

Fluorescent imaging of bacteria and friends: providing tools for enhancing microbiome and microbiological research

MERCK

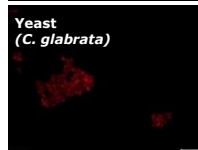
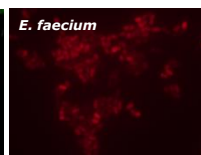
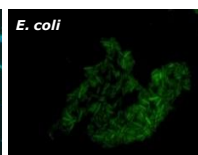
Nomi Barda, Avner Fink, Kezia Valyi, Cheli Khalif, Maya Aviram, Meirav Hyams, Alon Haim Sayer, Gil Yeroslavsky, Aviran Amir, Yael Vazana, Shirley Satanower.

Microbiome products department, Merck, Jerusalem, Israel

Fluorescent microbial imaging has emerged as an indispensable tool in microbiological research. The ability to identify and describe specific bacteria and other microorganisms has been instrumental in the emerging field of the microbiome. Furthermore, multiplexing combined with high resolution microscopy enables in-depth microbial community characterization in situ. Here, we examine different synthetic tools that we offer for the highly evolving microbiologist toolbox: fluorescent antibiotics, fluorescent metabolic labeling and specific FISH probes for bacteria and fungi. These fluorescent imaging tools are engineered to provide data on an extensive set of topics, ranging from bacterial colonization dynamics, host physiology and clinical diagnostics to antibiotics mode of action (MOA), antibiotic resistance and toxicity. We will focus on our currently available products and strategies, as well as on future perspectives in this field. We strongly believe that enhancing and diversifying high quality fluorescent imaging tools for microbiological research will inevitably open new venues for high-standard and complex microbiome-related research.

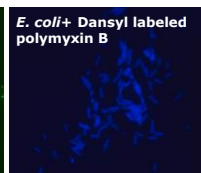
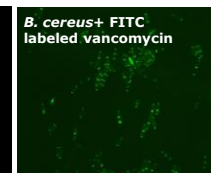
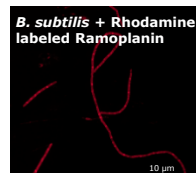
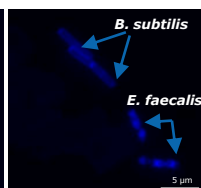
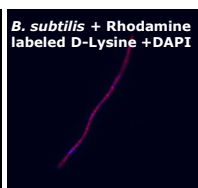
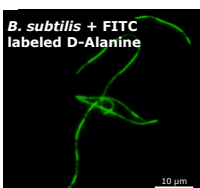
FISH probes for the detection of specific Bacteria and Fungi.

Specific FISH probes targeting bacteria or yeast were designed and optimized for highly specific staining. Some examples include (from left to right) *P. vulgaris* and *P. mirabilis* (a non-specific, all *Eubacteria* probe + DAPI), *E. coli* and *E. faecium* specific probes. Lower panel shows 2 candida species- *C. glabrata* (left) and *C. boidinii* (right) with a yeast specific probe. A recent paper highlighted the role of tumor intrinsic mycobiome in shaping a specific immune response¹.



Metabolic labeling: a novel tool to explore host-microbe interactions.

Using unique molecular 'building blocks' allows for broad range labeling of bacteria *in vivo*. This system has been used to show *B. fragilis* polysaccharide A trafficking *in vivo*² and specific interactions of bacteria with the immune system³. Recently, it was also shown that exposing a mixture of species to 3 different metabolic labels allows for the identification of each one by spectrometry *in vivo*⁴. Examples show *B. subtilis* with FITC-D-Alanine or Rhodamine-D-Lysine and DAPI (left and center, respectively). Pictures shows distinctively stained cell membrane, with intense signal at cell division sites. A mixed sample of *B. subtilis* and *E. faecalis* stained with Coumarin-D-Lysine enables bacterial differentiation (right).



References:

1. Narunsky-Haziza L. et al., Cell 2022 doi:10.1016/j.cell.2022.09.005.
2. Geva-Zatorsky N. et al., Nat. Med. 2015 doi: 10.1038/nm.3929
3. Hudak J.E. et al., Nat. Microbiol. 2017 doi: 10.1038/nmicrobiol.2017.99.
4. Hong S. et al. Chem. Sci. 2020. doi: 10.1039/d0sc01704c
5. Tiyanont K et al., PNAS 2022. doi: 10.1073/pnas.0600829103
6. Pereira P.M. et al., Antimicrob. Agents. Chemother. 2007 doi: 10.1128/AAC.00431-07

Antibiotics-conjugated fluorescent probes: Fluorescently labeled antibiotics provide a tool to rapidly stain live bacteria *in vitro* or *in vivo*, with specificity towards either gram negative or positive bacteria, allowing for monitoring of peptidoglycan biosynthesis⁵ and antibiotic resistance⁶.

[SigmaAldrich.com/microbiome](https://sigmaaldrich.com/microbiome)

FISH Probes:

Eubacteria probe MBD0032, MBD0033
E. coli MBD0042, MBD0042
E. faecium MBD0047, MBD0048
Yeast specific probe MBD0051

Antibiotics:

Rhodamine labeled Ramoplanin SBR00047
FITC labeled Vancomycin SBR00028
Dansyl labeled polymyxin-B SBR00029

Metabolic labeling:

FITC-D-alanine SBR00049
Rhodamine-D-Lysine SBR00069
Coumarin-D-Lysine SBR00070