transcriptional regulation (e.g., EL222) or two-component systems where a light receptor activates a separate regulator; the latter enables wavelength multiplexing but may require exogenous chromophores for non-blue activation [76, 77]. Applications of microbial optogenetics span synthetic biology, biotechnology, and fundamental research. In metabolic engineering, optical control can dynamically regulate metabolic flux in two-phase fermentations, such as switching pathways in *E. coli* to enhance muconic acid yield or in *S. cerevisiae* to boost β-carotene production [79]. Biofilm regulation is possible through light-controlled modulation of c-di-GMP levels or EPS biosynthesis, enabling inhibition or induction of biofilm formation [80]. In population control and spatial patterning, structured illumination can pattern multispecies biofilms or stabilize co-cultures via differential motility regulation [77, 80]. Optogenetics also facilitates gene expression studies by activating promoters at precise times to dissect regulatory networks under tightly controlled conditions [76, 77]. In biomedical and microbiome applications, engineered live microbial therapeutics can be made to deliver drugs or cytokines in response to light, enabling targeted interventions in the gut or tumor environments [78, 79]. Overall, optogenetics offers high spatial and temporal resolution, reversible control, multiplexing with multicolor systems, and minimal chemical interference. However, it does have limitations: the penetration depth of activation wavelengths, particularly blue light, is restricted in optically dense cultures or tissues; some systems require exogenous chromophores; continuous or high-intensity activation can impose a metabolic burden; and large-scale light delivery for industrial fermentations remains technically challenging [77–79].

5.2. Optical tweezers (OT)

OT use a tightly focused laser beam to create gradient forces that trap and manipulate microscopic dielectric particles in 3D [81, 82]. Particles with higher refractive indices than the surrounding medium are drawn to the focal point, counteracting scattering and radiation pressure for stable trapping. Trapping forces, adjustable via laser power and refractive index contrast, can be calibrated from pico- to nanonewton levels with ~100 aN sensitivity. Beam-shaping techniques (e.g., Bessel, Laguerre–Gaussian modes) allow multi-particle control, optical torque, and improved axial trapping depth [81, 82].

In microbiology, OT are primarily used for manipulation, with scattered light patterns measured by quadrant photodiodes (QPD) or similar detectors to quantify displacement and force [81, 82]. Coupling with fluorescence microscopy enables visualization of labeled structures [82], while "Raman tweezers" provide simultaneous manipulation and chemical fingerprinting

of single cells, revealing features such as cell walls, flagella, pili, intracellular organelles, and EPS in biofilms [83]. Modern OT use continuous-wave NIR lasers (typically 1,064 nm; 780 nm for specific applications) to minimize photodamage [84, 85]. High-Numerical aperture (NA) (>1.2) objectives focus the beam to a diffraction-limited spot, and spatial light modulators or diffractive optics enable holographic OT for multiple controllable traps [86]. Samples are held in liquid microchambers or microfluidic devices [85,86], with detection via back focal plane interferometry (QPD) for nanometer-scale displacement, high-speed cameras for imaging, and integrated Raman or fluorescence modules for molecular analysis [86,87]. Specialized beams, including Bessel for extended depth and Laguerre–Gaussian for particle rotation or torque, further enhance functionality [88]. OT enable precise, non-contact manipulation and measurement. They can hold single bacterial or microalgal cells for morphological, viability, and subcellular analysis [81], transport cells in microfluidic devices for sorting by optical or mechanical traits [85], and isolate biofilm cells to study adhesion, motility, and mechanical responses [85]. OT can arrange multiple cells into defined 2D/3D assemblies for synthetic biology [85,86] and measure forces such as pilus retraction, flagellar torque, cell-wall stiffness, and cell–surface adhesion. Raman tweezers allow concurrent trapping and label-free molecular fingerprinting for species identification or metabolic profiling, while integration with pulsed UV or Nd:YAG microbeams enables microsurgery, including membrane ablation and optoinjection [86–88]. OT offer noninvasive trapping in aqueous media, high spatial precision, attonewton-level force sensitivity, and compatibility with real-time imaging and spectroscopy [83]. Limitations include possible heating or photodamage from laser absorption, reduced trapping depth in turbid samples, difficulty manipulating cells in dense or mobile environments, and, in some cases, the need for fluorescent labeling for specificity [87–89]. Overall, OT combine precise physical manipulation with mechanical property measurement and, when coupled with spectroscopy, single-cell biochemical analysis [88].

5.3. Laser ablation and microbial interactions

Laser ablation uses focused laser pulses to remove or modify material, enabling the study and manipulation of microbial communities [90]. Applications include elemental mapping via LA-ICP-MS, revealing spatial element distributions in intact colonies (e.g., elevated manganese at *E. coli* colony edges) [91,92], and biofilm analysis [92]. Surface modification with femtosecond lasers (FSL) can create periodic structures on titanium or nano-microtextures on stainless steel, reducing adhesion and biofilm biomass by disrupting amyloid production and eDNA binding, with >60% antibacterial efficacy against *S. aureus* and *E. coli* [93,94]. Laser techniques also enable precise cell manipulation: Laser engineering of microbial systems (LEMS) isolates and cultivates single microbial cells from complex communities by fluorescence [95], Laser capture microdissection (LCM) separates mucosa-associated microbes for metagenomics [91,92], and laser-guided microfluidic systems or interference patterning support single-cell micropatterning and directional growth studies [92–95]. Limitations of laser ablation include the potential for contamination or toxicity during the interaction between the laser pulse and the targeted material [96, 97]. While FSL offer minimal thermal effects, other laser types might cause significant heating, affecting cell viability [95–97]. The choice of fabricated materials can be limited, and specific laser parameters (power, pulse width, repetition, interval, profile) significantly influence the desired outcomes [97].

6. Comparative Analyses

The diverse optical techniques reviewed—spanning advanced imaging, spectroscopy, optical sensing, and light-based manipulation—form a complementary toolbox for addressing the central challenges of microbial ecology. High-resolution imaging reveals spatial organization, morphology, and dynamic behavior from single cells to complex biofilms, while spectroscopic methods provide label-free, in situ chemical fingerprints of microbial metabolism and interactions. Optical biosensors extend these capabilities into portable, real-time monitoring of pathogens and community shifts in environmental and clinical settings. Light-based manipulation techniques add the ability to selectively control or perturb microbial processes, enabling causal studies and synthetic re-engineering of communities. Together, these modalities overcome the "Great Plate Count Anomaly" by enabling direct observation and functional analysis of both culturable and unculturable microorganisms in their native contexts, bridging structural, chemical, and functional perspectives. Importantly, the integration of these methods—for instance, coupling Raman spectroscopy or FTIR with confocal imaging and AI-enhanced analytics or linking quantum-scale sensing with lab-on-chip diagnostics—is driving a shift toward multifunctional, miniaturized platforms for real-time, ecologically relevant, and mechanistically precise microbial investigations. Figures 3 and 4 summarize technique trade-offs (resolution, speed, and field of view and capability criteria) to guide method selection for specific microbial questions. Methodology. Scores were derived from literature-reported performance ranges (resolution limits, acquisition rates, field of view), normalized to a common scale.

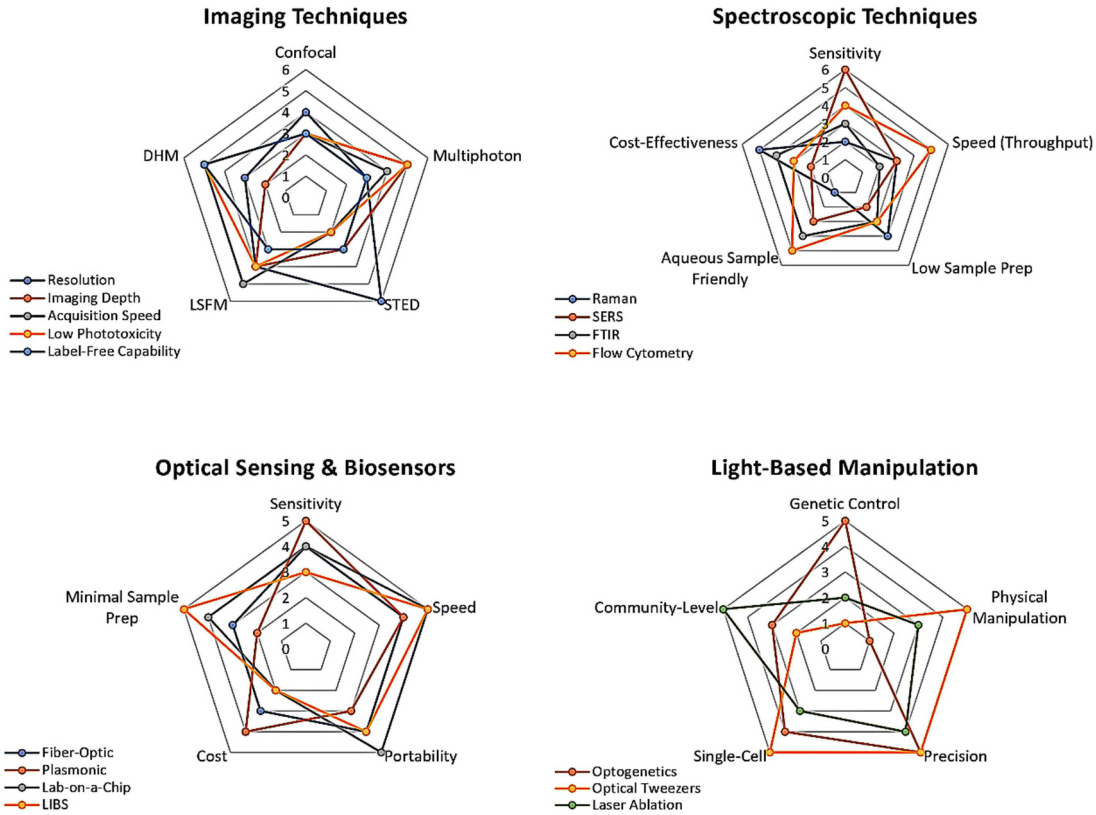
7. Emerging Trends and Future Directions

7.1. Artificial intelligence (AI)-enhanced optical imaging

AI, particularly deep learning and machine learning, is increasingly enhancing microbial optical imaging by delivering higher throughput and improved accuracy compared to manual or threshold-based analysis methods [98]. Beyond basic classification tasks, AI has been applied to diverse workflows. For example, convolutional neural networks have been used to detect antimicrobial resistance phenotypes by discriminating *E. coli* strains from subtle colony morphology differences in agar plate images [99]. In biofilm research, AI enables quantification of morphology from three-dimensional confocal laser scanning microscopy (CLSM) datasets, allowing measurement of biomass, roughness, and heterogeneity indices. In spectroscopic imaging, AI models have classified Raman spectra to differentiate closely related *Salmonella* serovars and to detect live and dead subpopulations within mixed microbial communities [99, 100]. Recent work has leveraged advanced vision architectures such as EfficientNetV2 for fine-grained microcolony feature extraction and domain adaptation techniques including Domain-Adversarial Neural Networks (DANNs) and multi-DANNs, which improve cross-instrument generalization across variations in magnification, illumination, and staining protocols [98, 99].

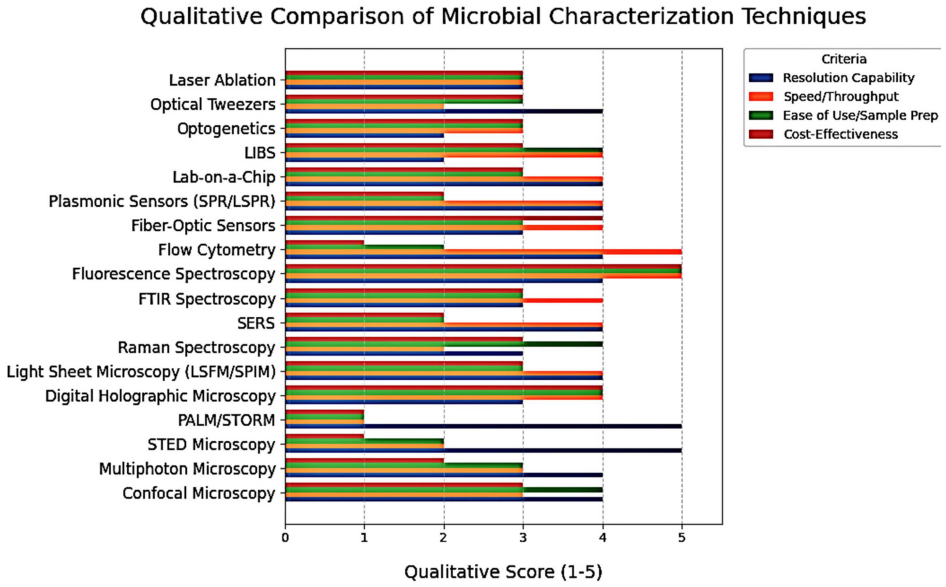
These AI methods integrate seamlessly with multimodal imaging workflows. In Raman–AI pipelines, automated feature extraction from hyperspectral data accelerates pathogen

Figure 3
A holistic comparison of the different techniques across five key capabilities



Note: Scores were assigned based on resolution/acquisition speed ranges, typical field of view, and representative applications from the literature after expert opinion. Values were normalized to a 1–5 scale, where higher scores represent better performance.

Figure 4
A comparison of microbial characterization techniques based on their typical performance across key criteria



Note: Scores are on a scale of 1 (lowest/worst) to 5 (highest/best). Scores were assigned based on published resolution/acquisition speed ranges, typical field of view, and representative applications from the cited literature after expert opinion.

identification [101]. In FTIR–AI approaches, functional group classification can be performed in real time for species or strain identification [102]. Likewise, combining DHM with AI enables automated extraction of microbial growth curves and detection of morphological changes at the single-cell level [103].

Despite these advances, several technical bottlenecks limit performance and adoption. A primary challenge is domain shift between datasets acquired on different microscopes or modalities, which can degrade accuracy. A scarcity of large, balanced, annotated microbial datasets—particularly for rare pathogens—further constrains model training, compounded by the high cost of expert annotation for microbial images and spectra [104]. Other limitations include model overfitting to lab-specific conditions in the absence of robust domain adaptation and the computational demands required to process high-resolution volumetric CLSM or LSFM datasets in real time [105].

Promising pathways forward include federated learning frameworks that enable training across institutions without sharing raw data—thus preserving privacy while increasing dataset diversity—cross-modality augmentation that integrates phase-contrast, fluorescence, and spectroscopic data, and the development of lightweight embedded AI models for portable Raman and FTIR units suited to field diagnostics in low-resource settings [93, 103]. Self-supervised learning represents another strategy to reduce reliance on labeled datasets. In terms of future timelines, short-term (≤5 years) priorities include integration of AI directly into instruments such as Raman, FTIR, and DHM systems, achieving robust cross-lab generalization, and enabling real-time biofilm morphometry [106, 107]. Mid-term goals (5–10 years) foresee clinical-grade AI capable of directly detecting resistance phenotypes from culture plates or portable optical biosensors [106, 107]. Long-term projections (>15 years) envision autonomous microbial monitoring stations that combine AI, multimodal imaging, and automated decision-making for remote or space habitats [106, 107].

7.2. Quantum sensing in microbiology

Quantum sensing leverages quantum-mechanical properties of matter—such as the spin-dependent fluorescence of nitrogen-vacancy (NV) centers in diamond and the size-dependent optical and electronic properties of quantum dots (QDs)—to achieve ultra-sensitive detection of environmental parameters at the nanoscale. NV centers function as atom-scale magneto-optical sensors: when illuminated with green light (~532 nm) and driven by microwaves, their spin states modulate red fluorescence in ways that depend on local magnetic and electric fields [108]. This quantum readout enables the detection of magnetic noise, temperature variations with millikelvin sensitivity, and subtle chemical changes at nanometer-scale resolution. QDs, on the other hand, provide tunable photoluminescence properties that can be engineered for nanoscale biochemical sensing via targeted conjugation [109].

In microbiology, such quantum tools have been applied to single-cell intracellular ion mapping—including pH, K^+, and Zn^{2+} gradients—within bacterial biofilms, as well as the quantification of reactive oxygen species (ROS) in *P. aeruginosa* under oxidative or antibiotic stress [110, 111]. They have been used to track enzyme secretion rates from bacteria encapsulated in microdroplet bioreactors, and QDs conjugated to nutrient analogs have enabled monitoring of nutrient uptake kinetics in nitrogen-fixing rhizobacteria during plant–microbe interaction studies [110, 111].

These capabilities can be integrated synergistically with other analytical modalities. For instance, combining Raman spectroscopy with quantum sensing allows correlative vibrational and magnetic-field mapping to link structure with function [112]. Pairing FTIR spectroscopy with NV sensing enables simultaneous nanoscale refractive index mapping and chemical fingerprinting to detect localized biochemical changes. Moreover, quantum sensing can work in parallel with AI pipelines to denoise signals and identify subtle dynamical changes in quantum time-series data [112].

Despite its promise, the current implementation of quantum sensing faces several technical bottlenecks. The fabrication of NV-diamond probes and the setup of quantum readout optics and electronics require high costs and specialized expertise [113]. Existing systems often depend on bulky, vibration-isolated platforms with carefully aligned microwave and optical components, limiting portability [113]. Sensitivity can degrade when transitioning from cryogenic, carefully shielded laboratory setups to ambient environmental or field conditions, and throughput remains a limitation for screening large microbial populations [113].

Future directions aim to address these challenges through on-chip photonic integration to miniaturize both optical readout and microwave control hardware, mass-production of nanodiamond sensors with reproducibly uniform NV distributions, and the development of hybrid quantum–plasmonic architectures to boost sensor response. Portable NV sensing modules with wireless data links could bring these systems into real-world and field applications. In terms of projected timelines, short-term goals (≤5 years) include laboratory-based NV sensing of microbial ionic and ROS dynamics and QD-based metabolic tracking in model microbial microcosms [114]. In the mid-term (5–10 years), pilot portable quantum biosensors may enable real-time monitoring of pathogen stress states in soil and water microbiomes [114]. In the long term (>15 years), a vision emerges for global microbial biosurveillance systems integrating AI analytics with ruggedized, space-deployable quantum photonic sensors for Earth and extraterrestrial habitats [114].

7.3. Space microbiology and biophotonics

Extreme terrestrial analogues such as the Atacama Desert and McMurdo Dry Valleys host microbes with pigment-based UV tolerance, diverse metabolic pathways, and rock-pore colonization—adaptations relevant to survival in extraterrestrial environments [115, 116]. Studying these extremophiles informs the search for alien biosignatures and evaluates biocontamination risks in planetary missions. Space microbiology employs advanced photonic tools to investigate these organisms: hyperspectral imaging characterizes cyanobacteria and cryptic communities via pigment spectra (chlorophyll *a*, carotenoids, scytonemin); fiber-optic fluorescence probes monitor *Deinococcus radiodurans* viability under simulated Martian UV [116, 117]; compact Raman spectrometers identify EPS composition in biofilms grown on regolith simulants; and LiDAR supports remote mapping of algal and microbial mats in polar sites. Portable plasmonic and fiber-optic biosensors also track microbial activity in International Space Station (ISS) environmental systems [115–117].

Deploying photonic systems in extraterrestrial or analog environments requires addressing significant engineering challenges. Radiation-induced darkening of silica fibers reduces optical throughput over long missions, while cosmic ray and solar proton exposure can impair detector sensitivity [118, 119]. Severe thermal cycling may cause optomechanical misalignment, and abrasive Martian dust can damage lenses and coatings. Limited communication bandwidth further necessitates onboard data preprocessing [118, 119]. Solutions include radiation-hardened fibers (e.g., doped

fused silica to limit color center formation), athermal designs using materials with matched thermal expansion, abrasion-resistant anti-static coatings, and AI-driven onboard compression and analysis to minimize data transfer [119, 120].

These technologies can be seamlessly integrated with other microbial detection and analysis techniques. Ruggedized Raman and FTIR micro-spectrometers can be used for in situ mineral–microbe biosignature mapping, while fiber-optic plasmonic biosensors can be adapted for real-time monitoring of microbial populations in closed-loop life support systems [121]. Coupling these sensing platforms with AI models enables real-time anomaly detection and classification within large, complex environmental optical datasets [121].

Looking ahead, short-term goals (≤5 years) include conducting Mars-analog field expeditions equipped with portable optical microbiology toolkits to refine in situ detection protocols [122]. In the mid-term (5–10 years), photonic microbial sensors are expected to be deployed on the ISS or lunar outposts for operational testing. In the long term (>15–20 years), autonomous, multimodal planetary biosurveillance arrays—integrating AI, quantum sensing, and micromanipulation tools—could be developed for extraterrestrial life detection and planetary habitat biosecurity [122].

8. Challenges and Opportunities

Current optical techniques for microbial research face six fundamental limitations that constrain their effectiveness: (1) Light scattering in dense biological samples like biofilms and soil matrices restricts practical imaging depth to approximately 100 μm, obscuring deeper structures [123, 124]. (2) Phototoxicity from intense illumination damages live cells during prolonged imaging, while photobleaching degrades fluorescent signals—challenges only partially mitigated by techniques like light sheet microscopy [125, 126]. (3) Throughput limitations arise in super-resolution methods (PALM/STORM) requiring thousands of frames for reconstruction [127, 128] and in hyperspectral Raman imaging where weak signals prolong acquisition times [129, 130]. (4) Fluorescence labeling, though widely used, can alter cellular phenotypes and proves particularly difficult to implement in live bacteria due to cell-wall permeability issues [131]. (5) The substantial costs of advanced systems (e.g., super-resolution, quantum sensing) and the need for specialized expertise create accessibility barriers [129, 131]. (6) AI-assisted imaging tools frequently lack generalizability across different experimental settings and institutions [128–131].

These challenges drive the development of next-generation solutions emphasizing deeper tissue penetration, reduced photodamage/phototoxicity, reduced scattering, higher throughput, label-free operation, cost reduction, and more robust analytical methods. AI integration enables automated microbial analysis and improved model generalizability, while open data repositories enhance reproducibility. Translational applications can bridge lab innovations with clinical solutions using cost-effective photonics.

9. Conclusion

Photonics and optics are enabling the study of previously inaccessible, unculturable microorganisms by breaking the diffraction barrier, enabling label-free chemical analysis and precise manipulation at the single-cell level. These capabilities are driving a fundamental shift toward real-time, in situ investigations of microbial physiology, interactions, and ecological functions. These benefits are further enhanced by the integration of AI, quantum sensing, and extraterrestrial studies. However, several challenges remain, including excessive light scattering, phototoxicity, and limited throughput. These challenges will lead to next-generation optical tools for practical advances in environmental monitoring, medical diagnostics, and biotechnological applications.

Ethical Statement

This study does not contain any studies with human or animal subjects performed by any of the authors.

Conflicts of Interest

The authors declare that they have no conflicts of interest to this work.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Author Contribution Statement

Abdul Rafay Rafiq: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration. **Muhammad Abdullah Farooq:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Hamza Tariq:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Ahsan Raza:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Zeeshan Zafar Iqbal:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization.

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