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PERSPECTIVE

Mobile Technologies for the Discovery, Analysis, and Engineering of the Global Microbiome

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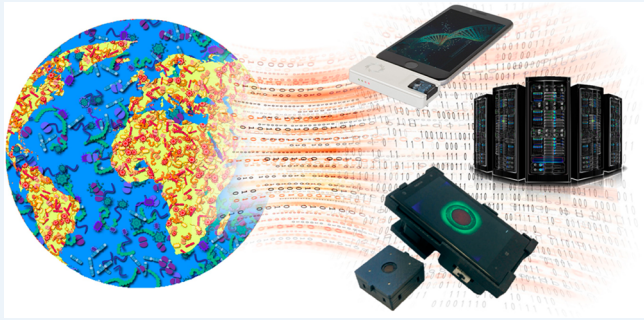
ABSTRACT: **The microbiome has been heralded as a gauge of and contributor to both human health and environmental conditions. Current challenges in probing, engineering, and harnessing the microbiome stem from its microscopic and nanoscopic nature, diversity and complexity of interactions among its members and hosts, as well as the spatiotemporal sampling and *in situ* measurement limitations induced by the restricted capabilities and norm of existing technologies, leaving some of the constituents of the microbiome unknown. To facilitate significant progress in the microbiome field, deeper understanding of the constituents' individual behavior, interactions with others, and biodiversity are needed. Also crucial is the generation of multimodal data from a variety of subjects and environments over time. Mobile imaging and sensing technologies, particularly through smartphone-based platforms, can potentially meet some of these needs in field-portable, cost-effective, and massively scalable manners by circumventing the need for bulky, expensive instrumentation. In this Perspective, we outline how mobile sensing and imaging technologies could lead the way to unprecedented insight into the microbiome, potentially shedding light on various microbiome-related mysteries of today, including the composition and function of human, animal, plant, and environmental microbiomes. Finally, we conclude with a look at the future, propose a computational microbiome engineering and optimization framework, and discuss its potential impact and applications.**



Microorganisms and viruses play profound roles around the globe. Bacteria, archaea, protists, and viruses inhabit the ocean, the soil, the air, and even our infrastructure, homes, and bodies, all together comprising the "global microbiome". Although estimates have varied, it has been reported that within the human body, the number of non-human cells is on the order of the number of human cells.[1,2] These microbes live on our skin, in our mouth, and in our gut, forming mysterious and complex ecosystems. Comprising upwards of 10,000 different species of bacteria,[3] with 500 or more distinct species in the gut alone, the composition of the human microbiome varies wildly among individuals, over time, and at different locations of the body.[4] The many microbiota of the natural environment are even more expansive, with an estimated 160 different species of bacteria in 1 mL of ocean water, and between 6,400 and 38,000 species per gram of soil.[5] The "built" environment, positioned at the nexus of the human and environmental microbiomes, is another unique landscape with specific microbial compositions, functions, and cultivation.[6] Because many of these bacteria cannot be cultured using traditional means, they have been essentially invisible until now, comprising what has been called microbial "dark matter". Only with the advent of DNA sequencing technology has the scientific community begun to appreciate fully the vastness of these microbial communities. Therefore, with this newfound knowledge and aided by emerging technology, we are tasked with discovery. What unique roles do these microbes play in our global health and the environment? How can we best monitor, facilitate, and cultivate or engineer a healthy microbiome and maintain it?

Already, dysfunction of the gut microbiome has been correlated with a number of disorders related to the digestive tract, such as Crohn's disease, irritable bowel syndrome (IBS), and ulcerative colitis.[7−10] Obesity has similarly come to be viewed through the optics of the microbiome. For example, a recent study reported that during a randomized, controlled, fiber and whole grain diet intervention, the ratio of *Prevotella* to *Bacteroides* bacteria in the participants' gut was found to be an important biomarker associated with loss of body fat.[11] Many other conditions outside the digestive system have also been linked to changes or dysbiosis of the microbiome. Patients with multiple sclerosis, allergies, acne, diabetes, and even cancer have

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been shown to possess microbiomes with different makeups than those of healthy individuals, painting an intricate picture of how microorganisms may influence, or be gauges of our health, beyond that of well-characterized pathogenic infections.[12−18] There is even evidence that the gut microbiome influences neural function through the so-called "gut−brain axis" and, by extension, our mental health, encompassing stress, anxiety, and mood as "microbial symptoms".[19−22] Even if microbes are not the sole cause of these disorders, the fact that such disorders and their progression impact the population distribution of microbes could pave the way for early detection of and improved treatments for various diseases through the analysis and engineering of the microbiome. In addition, many external factors have been shown to alter the makeup of a person's microbiome, such as age, geographic location, and a myriad of lifestyle choices.[23−28]

Parallel to investigations on the impact of the microbiome on human health, environmental health is also undergoing a period of microbiome-related discoveries.[29−34] Characterization of the microbiome within the permafrost, soil, ocean, and air are being performed to gain insight into the relationship between human health and our environment, as well as the effects of global warming and other anthropogenic disturbances.[35−44] Taken together, these ongoing discoveries of the microbiome will be important for the future of global health and environmental studies, as emphasized by the *U.S. National Microbiome Initiative* launched in 2016 by the White House Office of Science and Technology Policy (OSTP), which aims to advance microbiome science to benefit individuals, communities, and the planet.[45] Therefore, the technologies that enable these studies at a global scale will set the pace for transformative progress in our understanding and engineering of the global microbiome.

Here, we highlight some of the emerging mobile technologies that, enabled by the economies of scale accompanying consumer electronics (*e.g.*, mobile phones), provide highly sensitive, yet cost-effective sensing and microscopy tools that uniquely complement high-end laboratory equipment.[46,47] In addition, these mobile technologies can enable distributed data collection in a wide range of field settings as well as spatiotemporal mapping of the microbiome, leading to a wealth of global health information. Once scaled-up and made available to consumers, such technologies can help us dynamically monitor the state of a given microbiome and open up ways to probe the efficacy of therapies that target and engineer microbiome health and state. Through such mobile technologies, remote studies with extensive automated sensor networks can be more easily realized. The environmental sample processor (ESP), developed at the Monterey Aquarium Research Institute, is a good example of such an automated microbial monitoring system; it was designed for submersible exploration of ocean waters and can provide an early warning system for the development of harmful algae, or identification of negative effects of wastewater among other pollutants.[42−44,48] Mobile technologies such as the ESP, therefore, can facilitate "big data" solutions through coordinated, high-throughput, potentially crowd-sourced measurement efforts. If largely accessible, such technologies can even engage "citizen scientists" to contribute to meaningful microbial discoveries akin to the way amateur astronomers collectively observe the sky. However, most important is the imperative to democratize these measurement and analysis tools for improving global health, such that the benefit of discovery is wide-reaching and impactful.

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In this Perspective, we first discuss mobile sensing technologies that can aid in discovering the global microbiome, with a specific focus on DNA-sequencing technologies, DNA amplification, and quantification, along with the emerging molecular-sensing technologies, which can be used for measuring proteins and metabolites of a microbiome. Second, we discuss mobile imaging technologies. Here, we pay special attention to fluorescence read out, along with computational imaging techniques. Finally, we look into the future and propose a computational microbiome engineering and optimization framework that can be used to define and to quantify the state of a given microbiome and to transform it gradually into desired states in a controlled manner. Ultimately, such a framework can help us leverage the inherent measurement parameter space provided by cost-effective and mobile technologies to garner and to engineer meaningful health outcomes.

MOBILE SENSING TECHNOLOGIES FOR THE MICROBIOME

DNA Sequencing. High-throughput DNA sequencing, termed next-generation sequencing (NGS), can be considered the workhorse of microbiome discovery. Advances in sequencing technologies over the past two decades have dramatically lowered the cost per base pair, by nearly 6 orders of magnitude, heralding unprecedented flow of genomic data into the research community.[49−51] The Illumina HiSeq sequencing machines, for example, can process multiple samples in parallel, each collected from a unique microbial environment, sequencing the metagenome of the entire microbial ecosystem in 1 day. Such "snapshots" of the microbiome, coupled with relevant clinical data, have led to many revelatory findings.[14,52−55] For example, it has been shown that exposure to diverse microorganisms at an early age can reduce instances of asthma and other allergies, and that the vaginal microbiome becomes less diverse in preparation for childbirth.[55,56] In addition, maladaptation of the nominal microbiome composition of the stomach has been associated with carcinogenesis.[13] Such discoveries will have profound effects on global health, and have recently been bolstered by research initiatives such as the Unified Microbiome Initiative, the Human Microbiome Project, and the Earth Microbiome Project, among others.[49,57−59]

Next-generation sequencing technologies will continue to be foundational to these efforts, and have made significant strides in terms of proliferating into research laboratories. However, in their present form, these NGS devices are expensive and have bulky benchtop instrumentation, relegated to high-resource facilities. Illumina's NGS approach, called sequencing by synthesis, requires DNA to be fragmented into millions of strands, each around 150 bases in length, whereupon they are immobilized within a flow cell. A bridge amplification

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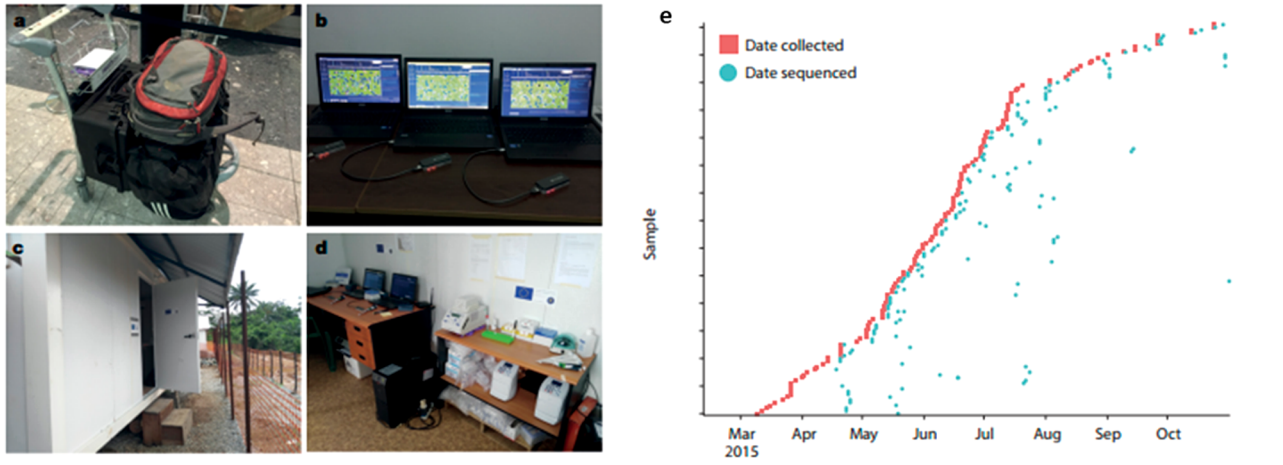

Figure 1. (a) All sequencing instruments and consumables packed into conventional luggage. (b–d) Genomic surveillance laboratory in Donka Hospital in Conakry, Guinea, and (e) collected and sequenced samples plotted as a function of time, showcasing high-throughput sequencing capabilities used for Ebola surveillance. Reprinted with permission from ref 63. Copyright 2016 Macmillan Publishers Limited.

procedure is then performed, using polymerase enzymes to copy the DNA fragments over many cycles. The actual sequencing is then performed, in which fluorescently tagged nucleotides bond to the fragmented single-stranded DNA and emit a wavelength-encoded fluorescence signal indicating the synthesized sequence. This process, known as "shotgun" sequencing, is massively parallelized, producing gigabytes of data that must then be processed to uncover the comprehensive sequence made up of the random fragments. As a result of this intricate and multistep approach, NGS technologies, despite significant progress, are still not cost-effective enough for ubiquitous use. The high research and development costs, specialized hardware required, such as high-end cameras and scanning components, as well as the need for relatively large volumes of costly reagents, are some of the areas for improvement as sequencing technologies evolve. In addition, eliminating the requirement for external laboratory equipment, trained personnel, and excess handling time will be key steps in moving toward democratization of this technology and its widespread use in microbiome analysis.

Despite the challenging prospect of sequencing DNA on a low-cost mobile platform, efforts toward this goal are vital to discovering the global microbiome, even if they come at the cost of shorter sequencing depths or lower throughput, among other trade-offs. For example, when using shotgun sequencing methods, as few as 20 base pairs can be sufficient to identify a micro-organism against the background ecosystem.[60] To this end, there are additional emerging sequencing techniques that offer practical advantages, which might lead to mobile implementations. For example, the Ion Torrent Personal Genome Machine (PGM) DNA sequencer uses scalable semiconductor technology to measure protons released by nucleotides as they bond to the target DNA, eliminating the need for fluorescence optics, high-end cameras, and scanning components.[51,61] Nanopore sequencing is another emerging technique, where single DNA strands are passed through a protein nanopore or an artificial nanopore while the electric current across the pore is continuously measured. The signal can be matched to the passing A-C-T-G nucleotides over long read lengths (>5 kbp) and at fast speeds (1 bp/ns) without the need for fluorescent read-out or a DNA amplification step. The MinION sequencer from Oxford Nanopore, for example, is a recently developed nanopore sequencer, which is roughly the size of a deck of cards, weighs 100 g, and can be entirely powered *via* a USB connection to a laptop. Although yielding higher error rates than the high-end NGS technologies, recent studies have successfully utilized the MinION sequencer in remote and challenging environments such as the Ecuadorian Choco Rainforest for the analyses of rare endemic fauna, and in remote hospitals in Guinea for genomic surveillance of the Ebola virus and its mutations (Figure 1).[62,63] Pushing the envelope further, Oxford Nanopore has begun the development of an even smaller, matchbox-sized, sequencer, the SmidgION, which will connect directly to a smartphone for field-based analysis.[64] Innovations such as these can build upon the progress made thus far to democratize DNA sequencing. Taken together, this "third generation" sequencing technology can open vast opportunities for microbiome analysis and discovery by leveraging mobility, robustness, affordability, and, in turn, ubiquity. Along with a genomic database curated by the greater research community, such mobile sequencing technologies could pinpoint characteristic aspects of the microbiome and, as a network, greatly expand our statistical, spatial, and temporal foundation for microbiome discovery and engineering.

Polymerase Chain Reaction and Microbiome Quantification. In addition to exciting developments in DNA sequencing using low-cost and mobile systems, sensing technologies that amplify, identify, or quantify specific DNA sequences are also being pushed to deliver mobile and cost-effective implementations. Polymerase chain reaction (PCR) is an important example of a ubiquitous DNA sensing technology that has a unique role to play in exploring the microbiome, and has recently been shown to be effective in a mobile capacity. During PCR, a DNA sample, in the presence of a PCR nucleotide mixture, is first heated to temperatures >90 °C such that the double stranded structure can "melt", or denature, leaving two complementary single strands of DNA. Next, the sample is cooled to a characteristic annealing temperature (usually around 60 °C) to enable hybridization between the single-stranded DNA and the chosen 'primer,' which consists of a sequence complementary to the target DNA. Lastly, by bringing the sample to a third characteristic temperature zone (*e.g.*, between 75 and 80 °C for Taq polymerase), the newly hybridized DNA is then elongated by polymerase enzymes, filling in the remaining sequence not complemented by the primer. This thermal cycle is repeated dozens of times,

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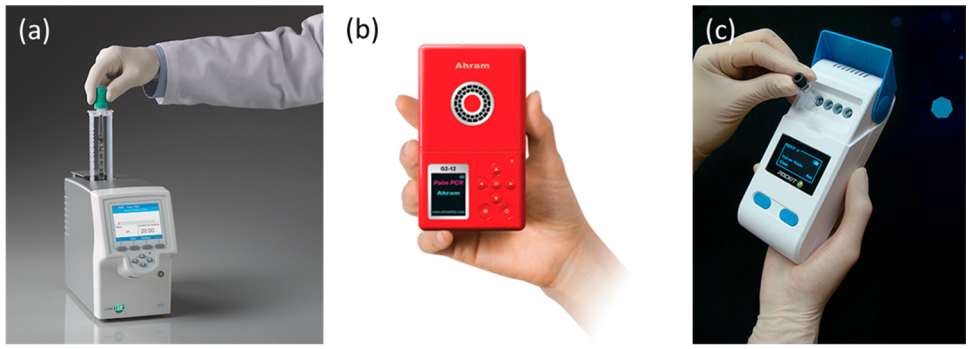

Figure 2. (a) cobas Liat polymerase chain reaction (PCR) system. Reprinted with permission from ref 67. Copyright 2018 Roche Diagnostics. (b) Palm PCR. Reprinted with permission from ref 68. Copyright 2017 Ahram Biosystems, Inc. (c) POCKIT nucleic analyzer (micro series). Reprinted with permission from ref 69. Copyright 2018 GeneReach Biotechnology Corp.

synthesizing millions of copies of the DNA sequence targeted by the primer. Thus, PCR can be used to amplify trace amounts of a specific microbial DNA sequence against a complex and varied genomic or metagenomic background, and is a powerful tool for the identification of harmful pathogens or other microbes of interest, among others. In addition, the inclusion of fluorophores, intercalated with amplified DNA strands, enables real-time quantification of the amplified sequence through the use of fluorescence imaging or sensing tools, resulting in quantitative PCR (qPCR).[65] Digital PCR is another extension of this technique, compartmentalizing the reaction to thousands of "on" or "off" signals, which, taken together, can yield more sensitive and robust quantification, without the use of standard calibration curves. Digital PCR provides exciting opportunities toward developing mobile technologies for microbiome analysis and, in fact, is already being used in the food industry to screen samples for *E. coli*, *Listeria*, *Campylobacter*, and *Salmonella*.[66]

One example of a mobile PCR platform is the cobas Liat PCR System developed by Roche Diagnostics, which was approved by the U.S. Food and Drug and Drug Administration (FDA) in 2015 (Figure 2a).[70] It is about the size of a small toaster, and can perform qPCR with sensitivities as high as 99.2% and with 100% for influenza A/B and *Streptococcus* specific DNA strands, respectively, as well as 100% specificity for both. This performance is far beyond that of the traditionally employed antigen-based rapid diagnostic tests (RDTs).[71] With integrated microfluidics for automated sample and reagent handling and convenient reagent loading strips, the cobas Liat PCR system requires ~5 min of hands-on time, and ~20 min of processing time until results are reported. Other examples of commercial PCR devices, marketed as highly sensitive, cost-effective, easy-to-use instruments include the Palm PCR by Ahram, and the POCKIT Nucleic Analyzer from Genereach (Figure 2).[72] These technologies realize DNA amplification in a mobile platform, providing powerful tools for discovering the global microbiome. As the scientific community learns more about the functions and the diversity of the human microbiome, diagnostic tests will emerge that perhaps require the quantification of statistically significant ratios of microbial DNA, or even characteristic relationships among a panel of microbes.[73] In fact, multiplexed PCR assays for the quantification of eight key bacteria have already been developed for the diagnosis of lower respiratory tract infections.[74] Screening microbial communities for pathogenic members will also advance some of the existing microbiome-centered treatments, such as fecal transplants. Mobile and cost-effective PCR technologies will therefore be vital for democratizing diagnosis and capitalizing on the knowledge gained from the microbiome. As we detail in the final section of this Perspective, these capabilities will also help us to quantify various states of the microbiome of interest using a panel of measurements, which might form the basis of an optimization framework to engineer and to probe microbiomes for specific outcomes.

Mobile and cost-effective PCR technologies will therefore be vital for democratizing diagnosis and capitalizing on the knowledge gained from the microbiome.

Toward the broad goal of low-cost and mobile DNA detection and quantification, several emerging technologies show promise in providing practical alternatives to the currently employed PCR-based techniques. For example, the thermocyclers typically used in PCR require relatively large amounts of power to achieve the desired heating and cooling speeds. One alternative approach has been to engineer a spatial domain PCR cycler, where the sample solution is physically moved to different thermal regions using fluidics or simple servos.[75,76] The microfluidics, fabricated as low-cost cartridges, can be carefully designed to minimize reagent use (to sub-picoliter levels), optimize heat exchange, and even automate sample pretreatment steps.[77] Polymerase chain reaction with isothermal operation is another attractive emerging technology. Eliminating the need for active heating and cooling of the sample, Priye *et al.* demonstrated isothermal PCR by utilizing a single localized heater, which induced the proper convection flow in an open chamber in order to cycle DNA in and out of the appropriate temperature zones.[78,79] This design, while employing complex fluid dynamics, provides a viable, cost-effective alternative to traditional thermocyclers, employing standard USB power, and a compact form factor, fitting onto a commercial drone.

Isothermal operation for DNA amplification can also be achieved by changing the underlying amplification chemistry. Techniques such as nucleic acid sequence-based amplification (NASBA), helicase-dependent amplification (HDA), rolling circle amplification (RCA), and strand displacement amplification (SDA) have demonstrated isothermal operation conducive to compact, battery-powered microbial DNA-sensing devices, forgoing the need for thermo-cycles altogether.[80,81] As another alternative, loop-mediated isothermal amplification (LAMP)

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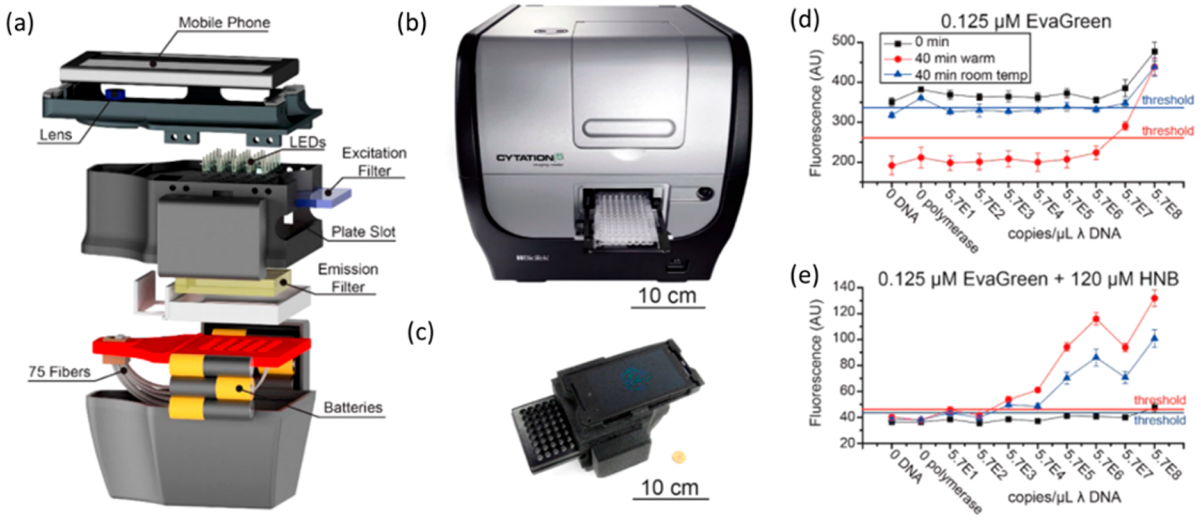

Figure 3. (a) Schematic of the mobile-phone based fluorescent well-plate reader for DNA quantification. (b) Commercial fluorescent well-plate reader alongside a (c) mobile-phone-based reader for comparison. (d) Fluorescent signal *versus* DNA concentration with EvaGreen intercalating dye only (c) and with hydroxynaphthol blue (HNB) indicator added to the EvaGreen intercalating dye. Reprinted from ref 84. Copyright 2017 American Chemical Society.

has emerged as a robust and promising isothermal amplification technique. In LAMP, DNA amplification is achieved at a single temperature ~60−65 °C through the use of several strand-displacing primers that self-hybridize, forming "loops" on their 3′ and 5′ ends, triggering an autocycling process, which, in turn, synthesizes millions of copies of double-stranded DNA at a constant temperature. Recent work has showcased the advantages of isothermal operation of LAMP, by demonstrating paper-based point-of-care tests.[82,83] In these works, the reagent handling is minimal, and the cost per assay is reported to be as low as $1.83. Connelly *et al* even demonstrated end-point read out with a hand-held UV source and a mobile phone, used to excite and to read the SYBR Green I intercalating dye, respectively, ultimately demonstrating a 500 cells/mL limit of detection for the *malB* gene of *E. coli*.

In addition, Kong *et al.* demonstrated lambda DNA quantification by utilizing LAMP in a simple "one-pot" assay, reading the fluorescence signal from intercalating dyes with a mobile phone-based well-plate reader (Figure 3).[84] They also reported that introducing Hydroxynaphthol blue (HNB), a commonly used colorimetric indicator, into the fluorescent LAMP assay can lead to a 20-fold increase in the fluorescence signal against the background, enabling a limit of detection as low as 25 copies/μL. With further advances in quantitative read-out schemes alongside the development of LAMP primers for microbes of interest, this type of mobile technology can bring ultrasensitive and multiplexed measurements to low-resource settings, including, for example, the home. Moreover, in addition to fluorophores as the status quo reporter, DNA amplification protocols that can leverage simple colorimetric indicator dyes, or turbidity-based tests for simpler and cheaper read-out instrumentation, should also be considered.

Proteomics and Metabolomics. Sensing and quantification of proteins and metabolites are also essential to microbiome discovery and analysis. These biomolecules carry out the microbiome function, and thus are vital to the life of the microbial community and, in turn, that of its host or environment. DNA sequencing of the microbiome is a powerful surveying tool; however, it is inadequate for providing functional information about specific pathways, interactions, and behavior. Therefore, mobile technologies that enable multiplexed proteomics or metabolomics testing will be invaluable tools alongside deep-sequencing technologies to uncover microbiome functions, operations, and overall health. Already, several multiomic studies have illuminated the intricate workings of the microbiota, their proteins, and metabolites. For example, colon cells use butyrate produced from the colon microbiome as an energy source, in order to help catalyze important steps in the metabolic and Krebs cycles.[85] The oceanic microbiome has even been documented to adapt to oil plumes, such as that created by the Deepwater Horizon oil spill in 2010,[86−88] through increased microorganism motility, chemotaxis, and hydrocarbon degradation *via* an alkane degradation pathway.[31]

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Even with a limited number of proteomic and metabolomic measurements, high-throughput, cost-effective, and mobile imaging and sensing technologies can populate a measurement parameter space in a massively parallel way. This parameter space can be used to quantify and to evaluate the underlying microbiome state and, in turn, indicate how best to perturb the given inputs to the complex microbiome network (*e.g.*, drugs, nutrition, *etc.*) to achieve a desired outcome. This iterative optimization framework, discussed in the final section of this Perspective, will largely benefit from mobile technologies that can measure and monitor the proteome and metabolome of the global microbiota, among other multiplexed information channels.

In addition, as the larger function and diversity of the microbiome are uncovered, novel diagnostic tests will emerge

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that rely on sensing the proteins or metabolites that are produced or consumed by, or otherwise interact with, the microbiota. Mobile technologies provide alternate tools for these indirect measurements and, therefore, play a role in democratizing diagnosis. One example of such a diagnostic test, the "*Helicobater pylori* (*H. pylori*) breath test" is commonly employed in hospitals to measure excessive *H. pylori* presence in the human gut. CO_2 is released by *H. pylori* and subsequently measured in the patient's breath.[89] This microorganism is commonly found in the human gut, but a large presence of *H. pylori* has been correlated with peptic ulcer disease, chronic heartburn, nausea, bloating, and even gastric adenocarcinomas. Therefore, quantifying *H. pylori* is essential knowledge for doctors when deciding a diagnosis and treatment. Of course, a fecal sample could be taken for genomic analysis, but CO_2 can be more readily measured, and therefore exists as a practical diagnostic biomarker. Taken together, any technology that can specifically lock onto a biomarker or a panel of biomarkers of the underlying microbiota state can be leveraged for better diagnostics as well as microbiome discovery and analysis.

It is important to note that proteomic and metabolomic technologies have existed for decades, employing a wide array of sensing and amplification strategies. In this Perspective, we do not attempt a comprehensive assessment of all of the progress made in this vital field. Instead, we highlight a few recently developed sensing technologies that show promise in uncovering the omics information pipeline. For instance, one recently developed technique, homogeneous entropy-driven biomolecular assay (HEBA), uses existing DNA amplification mechanisms as a means to transduce and to amplify a specific protein signal.[90] Through the inclusion of two catalyst-precursor oligonucleotides that are modified to have specific protein-binding elements, an entropy-driven DNA displacement assay was shown to create an amplified DNA signal proportional to the targeted protein concentration. Such an assay can be performed in a "one-pot" operation within 10 min, without the need for precise thermal control or cycling, and, therefore, is well-suited for point-of-care use. Thus, HEBA can leverage the DNA amplification and quantification technologies currently undergoing maturation for sequencing applications, while being broadly applicable to a number of proteins related to the microbiome. Such assays can be performed in a well-plate configuration, in line with traditionally employed high-throughput assays, as discussed below.

Well-plate-based tests/assays have long been a cornerstone of clinical microbiology and life sciences. They offer relatively high sensitivities using high-throughput and simple optical readout, and are therefore ideal for various applications such as antimicrobial susceptibility testing (AST), and colorimetric and fluorescent enzyme-linked immunosorbent assays (ELISAs). For example, AST is used to quantify a microbe's susceptibility to a range of antibiotics. A well-plate is used so that many drugs at different concentrations can be tested in parallel, enabling physicians to identify and to prescribe precise treatments that effectively target the pathogen with the minimum inhibitory concentration of the right antibiotic, helping to limit the growth of resistant strains. As another example, ELISA is one of the most common methods of protein sensing, leveraging the specificity of antigen–antibody interactions to quantify an analyte. However, despite the ubiquity of such microplate-based assays like AST and ELISA, they are largely relegated to well-equipped laboratories and clinics due to their reliance on costly reagents as well as bulky and expensive benchtop plate readers. In an effort to democratize this molecular-sensing technology, researchers have been working to make ELISA and its corresponding readout instrumentation mobile and cost-effective. Berg *et al.* demonstrated a mobile-phone-based ELISA reader, utilizing low-cost plastic optical fibers to sample each of the 96 wells on a conventional ELISA well-plate without the need for any mechanical scanning or large field-of-view optics.[84,91] All the fibers are bundled together and imaged through the use of a simple external lens and the smartphone camera. A similar design, but with fluorescent read-out capabilities, can be seen in Figure 3. Of course, reagents and their necessary storage is a drawback to such assays, along with the need to have trained personnel to pipet the substrates and wash buffers. To address these drawbacks, researchers are exploring inkjet-printing technologies to print reagents in microarrays, in an effort to store them on-chip without the need for refrigeration, among other approaches.[92]

Another strategy for reducing the reagent burden is to design label-free sensors. Without the need for additional steps after sample collection, such as fluorescent tagging and pipetting of the substrate or stop solution, label-free sensors can report molecular information at low cost and with simplified operation. Lateral flow assays, and paper-based antigen or antibody tests are perfect examples of such mobile sensing technologies; however, by and large, these tests come with severe limits in terms of sensitivity and specificity. One method of increasing the sensitivity in a label-free modality is through the use of surface-plasmon resonance. Surface plasmons, or the general field of plasmonics, are based on resonance phenomena, whereupon light, at a given resonant wavelength, excites collective electron oscillations at a metal–dielectric interface. These electron oscillations manifest as spectral features in the transmission and/or reflection spectra of the surface, responding to the presence of analytes at the sensor surface *via* resonant frequency changes. With the proper surface chemistry, plasmonics-based sensing elements can specifically capture a biomolecule of interest using antibody–antigen binding. Such elements can also be used to amplify Raman scattering (through surface-enhanced Raman scattering, SERS), fluorescence, or work in highly sensitive aggregation or nanoparticle degradation assays.[93−96] Plasmonic sensors have been used for direct detection of pathogenic microorganisms such as *E. coli*, *Salmonella* spp., *Listeria monocytogenes*, *Campylobacter jejuni*, and *Staphylococcus aureus*, among others, as well as nonpathogenic species such as *Bacillus subtilis* and *Helicobacter pylori*.[97,98] In addition, plasmonic techniques have been used for concentration measurements for a number of different large molecule biomarkers and even for viral load measurements in whole, unprocessed blood.[99,100] Combined with enzymatic reactions or responsive hydrogels, plasmonic sensors have demonstrated small molecule sensing of glucose and gas concentrations.[101−103] Surface-enhanced Raman scattering shows further promise as a technique to differentiate different bacterial species from their spectral signatures, although the weak Raman scattering signal often requires relatively expensive optics for read-out instrumentation.[104,105]

Another example of label-free optical sensors, called "holographic sensors", which work by exploiting interference phenomenon, have also shown promise for a number of microbiome proteins and metabolites, *e.g.*, protease, calcium dipicolinate, and exoenzymes produced during cell growth of *B. megaterium* and *B. subtilis*.[106−108] In addition, by selecting characteristic spectral bands (*e.g.*, employing light-emitting

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diodes, LEDs, or inexpensive laser diodes) and incorporating complementary metal−oxide semiconductor (CMOS) or photodiode technology, sensor readers that register a spectral response can be made cost effectively without sacrificing throughput.[109−111] Taken together, these label-free optical sensors offer a versatile technique for molecular sensing and will be increasingly useful in designing multiplexed mobile proteomics and metabolomics sensors.

These emerging sensing technologies, including plasmonics, will not only have to be demonstrated in proof-of-concept studies performed in laboratory settings, but must overcome sensitivity and specificity problems in the context of real biological and chemical environments, where the sensor fabrication variability[109] and end-user operational variance are serious concerns. In addition, the demand for expanded spatiotemporal resolution for proteomics and metabolomics places an extra burden on these technologies to operate as wearable, implantable, or ingestible sensors, potentially forming distributed sensing networks. Molecular sensors embedded in clothing, or on a wrist watch can physically integrate *in vivo* with the microbiome (*e.g.*, skin microbiome) and record real-time dynamics and responses to stimuli. Electrochemical sensors, for example, have already made headway in this capacity.[112] Recently, simultaneous, real-time sensing of glucose, lactate, sodium, potassium, and temperature was demonstrated in a flexible arm band for sweat analysis.[113] An eyeglasses-based metabolite sensor platform has also been reported.[114] Similarly, several disposable, "smart bandages" have been demonstrated, which are able to measure uric acid and pyocyanin, important metabolites of the microbiome that can act as biomarkers for wound healing status, for example.[115,116] The human gut, however, presents a formidable sensing environment, due to its acidity, darkness, and limited accessibility. Therefore, gut-based sensing technologies must aim toward a capsule-sized form factor, with wireless power and data transfer, as well as full biocompatibility of materials. Toward this goal, exciting preliminary studies by Kim *et al.* have demonstrated food material-based electrochemical sensors for the sensing of metabolites and molecules such as catechol, uric acid, ascorbic acid, dopamine, and acetaminophen.[117] Although not yet integrated with compact, body-friendly, wireless communications, such ingestible sensors could potentially provide an omic snapshot of the gut, at least partially, and prove transformative for microbiome discovery and analysis.

Regardless of the specific omics approach that is selected, quality control is a crucial requirement so that data from different spatiotemporal locations can be meaningfully compared. For example, data collection for the America Gut Project requires samples to be taken by volunteers and sent to a sequencing facility, and is therefore subject to days of travel under varying conditions that may affect the constituent microbes. The process of taking a sample is also vulnerable to variations among human subjects, requiring special sample collection kits and detailed instructions.[179] These sources of variability will need to be addressed in the next generation of large-scale microbiome investigations, perhaps through fully automated sample collection and pretreatment steps that are robust and repeatable even for nonprofessionals' use.

MOBILE IMAGING TECHNOLOGIES FOR THE MICROBIOME

Although the sequencing methods discussed earlier have become the primary tools used for microbiome discovery, optical imaging approaches offer several unique advantages. They can provide visual information about constituent microbes, enabling investigation of the relationships among bacteria and how they exchange resources with one another and with the environment. Because most bacteria cannot be cultured, it is clear that their interactions with other organisms *in situ* are critical for their survival. Imaging also lends itself to mobile, cost-effective, and nondestructive implementations, which could pave the way for democratized research into the microbiome and help us elucidate the complex relationships among microbes in a community by providing functional information (*e.g.*, motility or taxis, trajectories, and viability) *in vivo* and/or *in situ*. The development of ever-improving CMOS image sensor technology and exponentially decreasing computation costs have been the driving forces behind the recent growth in the number of field-portable imaging systems.[46]

Bacteria are typically cocci (round), bacilli (rod-shaped), or spirilla (curved). Their longest dimension can reach 10 μm, while the smallest dimension is often submicron. This means that conventional optical microscopy techniques, with resolutions defined by the diffraction limit, are on the bleeding edge in terms of seeing the microbial world. In other words, most bacteria can be seen or detected, however, they appear similar to each other under a bright-field microscope. Without the use of any labels, resolving characteristic nanoscale features such as flagella, key organelles, or cell wall structures remains a central challenge for optical imaging, requiring a combination of techniques to be employed in order to reveal functional information that is otherwise buried below the diffraction limit. These limitations become even more challenging when one considers the imaging and tracking of viruses within the microbiome, due to their nanoscopic size and the large sample volume that needs to be imaged.

Microscopes are typically designed to balance the required resolution and field of view (FOV) for the desired application. As the magnification and the numerical aperture of an objective lens are increased to enable smaller objects to be resolved, the FOV and the sample volume that are imaged shrink, limiting the number of individual objects that can be imaged at one time. This limited FOV presents a challenge for microbiome analysis because we are interested in microorganisms and viruses that are small and in many cases rare, requiring a large FOV and imaging volume to increase the odds of imaging and tracking the target objects.[3,118]

Next, we discuss a number of techniques, conducive to mobile and ubiquitous implementations, that have been developed to address these central challenges and to improve the utility of optical microscopy for discovery and analysis of the global microbiome.

Computational Microscopy Techniques. As the cost of computation has decreased dramatically,[119] mostly due to economies of scale driven by the consumer electronics industry, it has become feasible to replace bulky, expensive imaging optics with rapid, powerful algorithms employed during postprocessing and image reconstruction. Computation transforms the captured image—which may be noisy, aberrated, out of focus, or otherwise unusable in its captured state—to a result/image that can be better interpreted by the end user. Computation can also be used to design an optical imager or sensor, optimizing the raw data acquisition along with the reconstruction process.[109] Examples of computational imaging techniques that have proven valuable in the field of micro-

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biology include super-resolution,[120,121] light-field microscopy,[122,123] and compressive sensing or sampling.[124−126]

Perhaps one of the computational imaging approaches most adaptable to a mobile system is lens-free on-chip imaging. In lens-free on-chip imaging, a sample is placed directly on top of an imager (typically a CMOS imager chip) and illuminated with partially coherent light.[127,128] This method results in the formation of a characteristic interference pattern, called a hologram, created by the interference between the light scattered from the object and the partially coherent reference wave that is directly transmitted. If the illumination wavelength is known, then the recorded holograms can subsequently be computationally transformed onto the object plane, recovering a focused image of the samples. This approach, therefore, forgoes the need for bulky, expensive lenses or focusing stages, offering a robust and compact platform. It also boasts a large FOV, limited only to the total size of the sensor (typically greater than 10−20 mm^2), enabling much higher throughput than conventional lens-based microscopy. Because of their simple, in-line design, lens-free microscopes are compact and portable. The spatial resolution is tied to the pixel size of the sensor, but can be improved through pixel super-resolution techniques to submicron resolution.[129−131] One common approach involves capturing multiple images, each with unique information, and subsequently employing algorithms to fuse these images together, producing one image of higher resolution than any of the individual images. This higher resolution image can be achieved by capturing images while shifting the object with respect to the image sensor or *vice versa*,[129] varying the wavelength of the illumination light used,[130] or slightly changing the angle of illumination,[131] offering design flexibility for application-specific lens-free microscopes.[132]

One recent example of the significant potential of mobile lens-free imaging demonstrated environmental monitoring through spatiotemporal air-quality measurements.[133] In this work, a battery-powered, mobile pump captured particulate matter in air and deposited it onto a coverslip. Once immobilized, these particulates were rapidly imaged by a lens-free computational microscope. Employing further computation, a machine-learning algorithm discerned true particles from other artifacts, and used spatial features relating to particle geometry to determine their sizes with a high accuracy of ~93%. The device, termed c-Air, was used to gather samples from the neighborhoods adjacent to Los Angeles International Airport (LAX), measuring the effect of air traffic on the concentration of particulate matter as a function of both time and distance from the airport. Such a mobile, accurate system could be adapted to monitor the air microbiota for pathogens[134] or to deepen our understanding of the effect of microbes on climate.[135] It has been demonstrated that biological aerosols can travel across the globe,[136] and even play roles in ice nucleation leading to precipitation.[137] Particle sizing, as implemented in the c-Air platform, could enable the quantification of different strains of bacteria or mold based on geometrical features such as size and shape as well as spectral characteristics, which can be obtained by using multiband illumination.[138] For instance, an investigation into the particle size distribution of the air microbiota in the wake of a dust storm near the Mediterranean Sea found that *Firmicutes* were the most common at large (>3.3 μm) particle sizes, while *Actinobacteria* and *Bacteroidetes* (typical soil/environmental microbes) were dominant at smaller sizes (<3.3 μm).[134] Similar particle sizing approaches could offer further insight into the air microbiome and its effects on health and the environment. Although this type of investigation would not give a comprehensive breakdown of the constituent microbes in a sample, it could be used to track the relative frequencies of the more abundant types in the environmental microbiome, such as the phyla mentioned above, as well as the genera *Arthrobacter* or *Streptococcus*.[139]

As seen with the c-Air, machine learning is another emerging computational framework that can aid microscopy in elucidating the microbiome. Machine learning and, more recently, deep learning have become popular in the realm of image processing and analysis, not only because of the decrease in the cost of computation, but also due to the explosive amount of data that has become readily available from smartphones and digital cameras as well as the increased bandwidth to access the Internet. These changes are critical developments because the efficacy of machine learning for microbiology is predicated on the availability of a large volume of well-characterized training images. The vast quantities of data that could be generated from mobile imaging devices for the microbiome will be ripe for analysis using machine learning. It is also important to emphasize that machine-learning techniques require large computational capital only when algorithms are in training phases. Implementing previously trained algorithms requires only limited computational power, as readily available with smartphones, tablets, or laptop processors. Alternatively, data can be transferred over a network to enable remote servers to perform the computations and report the results. As an example, a portable implementation of machine learning for microbe viability analysis has been demonstrated for yeast cells, using a basic tablet and a lens-free microscope.[140] Such a platform, along with many others,[141,142] demonstrate how machine learning, alongside computational microscopy, can provide insight that cannot be obtained by sequencing approaches alone. Classification of different microorganisms based on their morphological and/or spectral signatures, *i.e.*, without DNA analysis, is therefore another central application of machine learning.[143] In fact, efforts to distinguish bacteria of the bacilli (rod) shape have already been successful, enabling automated diagnosis of tuberculosis using images of stained sputum samples,[144] as well as label-free classification of four different bacilli using quantitative phase imaging and light scattering.[145] A software package has also been developed that uses machine learning to determine bacterial cell boundaries, track cells between frames, and trace cell division events.[146] With the vast amount of data promised by various mobile imaging technologies, machine learning has a unique opportunity to address microbiota characterization, and may even be aided by "gamification" approaches, as has been demonstrated for protein folding[147] and malaria parasite detection,[148−150] among others.

With the vast amount of data promised by various mobile imaging technologies, machine learning has a unique opportunity to address microbiota characterization,

Fluorescence Microscopy. Fluorescence microscopy has been a cornerstone of microbiology research for decades. It

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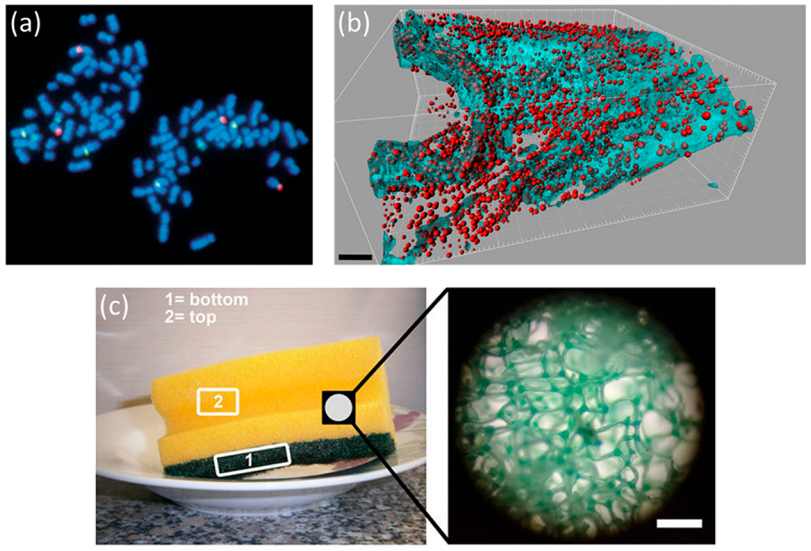

Figure 4. (a) Image captured with fluorescence *in situ* hybridization (FISH)-based microscopy. Reprinted with permission from ref 165. Copyright 2018 ThermoFisher Scientific. The red and green fluorescence indicates the location on the chromosome of specific sequences. Kitchen sponges are imaged (b) using FISH to show the spatial distribution of bacteria, and (c) under a bright-field microscope. Reprinted with permission from ref 163. Copyright 2017 Cardinale *et al.*

enables the imaging of a wide range of target particles, cells, or cellular components with high specificity. In fluorescence microscopy, the target object (*e.g.*, a virus, protein, or metabolite) is "tagged" with fluorescent molecules that emit light when illuminated (*i.e.*, excited), enabling the detection of specific targets even against a complex background. Tagging is performed with a fluorescent dye or quantum dot solution that is added to the sample, eventually binding specifically to the target of interest *via* antibody−antigen interactions or specific oligonucleotides. The specificity of this chemistry, therefore, determines the utility of fluorescence imaging for a given target−tag pair. The tagging process can be performed quickly, and ensures a stable, localized bond that is not disruptive to the target microbes. Furthermore, fluorophores of different emission wavelengths can be multiplexed to enable specific detection of many targets simultaneously.[151] Because of these advantages, fluorescence microscopy has been readily applied to genomics and proteomics as an invaluable tool for microbiome analysis.[152]

Fluorescence microscopy, in step with lens-free imaging, has already been implemented in portable, cost-effective devices for various global health applications.[81,153−157] A bright-field platform can be adapted for fluorescence imaging with the simple incorporation of excitation filters, which control the wavelength of the light responsible for exciting the fluorescent molecules, and emission filters, which transmit only the light emitted from the fluorescent molecules. For example, Koydemir *et al.* demonstrated a smartphone-based fluorescence microscope for *Giardia lamblia* cyst detection and quantification for rapid water quality screening.[157] The low-cost, field-portable fluorescence microscope design incorporates a three-dimensional printed mobile phone attachment that houses LEDs, batteries, a *z*-stage, an external lens, excitation and emission filters, and a sample cassette. A water sample is collected, filtered to remove large particulates, and then combined with a *Giardia lamblia*-specific fluorescent tag (antibodies conjugated with fluorescein dye). The sample collection, preparation, and fluorescence conjugation processes are all "field-portable" and can be implemented at or near the site where the water is being sampled, in less than 1 h. Images of labeled cysts are then captured with the smartphone microscope, sent to a remote server (or a local laptop) for processing using a machine-learning algorithm, and, in less than 2 min, a cyst count is sent back to the mobile phone. With a reported limit of detection of ∼12 cysts per 10 mL, this device demonstrates the utility of mobile fluorescence imaging technologies for quantifying microbial targets in the field, and, in principle, can be extended to any microorganism of interest, provided the proper fluorescent tag is avaialble.

In addition to using fluorescent tags that bind to the exterior of target cells, direct imaging of select genetic material itself can give further insight into the microbiome. Fluorescence *in situ* hybridization (FISH), for example, has been used to probe for specific DNA/RNA sequences, through fluorescent conjugation to a specific targeted gene or sequence (Figure 4a). Fluorescence *in situ* hybridization is a mature technique, with readily available kits and refined protocols,[158] and is already being leveraged for microbiome applications including imaging microbes that cannot be cultured,[159] marine bacteria,[160] as well as bacteria in fecal samples.[161,162] However, FISH has largely been supplanted for laboratory microbiome investigations by sequencing technologies, due to their ability to reveal similar genetic information with high throughput. However, as an imaging technique, FISH offers information about the spatial distributions of target genes (and, thus, the distribution of the microbes containing those genes), information that sequencing alone cannot provide. For example, FISH was used to reveal the spatial distribution of bacteria in kitchen sponges resulting from different sanitation levels (Figure 4b,c).[163] Although sequencing of the 16A rRNA genes obtained the population distribution of the sponge bacteria, FISH revealed that biofilms formed on the surface and in internal cavities of the sponge, and that bacterial concentrations in those areas reached up to 5.4×10^{10} cells per cubic cm. This type of spatial information is necessary for investigating how microbes interact with one another as well as with their environment. Although not yet demonstrated in a mobile, cost-effective capacity, FISH has been adapted to a microhole array chip, fabricated from silicon,

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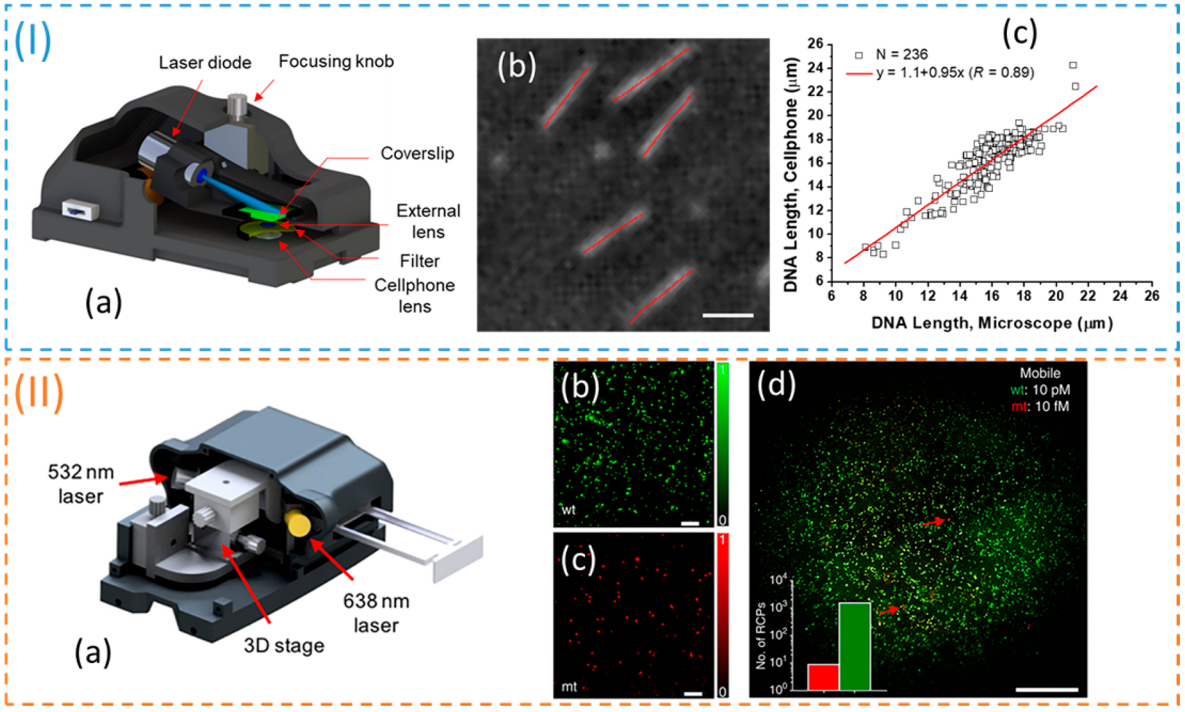

Figure 5. (I) Smartphone-based imager for DNA sizing. (a) Device schematic, featuring laser diode, focusing knob, sample coverslip, external lens, optical filter, and smartphone camera. (b) Image captured with this smartphone device. (c) DNA length measured with smartphone-based *versus* standard benchtop microscope. Reprinted from ref 156. Copyright 2014 American Chemical Society. (II) Multimodal smartphone-based imager for sequencing products and point-mutation analysis, with two laser diodes for exciting multiple fluorescence probes, as well as a white LED for bright-field microscopy. (a) Device schematic. (b) *KRAS* wild type image. (c) *KRAS* with codon 12 mutation image. (d) Smartphone image of a 1000:1 wild type to mutant ratio sample. Reprinted with permission from ref 81. Copyright 2017 Kühnemund *et al.*

enabling the capture of an individual cell at each hole in a 35-by-35 array configuration.[164] If the technique can be scaled up, leveraging established silicon microfabrication techniques, the reagent and labor costs of FISH could be dramatically reduced. When coupled with the previously discussed recent developments on field-portable fluorescence microscopy, FISH has the potential to become a cost-effective imaging tool for the exploration of the microbiome.

Similarly, several groups have developed mobile DNA imaging technologies that could be used for the analysis of the microbiome. For example, Wei *et al.* demonstrated a smartphone-based platform to measure the length of DNA strands with <1 kilobase pair (kbp) accuracy for strands of ≥10 kbp (Figure 5I).[156] Such DNA sizing measurements can reveal copy number variations in specific sequences, indicating unwanted mutations that have been correlated with drug resistance, cancers, and neurological diseases, among others. In addition, DNA length measurements can be an important presequencing step to ensure that the strands are all of comparable length for both short-read or long-read systems. Similarly, a multimodal smartphone-based fluorescent imaging system was used to quantify isothermal amplification products (rolling circle amplification, RCA) as well as to detect point mutations against a strong wild type background signal (Figure 5II).[81] These mobile implementations are able to perform certain steps of the sequencing process and to demonstrate the potential of field-compatible imaging techniques for investigating microbes' genetic material.

Other Optical Techniques. In addition to computational imaging techniques and fluorescence microscopy, other emerging optical technologies are providing exciting solutions to the aforementioned challenges of imaging the microbiome. One such technique is the use of self-assembled nanolenses.[166] These nanolenses enable the detection of nanoparticles or viruses as small as 30−40 nm over a large FOV, ultimately improving the detection signal-to-noise ratio of a diffraction limited imaging system. The sample preparation and formation of the nanolenses are fast and easy, with no need for equipment that could not be used in field settings. The objects to be imaged are diluted in a Tris-HCl/PEG 600 solution, sonicated, and pipetted onto a coverslip, which is then inverted and placed on a CMOS image sensor for lens-free imaging. A technique has also been developed to produce vapor-condensed nanolenses, enabling greater control over their formation, while maintaining a cost-effective, field-portable design.[167,168] Recently, nanolenses have been utilized in a field-portable system for the specific detection of herpes simplex virus.[169] The nanolens approach could conceivably be adapted to detect other pathogenic microbes or give counts of the more common bacteria. Because of the low cost, large FOV, and simple operation of the system, many information-rich tests can be performed in quick succession, potentially enabling high-throughput screening of diseases.

Another optical technique that is particularly suited to obtaining population profiles of cells in a sample is flow cytometry. This technique involves flowing large volumes of a given sample and recording the fluorescence and/or scattering signals of each object during the flow, and it was applied to the characterization of microbes long before NGS technologies matured.[170] Fluorescent tags are typically used to identify target cells or microbes as they flow past a detector, and as many as 17 different fluorophores can be multiplexed for flow cytometry,[171]

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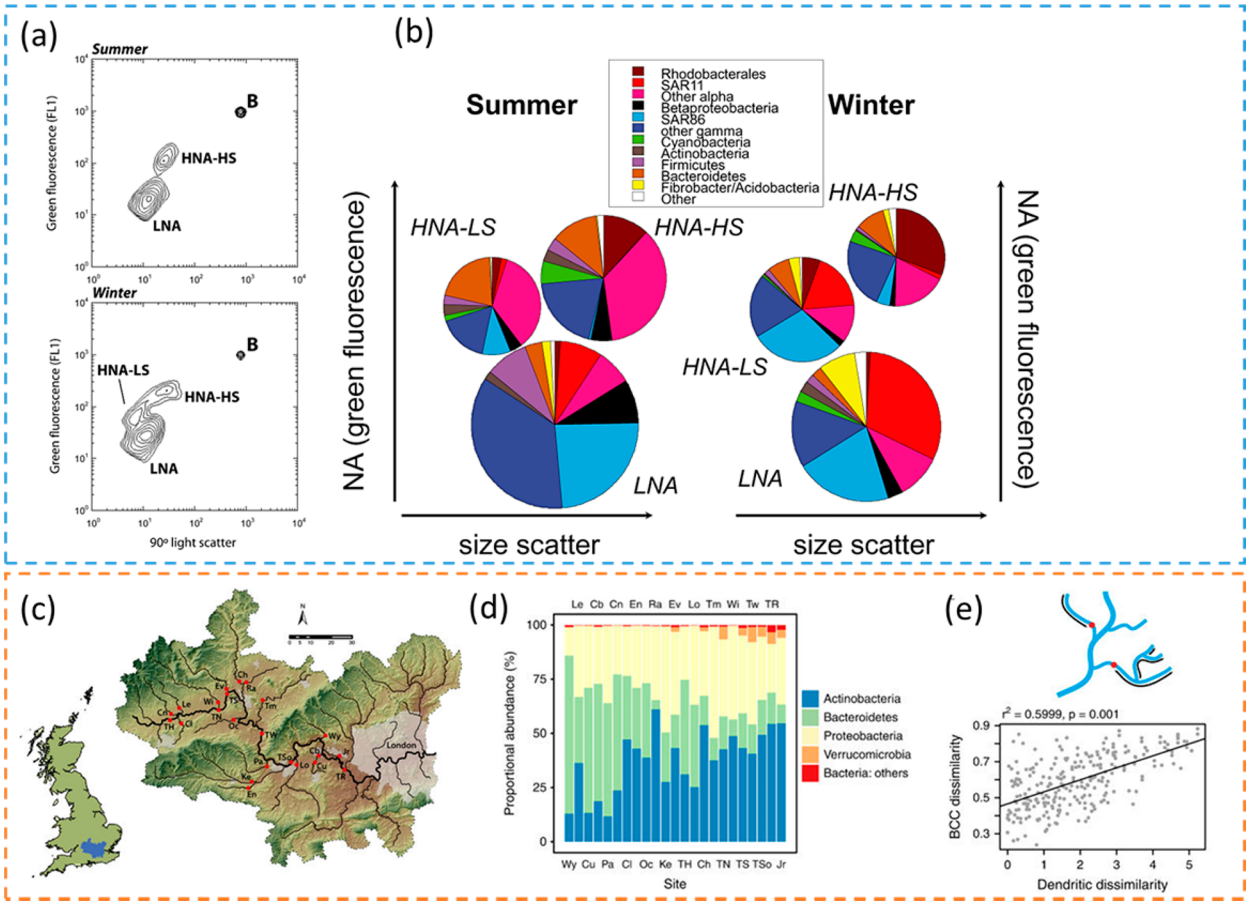

Figure 6. (a) Fluorescence and scattering distributions for bacteria in seawater samples in summer *versus* winter. Clustering is demonstrated based on high or low nucleic acid content (HNA/LNA) and scattering (HS/LS). (b) Bacteria population distributions in summer *versus* winter. The size of the graphs corresponds to the abundance of the groups, and the axes are the same as those in (a). Reprinted with permission from ref 174. Copyright 2012 Society for Applied Microbiology and Blackwell Publishing Ltd. (c) Map of a river basin and water sampling locations for flow cytometric and 16S rRNA measurements. (d) Bacteria population distribution at each sampling site. (e) Plots of population dissimilarity *versus* distance along the river between the sampling sites. Results show a linear relationship between the two. Reprinted with permission from ref 175. Copyright 2015 International Society for Microbial Ecology.

which could give tremendous insight into the microbial populations in fluids such as bodies of water, urine, or saliva, among many others. In one study, multiple fluorophores were used within a flow cytometer for the analysis of the microbiome inside a powdered infant formula facility, revealing microbial membrane integrity, metabolic activity, respiratory activity, and Gram characteristics within the analyzed samples.[172] Whereas 16S rRNA sequencing gave phylogenic population distributions for samples from different zones of the production facility, flow cytometry distinguished between viable and dead bacteria. This proved important because the zone in the facility with the highest total cell count differed from the zone with the highest viable cell count, possibly indicating that humidity plays a larger role in determining the density of viable microorganisms than do the hygiene practices in the production facility.

Flow cytometry has also been employed to investigate the populations of microbes in the natural environment, in particular in bodies of water.[173−175] The response of phytoplankton to ocean acidification, for example, has been investigated by flow cytometry.[173] In this study, phytoplankton were sorted into six distinct clusters based on their autofluorescence and side scatter signals, without the need for specific staining, while viruses and prokaryotes were studied using specific fluorescent tags. Flow cytometric measurements revealed that the population profile tended to shift toward smaller organisms after acidification. It was even shown, by comparison with 16S rRNA sequencing, that flow cytometric measurements of side scattering and nucleic acid content can track changes in the phylogenetic makeup of the ocean (Figure 6a,b).[174] Monitoring techniques such as this would be critical for engineering the microbiome because understanding the factors that influence any environmental microbiome will require temporal information to establish cause−effect relationships. These cytometry studies could be implemented to study other microbiota, such as those in river systems, which have been shown to vary, for example, from primarily *Bacteroidetes* in the headwaters to *Actinobacteria* downstream (Figure 6c−e).[175] It is important to note that all the flow cytometric studies mentioned above were performed on laboratory flow cytometers after sample collection in the field, however, low-cost, mobile flow cytometry tools have been demonstrated, raising the possibility of remote or autonomous sampling over unprecedented spatiotemporal landscapes.[176,177]

ENGINEERING THE MICROBIOME AND FUTURE OUTLOOK

In addition to the discovery of the global microbiome, various microbiome-related applications urgently need technologies for

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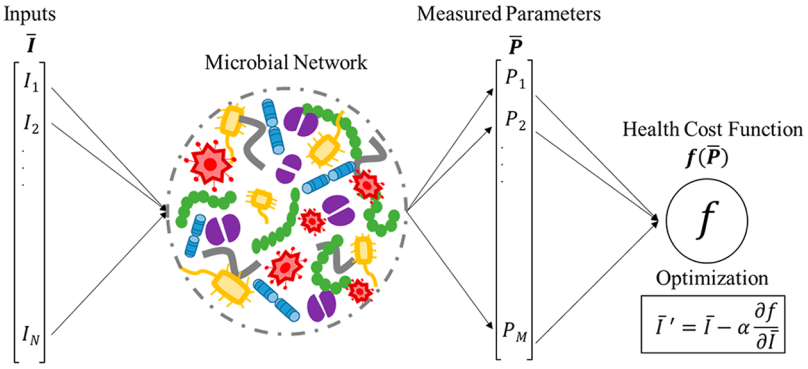

Figure 7. Schematic of the optimization framework for engineering the microbiome.

precise engineering, monitoring, and control of the state of a microbiome. The definition of "normal" for a human being and their microbiome has been an extensively researched topic with various exciting recent findings.[178−181] Despite all the progress, many unknowns remain: for example, what measurable parameter space can be used to cover the states of a specific microbiome, and what mathematical function of this multiparameter space can quantify these different states? If one can define such a quantifiable metric for the "microbiome state," what are the practical inputs that can be used to perturb and to engineer the microbiome, and what are the local gradients of the selected microbiome metric with respect to each input? The answers to these questions would help researchers in the microbiome field formulate an optimization framework that could gradually transform any given state of the microbiome into new states using small perturbations, which could lead to the optimization of the microbiome state with respect to a certain target metric or "health function". In addition, one could also then define different metrics or mathematical functions to be optimized depending on the desired outcome from an engineered microbiome. A simplified overview of such a computational microbiome engineering and optimization framework is shown in Figure 7.

Depending on the level of complexity and the size of the microbiome, its response time to small changes in each input, and the number of inputs and measurement parameters, the framework summarized in Figure 7 could gradually converge to desired states, even though it might eventually be a local optimum, and not necessarily the global optimal state of the microbiome, if one exists. Such a framework should intuitively remind the readers of an artificial neural network (used in machine learning) and its training or optimization. Unlike an artificial neural network that is typically engineered with a known connectivity map and activation function, the microbial community (Figure 7) defines a constantly evolving (as a function of both time and space) and an unknown network architecture between the inputs ($\bar{I} = [I_1, I_2, ..., I_N]$) and the measurement parameters ($\bar{P} = [P_1, P_2, ..., P_M]$) or consequently the health metric that is defined, $f(\bar{P})$. In deep learning, it is well-known that the small perturbations of a network following the gradients associated with a metric or cost function is the key for the network to perform extremely complicated tasks even though it does not necessarily converge at a global optimal point. From the perspective of microbiome engineering, the definition and quantification of the microbiome state through a multiparameter function, $f(\bar{P})$, that can be measured on demand, would be the key to engineer, to transform, and to monitor microbiomes through a controlled feedback process. This continuous feedback process could then "optimally" perturb the inputs to the microbiome using, for example, a gradient descent-based optimization framework, *i.e.*, $\bar{I}' = \bar{I} - \alpha \frac{\partial f}{\partial \bar{I}}$. Here, α is a perturbation or optimization parameter (or a vector), the magnitude of which depends on the desired severity of the perturbations to the input parameters. Depending on how $f(\bar{P})$ is defined, one can either minimize or maximize the selected metric, or aim to maintain it within a desired "healthy" range of values.

To be able to perform this level of engineering control and design of microbiome states *in situ*, we need advanced multimodal imaging and sensing technologies that are massively scalable, cost-effective, and ideally field-portable or mobile. In addition to on-demand and *in situ* evaluation of $f(\bar{P})$, we also need to integrate on the same platforms technologies that can control and perturb $\bar{I}$ in a desired manner. Depending on the microbiome of interest and its conditions *in situ*, what constitutes $\bar{I}$, $\bar{P}$, and $f(\bar{P})$ will widely vary. $\bar{I}$ can include various physical variables such as temperature or pH, as well as chemicals, prebiotics, nutrition (*e.g.*, glucose), antibiotics, or different species of microbes (*e.g.*, probiotics) that can be added into the microbial network in a controlled manner. Similarly, $\bar{P}$ can include a panel of proteomic, metabolomic, genomic and/or simple phenotypic measurements of the microbiome, including the spatial distribution of growth and inhibition of various species that constitute the microbiome. Importantly, this approach will work even if we do not specifically recognize the individual constituents of the microbiome, which is almost always the case.

In addition to engineering the microbiome, the same framework that is summarized in Figure 7 could also be used to engineer the dosage and composition of antimicrobials to be administered to patients, replacing the standard antimicrobial susceptibility testing (AST) that is conducted in clinical microbiology laboratories using microwell plates. The traditional AST method screens a predefined set of antibiotics at discrete concentrations on patient samples and, in this sense, it is suboptimal in terms of fine-tuning the antibiotic treatment to a specific patient's needs.[182−184] On the contrary, the optimization framework shown in Figure 7 might be used to converge to more optimized combinations of antibiotics and concentrations rapidly, tailored for the patient's sample. For such an application, $\bar{P}$ could simply involve turbidity measurements of the microbial network through bright-field and/or fluorescence imaging measurements, for example, and $\bar{I}$ could involve the concentrations of different antibiotics (or combination drugs) that are mixed with the cultured sample(s)

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taken from the patient in a controlled manner. In this specific example, the metric $f(\overline{P})$ would quantify the suppression of the growth of the pathogenic microbes by having a higher cost as the turbidity increases, whereas in other cases it could involve the quantification of a different desired outcome. To avoid overdosing the patient with many different types of antibiotics, the cost function/metric can also include in its definition a strong penalty term for a nonsparse drug combination, $\overline{I}$. For this purpose, among other possibilities, the L-1 norm of $\overline{I}$, *i.e.*, $\|\overline{I}\|_1$, can be used as a penalty term in a regularization formulation, i.e., $\overline{I}' = \arg\min_{\overline{I}}\{f(\overline{P}) + \lambda\|\overline{I}\|_1\}$, in order to force the optimization framework to converge on a single antibiotic type, or a sparse subset, with the minimum inhibitory concentration.[109,185] Here, λ represents a regularization, or "penalty" parameter, that can be tuned to increase or to decrease the sparsity of the types of antibiotics desired in the final treatment.

If the mobile, cost-effective, and multimodal imaging and sensing technologies discussed in this Perspective could reach the consumer and, for example, enter the home, researchers would have opportunities to track an individual's microbiome state continuously (*e.g.*, through skin, saliva, fecal matter, *etc.*) and to understand host−microbiome interactions. In addition, the soil, air, ocean, and freshwater microbiomes can similarly be monitored for human health and/or environmental health analysis. Ultimately, this information, together with the outlined framework, can be used to define and to quantify "healthy or normal" statistically, and to maintain its metric through controlled and informed dosing of drugs, nutrition, and other microbiome-based interventions.

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Notes

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