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Shedding light on the gut microbiome

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Learn how fluorescence microscopy can illuminate our gut microbiome and its role in cancer.

We live in a microbial world

A microbiome is a community of microorganisms present in any given environment. They inhabit nearly all habitats on the planet, from the upper atmosphere to the bottom of the ocean floor. They regulate carbon and nutrient cycling, animal (including human) and plant health, agriculture and the global food web.

Even our bodies are home to a vast microbiome consisting of trillions of bacteria, archaea, fungi and viruses. They help us digest our food, boost our immune system, produce vitamins[1] and even influence how we feel.[2] In fact, our microbiome is now considered an important organ we never knew we had.

The balancing act

Most of us dismiss microbes as germs that make us ill. In reality, most of our microbiome is friendly but some may become foes when disturbed. Let's imagine our microbiome as a sea of friendly and not-so-friendly microbes. A sea is calm unless a hurricane strikes. A healthy microbiome exists in harmony unless threatened by an external force, such as a pathogen or antibiotics. When this happens, the balance tips, which can lead to an overgrowth of bad bacteria – a condition called as dysbiosis. Dysbiosis has been linked to diseases such as diabetes, obesity, irritable bowel syndrome and cancer.[3] What exactly happens when our microbiome goes out of whack?

Piecing together the puzzle: a look into colorectal cancer and its link to the gut microbiome

The gut microbiome is the largest microbiome in our body. Colorectal cancer (CRC), or cancer of the gut, has intrigued scientists for decades because it is linked to a severely dysbiotic gut microbiome. With the help of sequencing technologies, scientists have been able to compare the gut microbiome of healthy people with that of CRC patients. Their results unanimously show an overgrowth of certain not-so-friendly microbial groups in the guts of the cancer patients.[4,5] Some of these bacteria were even found within the tumours of CRC patients, which suggests a breach in the intestinal barrier,[6,7] the lining of mucus and cells on the walls of the large intestine that prevents microbes from attacking our bodies.

Whether dysbiosis is a cause or symptom of CRC remains an unsolved puzzle. Gene sequencing technologies can tell us what microbes are within any given sample, but not where they are. Understanding the location and interactions of CRC microbes with cells in the colon is crucial to know how microbes break the intestinal barrier, migrate into colonic tis-

sue, and trigger cancerous signalling pathways that lead to the onset of CRC. Samples are broken down to extract nucleic acids prior to sequencing, which means that all information about location and tissue structure is lost. It is like acquiring all the pieces of a puzzle and trying to piece them back together in the dark.

Lighting up the gut microbiome

Fluorescence microscopy has the power to switch the lights on. By using a technique called fluorescence in-situ hybridisation (FISH), it is possible to light up different groups of microbes in different colours in a sample without destroying it. This is achieved by fluorescently labelling regions of bacterial ribosomal RNA that are unique to each group of bacteria.[8] The classic version of this technique uses one distinct fluorescent dye (fluorophore) per group.

Fluorescence microscopy

In fluorescence microscopy the sample is irradiated with light that matches the fluorophore excitation wavelength, which causes the fluorophore to emit light at a specific wavelength depending on its chemical structure. By filtering out the excitation light without blocking the emitted fluorescence, the fluorescence microscope selectively lights up the fluorescently labelled objects while the rest of the sample remains dark.

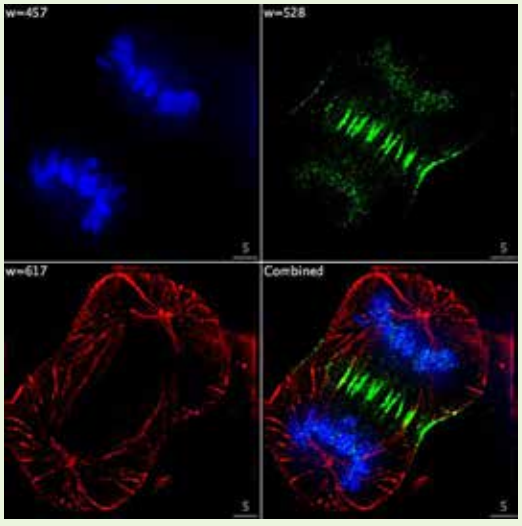
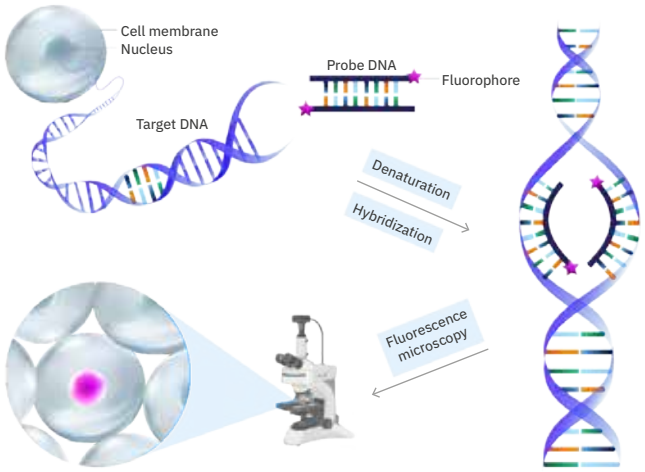


Image: F Lamiot/Wikimedia, CC BY-SA 4.0

Fluorescence microscopy images of dividing cells. The DNA has been labelled with a blue fluorophore, microtubules with a red fluorophore, and a protein that helps the chromosomes separate with a green fluorophore. In the final image, the three colour channels are combined.

Fluorescence in-situ hybridisation (FISH)

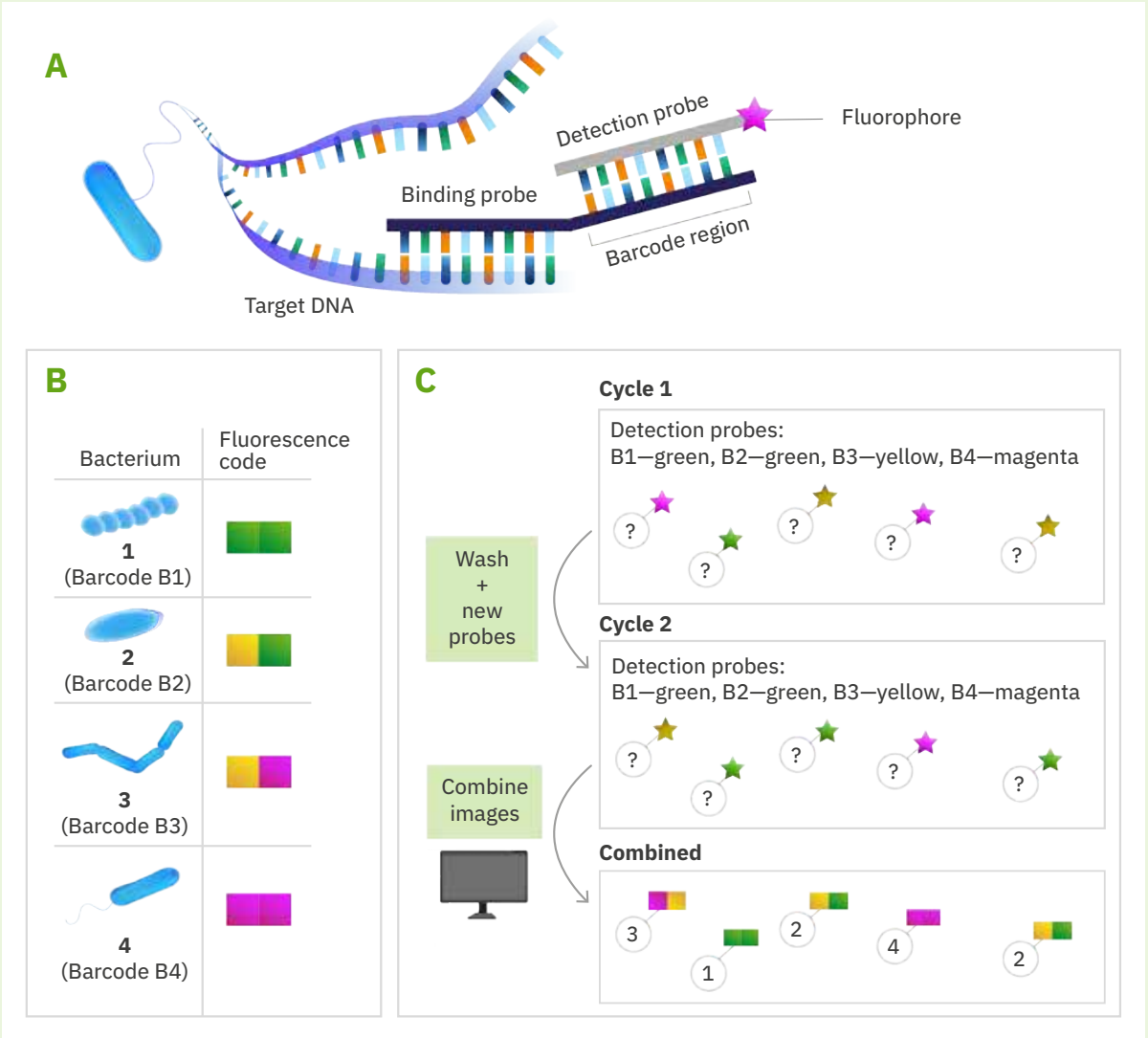
FISH is a fluorescence labelling method in which fluorophores are attached to DNA sequences (called probes) that are complementary to a target nucleic acid (e.g., a portion of a gene). The cell walls (for bacteria) and lipid membranes in the sample are permeabilized so that the probes can get into the cell/nucleus. The DNA is then denatured (the two strands are separated) so that the probes can bind. The fluorescently labelled probes are then added and hybridized with their complementary sequences through base pairing. If the target nucleic acid is RNA, it is first reverse transcribed to DNA by using reverse transcriptase enzymes.



However, the visible-light spectrum has a limited number of easily distinguished colours and since the efficiency of a fluorescence microscope relies on its ability to filter the excitation light from the emitted light, visualizing more than four different fluorophore colours simultaneously becomes a challenge. This limited colour palette is not sufficient to label a complex CRC microbiome with hundreds of different microbial groups. So, scientists came up with a way to harness the power of advanced fluorescence microscopy. Each bacterial group is labelled with a unique DNA sequence called a barcode, like the barcode on our supermarket products, and then unique sequences of fluorophore colours are used to light up each group of microbes to scan the barcode and identify the bacterial group.[9] The number of possible permutations is huge so the number of bacterial groups we can identify is no longer limited by the number of distinguishable fluorophore colours. For example, with four fluorophore colours in a sequence of five, the possible permutations are 4^5=1024!

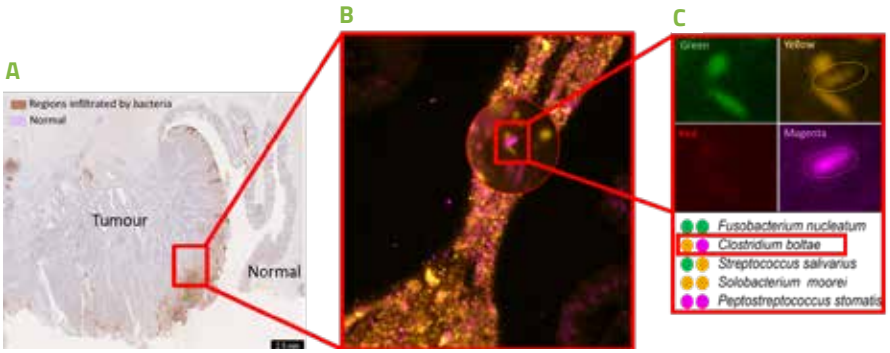
DNA-barcoded fluorescence microscopy

Instead of labelling the target nucleic acid with a single fluorophore, a specific DNA sequence called a barcode is used instead. Then to read the code, a set of fluorescently labelled detection probes is made for each barcode sequence. The barcode is decoded by using multiple cycles of hybridisation, with the detection probes removed before the next cycle. By choosing the colour of the detection probe for each barcode in each cycle and using a computer algorithm to combine the images at the end, you can assign each species a colour sequence to identify it in the sample.



A) A scheme showing the primary binding probe with its barcode and a secondary detection probe labelled with a fluorophore. Each barcode has a set of secondary probes of different colours.
B) If we want to detect four bacteria in a tissue sample, each one gets its own barcode DNA sequence (**B1**, **B2**, **B3**, **B4**). If you have three colours (red, blue, yellow), two cycles is enough so you assign each one a two-colour code, e.g., **B1**=green-green, **B2**=yellow-green, **B3**=yellow-magenta, **B4**=magenta-magenta.
C) The detection cycles for the example described in (B). In the first cycle, we add the secondary probes **B1**-red, **B2**-red, **B3**-blue, and **B4**-blue. The probes are washed off and in the second cycle we add **B1**-red, **B2**-yellow, **B3**-yellow and **B4**-blue. Computer software lines up the images to give the colour sequence for each spot of light and, based on that, we can identify the bacteria at each location.

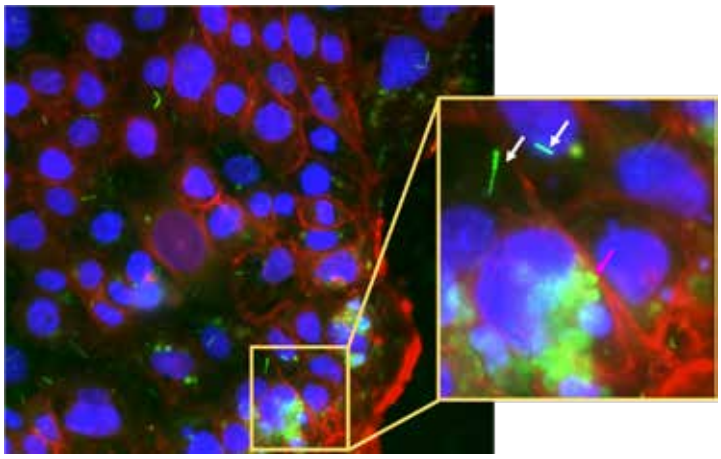
Using cutting-edge fluorescence microscopes, researchers at EMBL are now harnessing FISH to visualize hundreds or thousands of bacteria directly within a colon tumour. Bacterial groups relevant to CRC are assigned unique barcodes, fluorescently tagged with unique combinations of colours within the colon tumour tissue, and imaged on a fluorescence microscope. The images are decoded to reveal the identity and location of each bacterial group within the tumour.



A) Cross-section of a colorectal tumour with brown-stained regions indicating a gut bacteria invasion. B) An enlarged view of the boxed region in A, showing bacteria that have been labelled with FISH barcodes. C) An enlarged view of the circled region in B, showing FISH-barcoded bacteria. Four different fluorophores have been used for the barcodes. The circled bacterium can be seen only in the yellow and magenta channels. The FISH barcode list shows that this bacterium is Clostridium boltae, a prominent player in CRC.

Image courtesy of Sebastian Degner

This barcoded FISH method can also be used to view cancerous colonic cells and immune cells gate-crashing the party! The puzzle starts to come together. But wait, it gets better: what if we could study the whole system of microbes infecting colonic cells in the lab? Scientists have successfully grown mini-colons on a plate and infected them with fluorescently labelled cancer-associated bacteria to observe the processes of infection in real time by using fluorescence microscopy.[10] This can potentially take the puzzle to near completion.



A laboratory model for investigating the role of the gut microbiome in CRC. CRC-associated bacteria were labelled with green fluorescent dye and added to colon cells grown in a petri dish. The nuclei and cell membranes of the colon cells are labelled with blue (Hoechst) and red (Phalloidin) fluorescent dyes, respectively. The enlarged view shows green bacteria sticking to the surface of the cells (white arrows) and some that have already entered the cells (magenta arrow).

Image courtesy of the author

The exciting future of microbial fluorescence imaging

As amazing as it sounds, fluorescence microscopy does face some challenges. One major pitfall is the inability to distinguish true and false signals. False signals can come from fluorescent compounds inside a cell or artefacts introduced during sample processing. This issue can be tackled successfully with careful experimental design and efficient image-processing programs that can selectively remove background fluorescence. With its ability to illuminate the invisible world inside our guts, fluorescence microscopy has the power to visualize how microbes shape our bodies spatially, a dimension that is wholly absent in other approaches. An era of next-generation fluorescence microscopes with the ability to capture biological events in ultra-high definition within living organisms is approaching. It should soon be possible not only to visualize microbes colonizing your favourite model organ, but to zoom into each cell and witness cellular events simultaneously. It is like acquiring a cellular map with all necessary details to solve any biological puzzle. This enhanced understanding of how microbes contribute to cancer development could be used to develop new treatments or preventative measures. «

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Resources

- Watch this short video from the microbiology society about microbiomes.
- Watch a video about your own body's microbiome.
- Read about Bacterial rRNA FISH approaches.
- Read about the DNA-barcoded FISH approach that was used in this research and some of its applications.
- Try out fluorescence microscopy in school using the microscopes in action kit: Arevalagam H, Gaikwad S (2022) Colours in the dark: fluorescence microscopy for the classroom. *Science in School* **58**.
- Learn about the importance of animal use in research and some cutting-edge approaches to minimizing it: Schmerbeck S (2021) Organ-on-chip systems and the 3Rs. *Science in School* **54**.
- Read about antibiotic resistance and drug development: Fernandez MD, Soler ML, Godinho T (2021) Microbiology: Discovering antibacterial agents. Science in School **55**.
- Explore toxicology and the physiological effects of drugs using Daphnia as a model organism: Faria HM, Fonseca AP (2022) From drugs to climate change: hands-on experiments with Daphnia as a model organism. *Science in School* **59**.
- Teach students how to collect relevant data regarding a gene from biological databases: Grazioli C, Viale G (2022) A chromosome walk. *Science in School* **57**.

AUTHOR BIOGRAPHY

Ruby Priyadarshini Ponnudurai, a postdoctoral researcher at EMBL, Heidelberg, focuses on understanding how the human microbiome infiltrates colorectal tumours. She develops techniques to label and image microbes by using fluorescence labelling methods, with the aim of exploring how microbes interact with colon cells to trigger the development of colorectal cancer.