

# Enteric bacteria, Simple introduction With Numerical Optical Sectioning

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## Abstract

Human gastrointestinal microbiota, also known as gut flora or gut microbiota, are the microorganisms (generally bacteria and archaea), that live in the digestive tracts of humans. Many non-human animals, including insects, are hosts to numerous microorganisms that reside in the gastrointestinal tract as well. The human gastrointestinal metagenome is the aggregate of all the genomes of gut microbiota. The gut is one niche that human microbiota inhabit.

## 1 Introduction

In humans, the gut microbiota has the largest numbers of bacteria and the greatest number of species compared to other areas of the body. In humans, the gut flora is established at one to two years after birth, by which time the intestinal epithelium and the intestinal mucosal barrier that it secretes have co-developed in a way that is tolerant to, and even supportive of, the gut flora and that also provides a barrier to pathogenic organisms. The relationship between some gut flora and humans is not merely commensal (a non-harmful coexistence), but rather a mutualistic relationship. Some human gut microorganisms benefit the host by fermenting dietary fiber into short-chain fatty acids (SCFAs), such as acetic acid and butyric acid, which are then absorbed by the host. Intestinal bacteria also play a role in synthesizing vitamin B and vitamin K as well as metabolizing bile acids, sterols, and xenobiotics. The systemic importance of the SCFAs and other compounds they produce are like hormones and the gut flora itself appears to function like an endocrine organ, and dysregulation of

the gut flora has been correlated with a host of inflammatory and autoimmune conditions. The composition of human gut microbiota changes over time, when the diet changes, and as overall health changes. A systematic review from 2016 examined the preclinical and small human trials that have been conducted with certain commercially available strains of probiotic bacteria and identified those that had the most potential to be useful for certain central nervous system disorders.

While plant-based diets have some variation, vegetarian and vegan diets patterns are the most common. Vegetarian diets exclude meat products but still allow for eggs and dairy, while vegan diets exclude all forms of animal products. The diets of vegetarian and vegan individuals create a microbiome distinct from meat eaters, however there is not a significant distinction between the two. In diets that are centered around meat and animal products, there are high abundances of *Alistipes*, *Bifidobacteria* and *Bacteroides* which are all bile tolerant and may promote inflammation in the gut. In this type of diet, the group Firmicutes, which is associated with the metabolism of dietary plant polysaccharides, is found in low concentrations. Conversely, diets rich in plant-based materials are associated with greater diversity in the gut microbiome overall, and have a greater abundance of *Prevotella*, responsible for the long-term processing of fibers, rather than the bile tolerant species. Diet can be used to alter the composition of the gut microbiome in relatively short timescales. However, if wanting to change the microbiome to combat a disease or illness, long-term changes in diet have proven to be most successful.

#### Malnourishment

Malnourished human children have less mature and less diverse gut microbiota than healthy children, and changes in the microbiome associated with nutrient scarcity can in turn be a pathophysiological cause of malnutrition. Malnourished children also typically have more potentially pathogenic gut flora, and more yeast in their mouths and throats. Altering diet may lead to changes in gut microbiota composition and diversity.

#### Geography

Gut microbiome composition depends on the geographic origin of populations. Variations in a trade-off of *Prevotella*, the representation of the urease gene, and the representation of genes encoding glutamate synthase/degradation or other enzymes involved in amino acids degradation or vitamin biosynthesis show significant differences between populations from the US, Malawi or Amerindian origin. The US population has a high representation of enzymes encoding the degradation of glutamine and enzymes involved in vitamin and lipoic acid biosynthesis; whereas Malawi and Amerindian populations have a high representation of enzymes encoding glutamate synthase and they also have an overrepresentation of  $\alpha$ -amylase in their microbiomes. As the US population has a diet richer in fats than Amerindian or Malawian populations which have a corn-rich diet, the diet is probably the main determinant of the gut bacterial composition. Further studies have indicated a large difference in the composition of microbiota between European and rural African children. The fecal bacteria of children from Florence were compared to that of children from the small rural

village of Boulpon in Burkina Faso. The diet of a typical child living in this village is largely lacking in fats and animal proteins and rich in polysaccharides and plant proteins. The fecal bacteria of European children were dominated by Firmicutes and showed a marked reduction in biodiversity, while the fecal bacteria of the Boulpon children was dominated by Bacteroidetes. The increased biodiversity and different composition of gut flora in African populations may aid in the digestion of normally indigestible plant polysaccharides and also may result in a reduced incidence of non-infectious colonic diseases. On a smaller scale, it has been shown that sharing numerous common environmental exposures in a family is a strong determinant of individual microbiome composition. This effect has no genetic influence and it is consistently observed in culturally different populations.

#### Acquisition in human infants

The establishment of a gut flora is crucial to the health of an adult, as well as the functioning of the gastrointestinal tract. In humans, a gut flora similar to an adult's is formed within one to two years of birth as microbiota are acquired through parent-to-child transmission and transfer from food, water, and other environmental sources. The traditional view of the gastrointestinal tract of a normal fetus is that it is sterile, although this view has been challenged in the past few years. Multiple lines of evidence have begun to emerge that suggest there may be bacteria in the intrauterine environment. In humans, research has shown that microbial colonization may occur in the fetus with one study showing *Lactobacillus* and *Bifidobacterium* species were present in placental biopsies. Several rodent studies have demonstrated the presence of bacteria in the amniotic fluid and placenta, as well as in the meconium of babies born by sterile cesarean section. In another study, researchers administered a culture of bacteria orally to a pregnant dam, and detected the bacteria in the offspring, likely resulting from transmission between the digestive tract and amniotic fluid via the blood stream. However, researchers caution that the source of these intrauterine bacteria, whether they are alive, and their role, is not yet understood. During birth and rapidly thereafter, bacteria from the mother and the surrounding environment colonize the infant's gut. The exact sources of bacteria is not fully understood, but may include the birth canal, other people (parents, siblings, hospital workers), breastmilk, food, and the general environment with which the infant interacts. However, as of 2013, it remains unclear whether most colonizing arises from the mother or not. Infants born by caesarean section may also be exposed to their mothers' microflora, but the initial exposure is most likely to be from the surrounding environment such as the air, other infants, and the nursing staff, which serve as vectors for transfer.

Using synchrotron radiation Gabor and Fourier holograms have been demonstrated with spatial resolution below 100 nm at SXR wavelengths. Compact EUV sources based on high harmonic generation (HHG) were also used to demonstrate table-top in-line EUV holography with a spatial resolution of 7.9 nm and 0.8 nm. Time resolved holographic imaging, that exploits the short pulsewidth of the HHG sources, was also implemented to study the ultrafast dynamics of surface deformation with a lateral resolution of the order of 100 nm.

The recent development of compact coherent EUV laser sources has opened new opportunities for the implementation of novel imaging schemes with nanometer-scale resolution that fit on a table-top. In this paper, we present a proof of principle experiment in which we demonstrate that three dimensional imaging in a volume may be obtained from a single high numerical aperture (NA) hologram obtained with a table top EUV laser. Gabor holograms were numerically reconstructed over a range of image planes by sweeping the propagation distance. This numerical sectioning technique for holography is verified to produce a robust three dimension image of a test object.

Holographic imaging in the soft X-ray (SXR) and extreme ultraviolet (EUV) have been demonstrated in several experiments realized using EUV/SXR lasers and synchrotron sources. These include the first realization of soft X-ray laser holography at Lawrence Livermore National Laboratory using a large laser facility, and the holographic recording of biological samples and sub-micron structures using soft X-ray radiation from synchrotrons, among other experiments. A key idea in these experiments is to use coherent short wavelength illumination to achieve a spatial resolution beyond the reach of visible light.

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[width=30pc]mouse

Figure 1: (a) Diagram of the experimental set up. (b) Detail of the test object used.

[width=21pc]mouse

Figure 2: Lasing was obtained in the 46.9 nm 3s 1P1  $\rightarrow$  3p 1S0 transition of neon-like Ar by exciting Ar filled alumina capillaries 3.2 mm in diameter with a current pulse having an amplitude Lasing was obtained in the 46.9 nm 3s 1P1  $\rightarrow$  3p 1S0 transition of neon-like Ar by exciting Ar filled alumina capillaries 3.2 mm in diameter with a current pulse having an amplitude

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## 2 Experimental Details

The microbial composition of the gut microbiota varies across the digestive tract. In the stomach and small intestine, relatively few species of bacteria are generally present. The colon, in contrast, contains the highest microbial density recorded in any habitat on Earth with up to 10<sup>12</sup> cells per gram of intestinal content. These bacteria represent between 300 and 1000 different species. However, 99 % of the bacteria come from about 30 or 40 species. As a consequence of their abundance in the intestine, bacteria also make up to 60% of the dry mass of feces. Fungi, protists, archaea, and viruses are also present in the gut flora, but less is known about their activities. Over 99 % of the bacteria in the gut are anaerobes, but in the cecum, aerobic bacteria reach high densities. It is estimated that these gut flora have around a hundred times as many genes in total as there are in the human genome.

Many species in the gut have not been studied outside of their hosts because most cannot be cultured. While there are a small number of core species of microbes shared by most individuals, populations of microbes can vary widely among different individuals. Within an individual, microbe populations stay fairly constant over time, even though some alterations may occur with changes in lifestyle, diet and age. The Human Microbiome Project has set out to better describe the microflora of the human gut and other body locations.

The four dominant bacterial phyla in the human gut are Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria. Most bacteria belong to the genera *Bacteroides*, *Clostridium*, *Faecalibacterium*, *Eubacterium*, *Ruminococcus*, *Peptococcus*, *Peptostreptococcus*, and *Bifidobacterium*. Other genera, such as *Escherichia* and *Lactobacillus*, are present to a lesser extent. Species from the genus *Bacteroides* alone constitute about 30% of all bacteria in the gut, suggesting that this genus is especially important in the functioning of the host. Fungal genera that have been detected in the gut include *Candida*, *Saccharomyces*, *Aspergillus*, *Penicillium*, *Rhodotorula*, *Trametes*, *Pleospora*, *Sclerotinia*, *Bullera*, and *Galactomyces*, among others. *Rhodotorula* is most frequently found in individuals with inflammatory bowel disease while *Candida* is most frequently found in individuals with hepatitis B cirrhosis and chronic hepatitis B. Archaea constitute another large class of gut flora which are important in the metabolism of the bacterial products of fermentation.

Industrialization is associated with changes in the microbiota and the reduction of diversity could drive certain species to extinction; in 2018, researchers proposed a biobank repository of human microbiota.

### 2.1 Some Extra Details

The small intestine contains a trace amount of microorganisms due to the proximity and influence of the stomach. Gram-positive cocci and rod-shaped bacteria are the predominant microorganisms found in the small intestine. However, in the distal portion of the small intestine alkaline conditions support gram-negative bacteria of the Enterobacteriaceae. The bacterial flora of the small

intestine aid in a wide range of intestinal functions. The bacterial flora provide regulatory signals that enable the development and utility of the gut. Overgrowth of bacteria in the small intestine can lead to intestinal failure. In addition the large intestine contains the largest bacterial ecosystem in the human body. About 99% of the large intestine and feces flora are made up of obligate anaerobes such as *Bacteroides* and *Bifidobacterium*. Factors that disrupt the microorganism population of the large intestine include antibiotics, stress, and parasites. Bacteria make up most of the flora in the colon and 60% of the dry mass of feces. This fact makes feces an ideal source of gut flora for any tests and experiments by extracting the nucleic acid from fecal specimens, and bacterial 16S rRNA gene sequences are generated with bacterial primers. This form of testing is also often preferable to more invasive techniques, such as biopsies. Somewhere between 300 and 1000 different species live in the gut, with most estimates at about 500. However, it is probable that 99% of the bacteria come from about 30 or 40 species, with *Faecalibacterium prausnitzii* being the most common species in healthy adults. Fungi and protists also make up a part of the gut flora, but less is known about their activities. The virome is mostly bacteriophages. Research suggests that the relationship between gut flora and humans is not merely commensal (a non-harmful coexistence), but rather is a mutualistic, symbiotic relationship. Though people can survive with no gut flora, the microorganisms perform a host of useful functions, such as fermenting unused energy substrates, training the immune system via end products of metabolism like propionate and acetate, preventing growth of harmful species, regulating the development of the gut, producing vitamins for the host (such as biotin and vitamin K), and producing hormones to direct the host to store fats. Extensive modification and imbalances of the gut microbiota and its microbiome or gene collection are associated with obesity. However, in certain conditions, some species are thought to be capable of causing disease by causing infection or increasing cancer risk for the host.

### 2.1.1 Even more details

It has been demonstrated that there are common patterns of microbiome composition evolution during life. In general, the diversity of microbiota composition of fecal samples is significantly higher in adults than in children, although interpersonal differences are higher in children than in adults. Much of the maturation of microbiota into an adult-like configuration happens during the three first years of life. As the microbiome composition changes, so does the composition of bacterial proteins produced in the gut. In adult microbiomes, a high prevalence of enzymes involved in fermentation, methanogenesis and the metabolism of arginine, glutamate, aspartate and lysine have been found. In contrast, in infant microbiomes the dominant enzymes are involved in cysteine metabolism and fermentation pathways.

**A continuous** Studies and statistical analyses have identified the different bacterial genera in gut microbiota and their associations with nutrient intake.

Gut microflora is mainly composed of three enterotypes: Prevotella, Bacteroides, and Ruminococcus. There is an association between the concentration of each microbial community and diet. For example, Prevotella is related to carbohydrates and simple sugars, while Bacteroides is associated with proteins, amino acids, and saturated fats. Specialist microbes that break down mucin survive on their host's carbohydrate excretions. One enterotype will dominate depending on the diet. Altering the diet will result in a corresponding change in the numbers of species.

Humanoid robots have received much attention recently. Although they are able to walk without falling, their movements are not natural looking. In order for these robots to move more like humans, two technological obstacles need to be addressed: Firstly, there is a lack of flexibility in their body torsos. In all human activities, movements from the spine are involved. Yet, this important factor has been neglected by the majority of humanoid robotic researchers. One of the main reasons is that the added degrees of freedom (DOF) make it more costly and difficult to build a robot. Another key issue is that it is very difficult to program these types of robots so that they can maintain balance. This numerical sectioning technique for holography is verified to produce a robust three dimension image of a test object. This numerical sectioning technique for holography is verified to produce a robust three dimension image of a test object. In order to address the above technological challenges, we need to develop flexible spine humanoid robots as experimental platforms. Recently, a few researchers have developed several spinal robots based on the anatomy of the human skeleton, Mizuuchi built a tendon-driven robot called "Kenta" [?, ?]. Although the robot has a spine, there has been no data to show that it can move in a flexible way. Also, the robot cannot stand up without external support because the upper body is too heavy [?]. Mizuuchi later improved his prototype. However, it is also unable to stand up without external support [?, ?]. Vegetarian and vegan diets

While plant-based diets have some variation, vegetarian and vegan diets patterns are the most common. Vegetarian diets exclude meat products but still allow for eggs and dairy, while vegan diets exclude all forms of animal products. The diets of vegetarian and vegan individuals create a microbiome distinct from meat eaters, however there is not a significant distinction between the two. In diets that are centered around meat and animal products, there are high abundances of Alistipes, Bilophila and Bacteroides which are all bile tolerant and may promote inflammation in the gut. In this type of diet, the group Firmicutes, which is associated with the metabolism of dietary plant polysaccharides, is found in low concentrations. Conversely, diets rich in plant-based materials are associated with greater diversity in the gut microbiome overall, and have a greater abundance of Prevotella, responsible for the long-term processing of fibers, rather than the bile tolerant species. Diet can be used to alter the composition of the gut microbiome in relatively short timescales. However, if wanting to change the microbiome to combat a disease or illness, long-term changes in diet have proven to be most successful.



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Gut microbiome composition depends on the geographic origin of populations. Variations in a trade-off of Prevotella, the representation of the urease gene, and the representation of genes encoding glutamate synthase/degradation or other enzymes involved in amino acids degradation or vitamin biosynthesis show significant differences between populations from the US, Malawi or Amerindian origin. The US population has a high representation of enzymes encoding the degradation of glutamine and enzymes involved in vitamin and lipoic acid biosynthesis; whereas Malawi and Amerindian populations have a high representation of enzymes encoding glutamate synthase and they also have an overrepresentation of  $\alpha$ -amylase in their microbiomes. As the US population has a diet richer in fats than Amerindian or Malawian populations which have a corn-rich diet, the diet is probably the main determinant of the gut bacterial composition. Further studies have indicated a large difference in the composition of microbiota between European and rural African children. The fecal bacteria of children from Florence were compared to that of children from the small rural village of Boulpon in Burkina Faso. The diet of a typical child living in this village is largely lacking in fats and animal proteins and rich in polysaccharides and plant proteins. The fecal bacteria of European children were dominated by Firmicutes and showed a marked reduction in biodiversity, while the fecal bacteria of the Boulpon children was dominated by Bacteroidetes. The increased biodiversity and different composition of gut flora in African populations may aid in the digestion of normally indigestible plant polysaccharides and also may result in a reduced incidence of non-infectious colonic diseases. On a smaller scale, it has been shown that sharing numerous common environmental exposures in a family is a strong determinant of individual microbiome composition. This effect has no genetic influence and it is consistently observed in culturally different populations.

### Acquisition in human infants

The establishment of a gut flora is crucial to the health of an adult, as well the functioning of the gastrointestinal tract. In humans, a gut flora similar to an adult's is formed within one to two years of birth as microbiota are acquired through parent-to-child transmission and transfer from food, water, and other environmental sources. The traditional view of the gastrointestinal tract of a normal fetus is that it is sterile, although this view has been challenged in the past few years. Multiple lines of evidence have begun to emerge that suggest there may be bacteria in the intrauterine environment. In humans, research has shown that microbial colonization may occur in the fetus with one study showing *Lactobacillus* and *Bifidobacterium* species were present in

placental biopsies. Several rodent studies have demonstrated the presence of bacteria in the amniotic fluid and placenta, as well as in the meconium of babies born by sterile cesarean section. In another study, researchers administered a culture of bacteria orally to a pregnant dam, and detected the bacteria in the offspring, likely resulting from transmission between the digestive tract and amniotic fluid via the blood stream. However, researchers caution that the source of these intrauterine bacteria, whether they are alive, and their role, is not yet understood. During birth and rapidly thereafter, bacteria from the mother and the surrounding environment colonize the infant's gut. The exact sources of bacteria is not fully understood, but may include the birth canal, other people (parents, siblings, hospital workers), breastmilk, food, and the general environment with which the infant interacts. However, as of 2013, it remains unclear whether most colonizing arises from the mother or not. Infants born by caesarean section may also be exposed to their mothers' microflora, but the initial exposure is most likely to be from the surrounding environment such as the air, other infants, and the nursing staff, which serve as vectors for transfer.

During the first year of life, the composition of the gut flora is generally simple and changes a great deal with time and is not the same across individuals. The initial bacterial population are generally facultative anaerobic organisms; investigators believe that these initial colonizers decrease the oxygen concentration in the gut, which in turn allows obligately anaerobic bacteria like Bacteroides, Actinobacteria, and Firmicutes to become established and thrive. Breast-fed babies become dominated by bifidobacteria, possibly due to the contents of bifidobacterial growth factors in breast milk, and by the fact that breast milk carries prebiotic components, allowing for healthy bacterial growth. In contrast, the microbiota of formula-fed infants is more diverse, with high numbers of Enterobacteriaceae, enterococci, bifidobacteria, Bacteroides, and clostridia. Caesarean section, antibiotics, and formula feeding may alter the gut microbiome composition. Children treated with antibiotics have less stable, and less diverse floral communities. Caesarean sections have been shown to be disruptive to mother-offspring transmission of bacteria, which impacts the overall health of the offspring by raising risks of disease such as celiacs, asthma, and type 1 diabetes. This further evidences the importance of a healthy gut microbiome. Various methods of microbiome restoration are being explored, typically involving exposing the infant to maternal vaginal contents, and oral probiotics.

#### Functions

When the gut flora first started to be studied, it was thought to have three key roles: directly defending against pathogens, fortifying host defense by its role in developing and maintaining the intestinal epithelium and inducing antibody production there, and metabolizing otherwise indigestible compounds in food; subsequent work discovered its role in training the developing immune system, and yet further work focused on its role in the gut-brain axis.

#### Direct inhibition of pathogens

The gut flora community plays a direct role in defending against pathogens by fully colonizing the space, making use of all available nutrients, and by secret-

ing compounds that kill or inhibit unwelcome organisms that would compete for nutrients with it. Disruption of the gut flora allows competing organisms like *Clostridium difficile* to become established that otherwise are kept in abeyance.

#### Development of enteric protection and immune system

In humans, a gut flora similar to an adult's is formed within one to two years of birth. As the gut flora gets established, the lining of the intestines the intestinal epithelium and the intestinal mucosal barrier that it secretes develop as well, in a way that is tolerant to, and even supportive of, commensalistic microorganisms to a certain extent and also provides a barrier to pathogenic ones. Specifically, goblet cells that produce the mucosa proliferate, and the mucosa layer thickens, providing an outside mucosal layer in which "friendly" microorganisms can anchor and feed, and an inner layer that even these organisms cannot penetrate. Additionally, the development of gut-associated lymphoid tissue (GALT), which forms part of the intestinal epithelium and which detects and reacts to pathogens, appears and develops during the time that the gut flora develops and established. The GALT that develops is tolerant to gut flora species, but not to other microorganisms. GALT also normally becomes tolerant to food to which the infant is exposed, as well as digestive products of food, and gut flora's metabolites (molecules formed from metabolism) produced from food. The human immune system creates cytokines that can drive the immune system to produce inflammation in order to protect itself, and that can tamp down the immune response to maintain homeostasis and allow healing after insult or injury. Different bacterial species that appear in gut flora have been shown to be able to drive the immune system to create cytokines selectively; for example *Bacteroides fragilis* and some *Clostridia* species appear to drive an anti-inflammatory response, while some segmented filamentous bacteria drive the production of inflammatory cytokines. Gut flora can also regulate the production of antibodies by the immune system. One function of this regulation is to cause B cells to class switch to IgA. In most cases B cells need activation from T helper cells to induce class switching; however, in another pathway, gut flora cause NF- $\kappa$ B signaling by intestinal epithelial cells which results in further signaling molecules being secreted. These signaling molecules interact with B cells to induce class switching to IgA. IgA is an important type of antibody that is used in mucosal environments like the gut. It has been shown that IgA can help diversify the gut community and helps in getting rid of bacteria that cause inflammatory responses. Ultimately, IgA maintains a healthy environment between the host and gut bacteria. These cytokines and antibodies can have effects outside the gut, in the lungs and other tissues. The immune system can also be altered due to the gut bacteria's ability to produce metabolites that can affect cells in the immune system. For example short-chain fatty acids (SCFA) can be produced by some gut bacteria through fermentation. SCFAs stimulate a rapid increase in the production of innate immune cells like neutrophils, basophils and eosinophils. These cells are part of the innate immune system that try to limit the spread of infection.

#### Metabolism

Without gut flora, the human body would be unable to utilize some of the

undigested carbohydrates it consumes, because some types of gut flora have enzymes that human cells lack for breaking down certain polysaccharides. Rodents raised in a sterile environment and lacking in gut flora need to eat 30

#### Pharmacomicrobiomics

The human metagenome (i.e., the genetic composition of an individual and all microorganisms that reside on or within the individual's body) varies considerably between individuals. Since the total number of microbial and viral cells in the human body (over 100 trillion) greatly outnumbers *Homo sapiens* cells (tens of trillions), there is considerable potential for interactions between drugs and an individual's microbiome, including: drugs altering the composition of the human microbiome, drug metabolism by microbial enzymes modifying the drug's pharmacokinetic profile, and microbial drug metabolism affecting a drug's clinical efficacy and toxicity profile. Apart from carbohydrates, gut microbiota can also metabolize other xenobiotics such as drugs, phytochemicals, and food toxicants. More than 30 drugs have been shown to be metabolized by gut microbiota. The microbial metabolism of drugs can sometimes inactivate the drug.

#### Gut-brain axis

The gut-brain axis is the biochemical signaling that takes place between the gastrointestinal tract and the central nervous system. That term has been expanded to include the role of the gut flora in the interplay; the term "microbiome-gut-brain axis" is sometimes used to describe paradigms explicitly including the gut flora. Broadly defined, the gut-brain axis includes the central nervous system, neuroendocrine and neuroimmune systems including the hypothalamo-pituitary-adrenal axis (HPA axis), sympathetic and parasympathetic arms of the autonomic nervous system including the enteric nervous system, the vagus nerve, and the gut microbiota. A systematic review from 2016 examined the preclinical and small human trials that have been conducted with certain commercially available strains of probiotic bacteria and found that among those tested, *Bifidobacterium* and *Lactobacillus* genera (*B. longum*, *B. breve*, *B. infantis*, *L. helveticus*, *L. rhamnosus*, *L. plantarum*, and *L. casei*), had the most potential to be useful for certain central nervous system disorders.

#### Alterations in flora balance

##### Effects of antibiotic use

Altering the numbers of gut bacteria, for example by taking broad-spectrum antibiotics, may affect the host's health and ability to digest food. Antibiotics can cause antibiotic-associated diarrhea (AAD) by irritating the bowel directly, changing the levels of gut flora, or allowing pathogenic bacteria to grow. Another harmful effect of antibiotics is the increase in numbers of antibiotic-resistant bacteria found after their use, which, when they invade the host, cause illnesses that are difficult to treat with antibiotics. Changing the numbers and species of gut flora can reduce the body's ability to ferment carbohydrates and metabolize bile acids and may cause diarrhea. Carbohydrates that are not broken down may absorb too much water and cause runny stools, or lack of SCFAs produced by gut flora could cause diarrhea. A reduction in levels of native bacterial species also disrupts their ability to inhibit the growth of harmful species

such as *C. difficile* and *Salmonella kedougou*, and these species can get out of hand, though their overgrowth may be incidental and not be the true cause of diarrhea. Emerging treatment protocols for *C. difficile* infections involve fecal microbiota transplantation of donor feces. (see Fecal transplant). Initial reports of treatment describe success rates of 90

#### Pregnancy

Women's gut microbiota change as pregnancy advances, with the changes similar to those seen in metabolic syndromes such as diabetes. The change in gut flora causes no ill effects. The newborn's gut biota resemble the mother's first-trimester samples. The diversity of the flora decreases from the first to third trimester, as the numbers of certain species go up.

#### Probiotics, prebiotics, synbiotics, and pharmabiotics

Probiotics are microorganisms that are believed to provide health benefits when consumed. With regard to gut flora, prebiotics are typically non-digestible, fiber compounds that pass undigested through the upper part of the gastrointestinal tract and stimulate the growth or activity of advantageous gut flora by acting as substrate for them. Synbiotics refers to food ingredients or dietary supplements combining probiotics and prebiotics in a form of synergism. The term "pharmabiotics" is used in various ways, to mean: pharmaceutical formulations (standardized manufacturing that can obtain regulatory approval as a drug) of probiotics, prebiotics, or synbiotics; probiotics that have been genetically engineered or otherwise optimized for best performance (shelf life, survival in the digestive tract, etc.); and the natural products of gut flora metabolism (vitamins, etc.). There is some evidence that treatment with some probiotic strains of bacteria may be effective in irritable bowel syndrome and chronic idiopathic constipation. Those organisms most likely to result in a decrease of symptoms have included:

- Enterococcus faecium
- Lactobacillus plantarum
- Lactobacillus rhamnosus
- Propionibacterium freudenreichii
- Bifidobacterium breve
- Lactobacillus reuteri
- Lactobacillus salivarius
- Bifidobacterium infantis
- Streptococcus thermophilus

#### Research

Tests for whether non-antibiotic drugs may impact human gut-associated bacteria were performed by in vitro analysis on more than 1000 marketed drugs against 40 gut bacterial strains, demonstrating that 24

#### Role in disease

Bacteria in the digestive tract can contribute to and be affected by disease in various ways. The presence or overabundance of some kinds of bacteria may contribute to inflammatory disorders such as inflammatory bowel disease. Additionally, metabolites from certain members of the gut flora may influence host signalling pathways, contributing to disorders such as obesity and colon

cancer. Alternatively, in the event of a breakdown of the gut epithelium, the intrusion of gut flora components into other host compartments can lead to sepsis.

#### Ulcers

*Helicobacter pylori* can cause stomach ulcers by crossing the epithelial lining of the stomach. Here the body produces an immune response. During this response, parietal cells are stimulated and release extra hydrochloric acid (HCl+) into the stomach. However, the response does not stimulate the mucus-secreting cells that protect and line the epithelium of the stomach. The extra acid sears holes into the epithelial lining of the stomach, resulting in stomach ulcers.

#### Bowel perforation

Normally-commensal bacteria can harm the host if they extrude from the intestinal tract. Translocation, which occurs when bacteria leave the gut through its mucosal lining, can occur in a number of different diseases. If the gut is perforated, bacteria invade the interstitium, causing a potentially fatal infection.

#### Inflammatory bowel diseases

The two main types of inflammatory bowel diseases, Crohn's disease and ulcerative colitis, are chronic inflammatory disorders of the gut; the causes of these diseases are unknown and issues with the gut flora and its relationship with the host have been implicated in these conditions. Additionally, it appears that interactions of gut flora with the gut-brain axis have a role in IBD, with physiological stress mediated through the hypothalamic-pituitary-adrenal axis driving changes to intestinal epithelium and the gut flora in turn releasing factors and metabolites that trigger signaling in the enteric nervous system and the vagus nerve. The diversity of gut flora appears to be significantly diminished in people with inflammatory bowel diseases compared to healthy people; additionally, in people with ulcerative colitis, Proteobacteria and Actinobacteria appear to dominate; in people with Crohn's, *Enterococcus faecium* and several Proteobacteria appear to be over-represented. There is reasonable evidence that correcting gut flora imbalances by taking probiotics with Lactobacilli and Bifidobacteria can reduce visceral pain and gut inflammation in IBD.

#### Irritable bowel syndrome

Irritable bowel syndrome is a result of stress and chronic activation of the HPA axis; its symptoms include abdominal pain, changes in bowel movements, and an increase in proinflammatory cytokines. Overall, studies have found that the luminal and mucosal microbiota are changed in irritable bowel syndrome individuals, and these changes can relate to the type of irritation such as diarrhea or constipation. Also, there is a decrease in the diversity of the microbiome with low levels of fecal Lactobacilli and Bifidobacteria, high levels of facultative anaerobic bacteria such as *Escherichia coli*, and increased ratios of Firmicutes: Bacteroidetes.

#### Other inflammatory or autoimmune conditions

Allergy, asthma, and diabetes mellitus are autoimmune and inflammatory disorders of unknown cause, but have been linked to imbalances in the gut flora and its relationship with the host. As of 2016 it was not clear if changes to the gut flora cause these auto-immune and inflammatory disorders or are a product

of or adaptation to them.

#### Asthma

With asthma, two hypotheses have been posed to explain its rising prevalence in the developed world. The hygiene hypothesis posits that children in the developed world are not exposed to enough microbes and thus may contain lower prevalence of specific bacterial taxa that play protective roles. The second hypothesis focuses on the Western pattern diet, which lacks whole grains and fiber and has an overabundance of simple sugars. Both hypotheses converge on the role of short-chain fatty acids (SCFAs) in immunomodulation. These bacterial fermentation metabolites are involved in immune signalling that prevents the triggering of asthma and lower SCFA levels are associated with the disease. Lacking protective genera such as *Lachnospira*, *Veillonella*, *Rothia* and *Faecalibacterium* has been linked to reduced SCFA levels. Further, SCFAs are the product of bacterial fermentation of fiber, which is low in the Western pattern diet. SCFAs offer a link between gut flora and immune disorders, and as of 2016, this was an active area of research. Similar hypotheses have also been posited for the rise of food and other allergies.

#### Diabetes mellitus type 1

The connection between the gut microbiota and diabetes mellitus type 1 has also been linked to SCFAs, such as butyrate and acetate. Diets yielding butyrate and acetate from bacterial fermentation show increased Treg expression. Treg cells downregulate effector T cells, which in turn reduces the inflammatory response in the gut. Butyrate is an energy source for colon cells. butyrate-yielding diets thus decrease gut permeability by providing sufficient energy for the formation of tight junctions. Additionally, butyrate has also been shown to decrease insulin resistance, suggesting gut communities low in butyrate-producing microbes may increase chances of acquiring diabetes mellitus type 2. Butyrate-yielding diets may also have potential colorectal cancer suppression effects.

#### Obesity and metabolic syndrome

The gut flora has also been implicated in obesity and metabolic syndrome due to the key role it plays in the digestive process; the Western pattern diet appears to drive and maintain changes in the gut flora that in turn change how much energy is derived from food and how that energy is used. One aspect of a healthy diet that is often lacking in the Western-pattern diet is fiber and other complex carbohydrates that a healthy gut flora require flourishing; changes to gut flora in response to a Western-pattern diet appear to increase the amount of energy generated by the gut flora which may contribute to obesity and metabolic syndrome. There is also evidence that microbiota influence eating behaviours based on the preferences of the microbiota, which can lead to the host consuming more food eventually resulting in obesity. It has generally been observed that with higher gut microbiome diversity, the microbiota will spend energy and resources on competing with other microbiota and less on manipulating the host. The opposite is seen with lower gut microbiome diversity, and these microbiotas may work together to create host food cravings. Additionally, the liver plays a dominant role in blood glucose homeostasis by maintaining a balance

between the uptake and storage of glucose through the metabolic pathways of glycogenesis and gluconeogenesis. Intestinal lipids regulate glucose homeostasis involving a gut-brain-liver axis. The direct administration of lipids into the upper intestine increases the long chain fatty acyl-coenzyme A (LCFA-CoA) levels in the upper intestines and suppresses glucose production even under subdiaphragmatic vagotomy or gut vagal deafferentation. This interrupts the neural connection between the brain and the gut and blocks the upper intestinal lipids' ability to inhibit glucose production. The gut-brain-liver axis and gut microbiota composition can regulate the glucose homeostasis in the liver and provide potential therapeutic methods to treat obesity and diabetes. Just as gut flora can function in a feedback loop that can drive the development of obesity, there is evidence that restricting intake of calories (i.e., dieting) can drive changes to the composition of the gut flora.

#### Liver disease

As the liver is fed directly by the portal vein, whatever crosses the intestinal epithelium and the intestinal mucosal barrier enters the liver, as do cytokines generated there. Dysbiosis in the gut flora has been linked with the development of cirrhosis and non-alcoholic fatty liver disease.

#### Cancer

Some genera of bacteria, such as *Bacteroides* and *Clostridium*, have been associated with an increase in tumor growth rate, while other genera, such as *Lactobacillus* and *Bifidobacteria*, are known to prevent tumor formation. As of December 2017 there was preliminary and indirect evidence that gut microbiota might mediate response to PD-1 inhibitors; the mechanism was unknown.

#### Neuropsychiatric

Interest in the relationship between gut flora and neuropsychiatric issues was sparked by a 2004 study showing that germ-free mice showed an exaggerated HPA axis response to stress compared to non-GF laboratory mice. As of January 2016, most of the work that has been done on the role of gut flora in the gut-brain axis had been conducted in animals, or characterizing the various neuroactive compounds that gut flora can produce, and studies with humans measuring differences between people with various psychiatric and neurological differences, or changes to gut flora in response to stress, or measuring effects of various probiotics (dubbed "psychobiotics in this context), had generally been small and could not be generalized; whether changes to gut flora are a result of disease, a cause of disease, or both in any number of possible feedback loops in the gut-brain axis, remained unclear. A systematic review from 2016 examined the preclinical and small human trials that have been conducted with certain commercially available strains of probiotic bacteria and found that among those tested, the genera *Bifidobacterium* and *Lactobacillus* (*B. longum*, *B. breve*, *B. infantis*, *L. helveticus*, *L. rhamnosus*, *L. plantarum*, and *L. casei*) had the most potential to be useful for certain central nervous system disorders.

#### Other animals

The composition of the human gut microbiome is similar to that of the other great apes. However, humans gut biota has decreased in diversity and changed in composition since our evolutionary split from Pan. Humans display increases in



Bacteroidetes, a bacterial phylum associated with diets high in animal protein and fat, and decreases in Methanobrevibacter and Fibrobacter, groups that ferment complex plant polysaccharides. These changes are the result of the combined dietary, genetic, and cultural changes humans have undergone since evolutionary divergence from Pan.

In addition to humans and vertebrates, some insects also possess complex and diverse gut microbiota that play key nutritional roles. Microbial communities associated termites can constitute a majority of the weight of the individuals and perform important roles in the digestion of lignocellulose and nitrogen fixation. These communities are host-specific, and closely related insect species share comparable similarities in gut microbiota composition. In cockroaches, gut microbiota have been shown to assemble in a deterministic fashion, irrespective of the inoculum; the reason for this host-specific assembly remains unclear. Bacterial communities associated with insects like termites and cockroaches are determined by a combination of forces, primarily diet, but there is some indication that host phylogeny may also be playing a role in the selection of lineages. For more than 51 years it has been known that the administration of low doses of antibacterial agents promotes the growth of farm animals to increase weight gain. In a study performed on mice by Ilseung Cho, the ratio of Firmicutes and Lachnospiraceae was significantly elevated in animals treated with subtherapeutic doses of different antibiotics. By analyzing the caloric content of faeces and the concentration of small chain fatty acids (SCFAs) in the GI tract, they concluded that the changes in the composition of microbiota lead to an increased capacity to extract calories from otherwise indigestible constituents, and to an increased production of SCFAs. These findings provide evidence that antibiotics perturb not only the composition of the GI microbiome but also its metabolic capabilities, specifically with respect to SCFAs.

### 3 Results

We adjusted the<sup>1</sup> exposure so that the photoresist operated in a linear response regime. With exposure by the EUV laser, the holographic interference pattern generated by the reference and the object beams was recorded in the photoresist and converted to a surface modulation after the development. Thus, the holograms were recorded as a relief pattern in the surface of a photoresist deposited on a Si wafer.

- Weight parameters for the simulated robot.
- Length of each body link.

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<sup>1</sup>The aluminum foil contours over the semicircular aperture to produce a variable height surface with the desirable characteristics for this test. The 100 nm aluminum foil has a transmission of approximately 35% at  $\alpha = 46.9$  nm considering the layer of native oxide 19 and effectively cuts the lower photon energy plasma emission from the Ar discharge in the laser source.

- Specifications of body joints. Upper Torso means the spine. Due to symmetry, body parts from the right are not shown.
- Neuron parameters.  $\Theta$  is the threshold,  $\Gamma$  is the gain.  $\tau_D$  and  $\tau_A$  are respectively the time constant of the dendritic sums and that of the frequency adaptation.  $\mu$  is the coefficient of frequency adaptation.

Holograms recorded in such a fashion can not be reconstructed in the conventional way with an optical reconstruction beam. In order to numerically reconstruct the holograms, the surface modulation was digitized with a Novascan atomic force microscope (AFM) operated in tapping mode. Holograms recorded in such a fashion can not be reconstructed in the conventional way with an optical reconstruction beam. In order to numerically reconstruct the holograms, the surface modulation was digitized with a Novascan atomic force microscope (AFM) operated in tapping mode.

1. Screenshot of the Webots simulation environment.
2. Schematic diagram of the simulated robot.
3. Schematic of the model lamprey CPG. Connections with a dot ending represent inhibitory connections while those with an arrow ending represent excitatory connections.
4. Sample output of a segmental oscillator (the 20th segment from the CPG). MNI and MNr respectively represents the output from the left and right motoneurons. Note the regularity of the neural pulses.

Two holograms digitized in this manner are displayed in Fig. 2. The digital reconstruction of the hologram digitized by the AFM is based on a numerical Fresnel propagator in Table ?? and ?. To obtain the amplitude and the phase distribution of the field in the image plane, the field emerging from the hologram illuminated by a plane wave is back propagated with the Fresnel-Kirchhoff integral. The integral was evaluated by the product of the spatial frequency representation of the hologram obtained through a two dimensional fast Fourier transformation and the quadratic phase free space Fresnel propagator in the spatial frequency domain.

**Theorem 1 (Einstein-Podolsky-Rosenberg)** *The back-propagation distance is determined by calculating the Fresnel zone plate (FZP) focal distance for the specific hologram geometry. For the specific geometry employed in this experiment, the FZP focal length is approximately the distance between the object and the recording medium. The digital images of the holograms processed with the Fresnel propagation code generated the reconstructed images shown in Fig. 2.*

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## 4 Conclusions

We have demonstrated that through detailed processing of the reconstructed holographic images, performed by changing the object-hologram distance in the reconstruction code, it is possible to discriminate depth in the object. Using a specially fabricated object composed of spherical markers 465 nm in diameter spread on a tilted transparent surface, the reconstruction and analysis of the hologram allowed to map the surface topography with a resolution close to 2  $\mu$ m, with such resolution depending on the particular NA of the exposure.

The lateral resolution of the image obtained by numerical reconstruction was assessed utilizing a wavelet image decomposition and image correlation. The best lateral resolution obtained with a high NA recording, 164 nm, represents an improvement of more than a factor two relative to previously published results.

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Review articles Maranduba, Carlos Magno da Costa; De Castro, Sandra Bertelli Ribeiro; Souza, Gustavo Torres de; Rossato, Cristiano; Da Guia, Francisco Carlos; Valente, Maria Anete Santana; Rettore, Joo Vitor Paes; Maranduba, Claudinia Pereira; Souza, Camila Maurmann de; Carmo, Antnio Mrcio Resende do; MacEdo, Gilson Costa; Silva, Fernando de S (2015). "Intestinal Microbiota as Modulators of the Immune System and Neuroimmune System: Impact on the Host Health and Homeostasis". *Journal of Immunology Research*. 2015: 931574. doi:10.1155/2015/931574. PMC 4352473. PMID 25759850.

De Preter, Vicky; Hamer, Henrike M; Windey, Karen; Verbeke, Kristin (2011). "The impact of pre- and/or probiotics on human colonic metabolism: Does it affect human health?". *Molecular Nutrition Food Research*. 55 (1): 4657. doi:10.1002/mnfr.201000451. PMID 21207512.

Prakash, Satya; Rodes, Laetitia; Coussa-Charley, Michael; Tomaro-Duchesneau, Catherine; Tomaro-Duchesneau, Catherine; Coussa-Charley; Rodes (2011). "Gut microbiota: Next frontier in understanding human health and development of biotherapeutics". *Biologics: Targets and Therapy*. 5: 7186. doi:10.2147/BTT.S19099. PMC 3156250. PMID 21847343.

Wu, G. D.; Chen, J.; Hoffmann, C.; Bittinger, K.; Chen, Y.-Y.; Keilbaugh, S. A.; Bewtra, M.; Knights, D.; Walters, W. A.; Knight, R.; Sinha, R.; Gilroy, E.; Gupta, K.; Baldassano, R.; Nessel, L.; Li, H.; Bushman, F. D.; Lewis, J.D. (2011). "Linking Long-Term Dietary Patterns with Gut Microbial Enterotypes". *Science*. 334 (6052): 10508. Bibcode:2011Sci...334..105W. doi:10.1126/science.1208344. PMC 3368382. PMID 21885731.