FISHing of gut microbiota with the NanoZoomer S60

Risson A., Tamellini A., Darnaud M.

BIOASTER, a new model for technology innovation in microbiology

The BIOASTER Technology Research Institute (TRI) was created in April 2012 by the Institut Pasteur and Lyonbiopole health competitiveness cluster, following the initiative of the French Government through the "Investissements d'Avenir" Program.

BIOASTER is leading collaborative projects that bring together academics, start-ups, SMEs and industrial groups, and is developing a unique technological and innovative model in order to overcome technological bottlenecks and explore new avenues applied to microbiology in health and diseases. In this context, the Preclinical Models & Imaging Unit tested the Hamamatsu NanoZoomer S60 with its fluorescence module, to evaluate its relevance for bacteria imaging in relation to the study of host-microbiota and microbes-microbes interactions.

Bringing the gut microbiota into focus

Histological processing can alter tissues so several sections are often placed on the same microscopy slide in order to maximize the acquisition of views. One great advantage of the NanoZoomer is its capacity to scan the whole microscopy slide with the possibility afterwards to zoom anywhere within the scanned image to look just as well at a whole tissue section (Figure 1) as at a specific subcellular site (Figure 3).

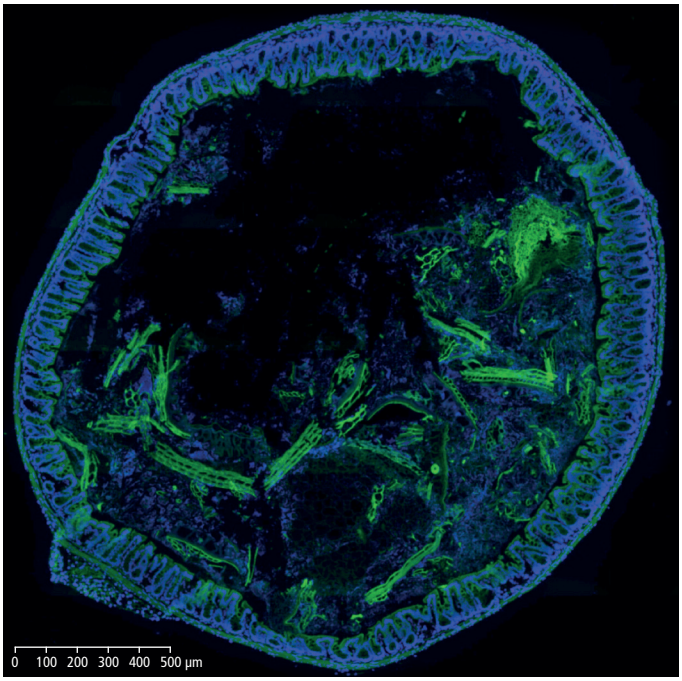

Figure 1: Whole mouse colon section stained with DAPI (blue) showing high level of green autofluorescence from the gut epithelium and lumen, which can be a limitation for imaging samples with green channel (FITC). 20x objective, 4x view.

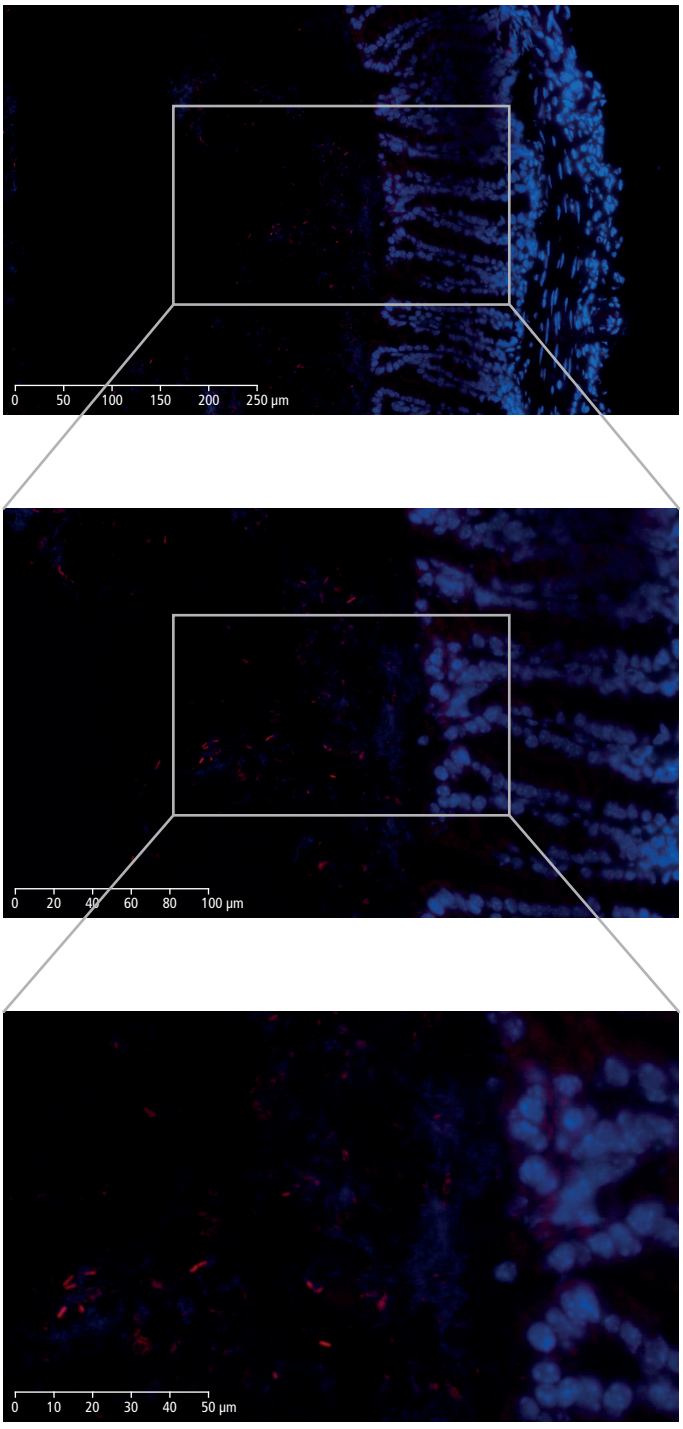

Figure 2: Mouse colon section showing epithelial cell nuclei stained with DAPI (blue) and Lactobacilli distribution within the lumen, detected by the FISH probe LGC0355 labeled with the fluorophore CY5 (red). 20x objective (above), magnification to 40x (middle) and additional numerical zoom to 80x (below).

Hamamatsu Photonics Europe GmbH
Phone: +49 (0)8152 375-0 · Fax: +49 (0) 8152 2658
www.hamamatsu.eu · E-mail: info@hamamatsu.eu

In addition to high quality magnification, the NanoZoomer offers two essential features, an automated focus with the possibility to delete or add manually focus check points on the epithelium, and Z-stacking for samples with 3D structures such as bacteria. We found that 0.5 µm z-steps travelling from -0.5 µm (below focus point) to +1.5 µm (above focus point) were optimal for mouse colon sections of 4 µm.

Another great value of the NanoZoomer is the possibility to detect 6 different fluorescent probes or more, optimize individual intensity and potentially modify colors to improve clarity, allowing for co-staining of different targets, but it also makes it possible to overcome some limitations due to the green autofluorescence of the intestinal epithelium (1) and lumen, which might mask bacteria specific signals (Figure 1).

Imaging of Lactobacilli detected by specific fluorescent in situ hybridization (FISH) in mouse colon sections

Increased evidence that gut microbiota is a key factor in human health and diseases opens a new golden age in microbiology. From pathologies to phenotypical features, its range of action holds promises which have to be unveiled in the near future. However, the gut microbiota is an ecosystem that has proved itself to be a lot more complicated than what was expected. As a result, the development of simplified models, but conserving the key features of original models, is of high necessity in enabling scientists to study the causal link between microbiota changes and the host response (2, 3).

In the context of an internal innovation project at BIOASTER which aimed to establish such a gnotobiotic mouse model, we set up FISH imaging activities in order to highlight host-microbiota and microbes-microbes interactions. Hereinafter, we show images of a mouse colon section stained with the FISH probe LGC0355 targeting Lactobacilli, Firmicutes (Figure 2), which are among the most common bacteria in the mouse gut, but also in the intestine of humans and widely used as probiotics.

Combining immunofluorescence (IF) and bacterial FISH to highlight the intestinal barrier

Next, we additionally stained the mucosal barrier in order to inspect the border between the intestinal epithelium and the microbes (Figure 3). The mucus secreted by Goblet cells protects the epithelial cells against damage, but also accommodates Lactobacilli which have adhesion properties on mucins, allowing suitable interaction with the host to confer health benefits (4).

In conclusion, the NanoZoomer S60 provides high quality fluorescence imaging of gut microbiota comparable to confocal microscopy, with improved reproducibility and time management thanks to automated scanning and a proper slide capacity. On top of that, the same equipment allows brightfield imaging, and easy-to-use software offers full options analysis, including interactive data sharing with collaborators.

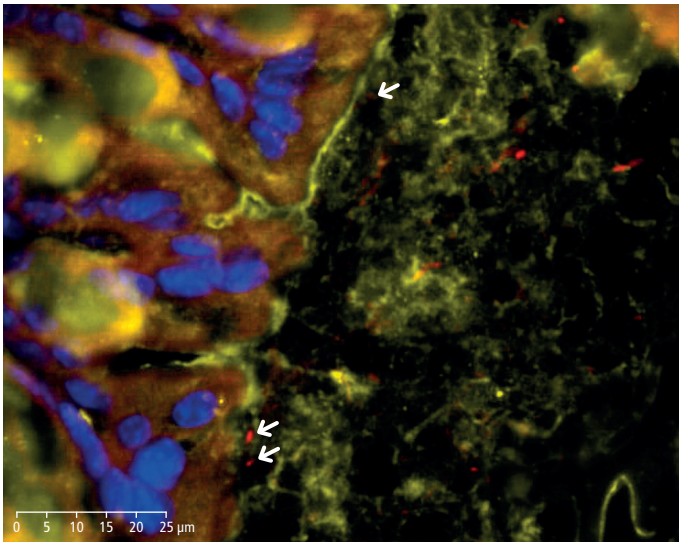

Figure 3: IF staining of Muc2 secreted by Goblet cells (orange, TxRed) in mouse colon section showing the adhesion of Lactobacilli (red, CY5) to host surfaces (epithelial cell nuclei stained with DAPI, blue) through mucus-microbe interactions (arrows). 20x objective, 80x view.

For further information see:

[1] Monici M. (2005) "Cell and tissue autofluorescence research and diagnostic applications." Biotechnol Annu Rev 11, 227.

[2] Stappenbeck TS, Virgin HW. (2016) "Accounting for reciprocal host-microbiome interactions in experimental science." Nature 534, 191.

[3] Martin R, Bermudez-Humaran LG, Langella P. (2016) "Gnotobiotic Rodents: An In Vivo Model for the study of Microbe-Microbe Interactions." Front. Microbiol. 7, 409.

[4] Van Tassell ML, Miller MJ. (2011) "Lactobacillus Adhesion to Mucus." Nutrients 3, 613.

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Phone: +49 (0)8152 375-0 · Fax: +49 (0) 8152 2658
www.hamamatsu.eu · E-mail: info@hamamatsu.eu