W6.2 | Combining nucleic acid mimics and spectral imaging with fluorescence *in situ* hybridization for the analysis of the gastric micro-biogeography

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Nucleic acid mimics (NAMs)-based assays, such as locked nucleic acid/2′-O-methyl-RNA-fluorescence *in vivo* hybridization (LNA/2′OMe-FISH), have been developed for the identification and spatial location of *Helicobacter pylori* directly in the stomach. While *H. pylori* is considered the main gastric pathogen, there is a diverse range of stomach colonizers that may be associated with disease in the stomach.

In this work, giving the enhanced hybridization properties of NAMs, we intend to combine them with FISH and spectral imaging, in one technique. This technique, designated as NAM-CLASI-FISH, will allow the evaluation of the gastric micro-biogeography.

We have selected 8 fluorochromes with distinct spectral properties and 8 mouse gastric bacterial species. To control thermodynamic parameters, LNA/2′OMe probes coupled with the different fluorochromes were used. Universal Eubacteria LNA/2′OMe probe sequences were used to rank the species/fluorochromes. Mixed samples were analyzed by Leica TCS SP5 Confocal; a linear unmixing algorithm was applied to identify the fluorochromes present in each pixel of the image. Lastly, the procedure was validated using mixed bacterial populations to evaluate its potential for quantifying different targets in a sample.

A strong variation on the fluorescence intensities was found between species and between fluorochromes, which were balanced by matching “weaker” species with “stronger” fluorochromes and vice versa. Validation tests with different proportions of bacteria labelled with the different fluorochromes have shown the method ability to correctly distinguish the different relative proportions of bacteria. Future work will focus on imaging and unmixing of unknown gastric samples labeled with the fluorochromes tested.

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