

# FISHing

A circular inset in the upper left portion of the image shows a school of four fish swimming in an aquarium. The fish are silver with prominent orange and red stripes on their sides and tails. They are swimming in clear water with green, feathery artificial plants in the background. The rest of the image has a dark blue background with a faint, repeating pattern of fish scales.

For  
Answers

**GEO**

# FISH

(Fluorescence In-Situ Hybridization)



"**FISH**" (Fluorescence in-situ Hybridization) is a powerful tool for investigating the relative abundance and physical characteristics of different populations of microorganisms. **FISH** combines fluorescence microscopy with oligonucleotide probes--precisely designed DNA sequences conjugated with fluorescent molecules that are used to "tag," visualize and quantify specific microbes of interest.

Geovation's multi-color **FISH** ("mFISH") lab uses multiple probes with different color fluorochromes to simultaneously provide quantitative information on different sub-populations in the same sample. Geovation offers several mFISH assays to provide relatively rapid and cost-effective enumeration of important groups of microorganisms relative to other methods. mFISH also enables the simultaneous visualization of the spatial arrangements of microbes in mixed consortia to investigate the potential for mutualistic or syntrophic interactions among different organisms.

**FISH** provides data useful for applications ranging from biological monitoring of monitored natural attenuation ("MNA") programs, bioremediation monitoring and process optimization, bacterial source tracking ("BST"), medical and public-health microbiology, and problem solving and process monitoring for waste-water treatment.

## Capabilities & Utility of Geovation's mFISH Lab Services

Quantify total microbial cells, specific microbes and groups of microbes at different taxonomic levels: **Domain - Phylum - Class - Order - Family - Genus - Species**

Spatial mapping of population abundance (e.g., ground-water plumes, BST investigations)

Evaluate shifts in target populations over time

Detection limits: approx.  $10^4$  cells / mL (2 mL sample aliquot)

Investigative problem-solving in microbial ecology:

Bioremediation and MNA programs

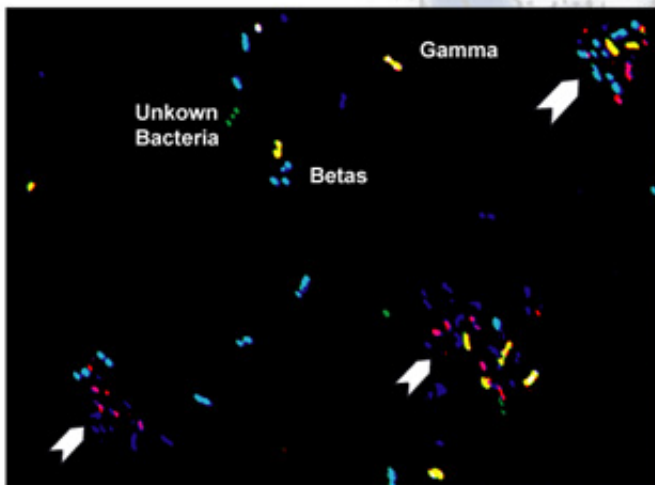
Waste-Water Treatment

Bacterial Source Tracking ("BST")

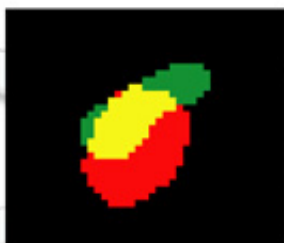
Public Health Microbiology

Gut microbiology (investigative tool for

ulcerative colitis, Crohn's disease, ulcers)

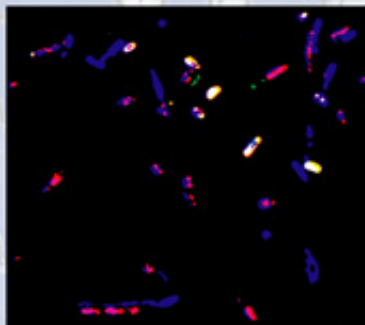


False-color RGB composite bitmap image of a "MP1" mFISH assay showing denitrifying bacteria from a gasoline-contaminated aquifer. Aqua cells are beta-proteobacteria (dual hybridizations with 'blue' BET42a and 'green' EUB338 probes), yellow cells are gamma-proteobacteria (dual hybridizations with 'red' GAM42a and 'green' EUB338 probes) and green cells are undifferentiated bacteria (single hybridizations with 'green' EUB338 probe). Red, purple, and blue objects are noise from cells within biofilms (arrows).



**Evidence of Syntrophism?** Rod-vibrio shaped delta-proteobacteria cell (Green) attached to coccoid *Dehalococcoides ethenogenes* ("DHE") cell (Red). Yellow area is where cells overlap. False-color composite image, "MP5" mFISH assay, sample from CVOC contaminated aquifer undergoing active anaerobic bioremediation. DHE cells represent about 9% of total DAPI-stained cells.





#### **Abundant Archaea?**

Tot. Bacteria (Red-Violet) and beta-proteobacteria (Yellow-White) cells are differentiated from other DAPI-stained cells (Blue) that did not hybridize with either the EUB338 or BET42a probes. *False-color composite image from a "MP1" mFISH assay performed on a ground-water sample from a TCE-contaminated aquifer. Bacteria represent about 70% of total DAPI-stained cells, beta-proteobacteria about 30%.*

*Many of the 30% of DAPI-stained cells that did not hybridize with EUB338 may be archaea. Little is currently known about psychrotolerant archaea in the environment and their role in biodegradation and bioremediation.*

## **Geovation's FISH Lab Fast-Facts - What is it all about?**

### **Procedures**

Sterile sample vials with fixative / preservative are shipped to the client under standard chain-of-custody procedures

Water and ground-water samples are collected and "fixed" in the field, kept cool and returned via overnight courier to Geovation (soil samples or samples containing solids require special handling and preparation procedures - additional charges may apply)

In the hybridization lab, samples are filtered, hybridized with fluorochrome-labeled oligonucleotide probes (multiple probes & colors for mFISH assays), washed, DAPI stained and mounted onto slides

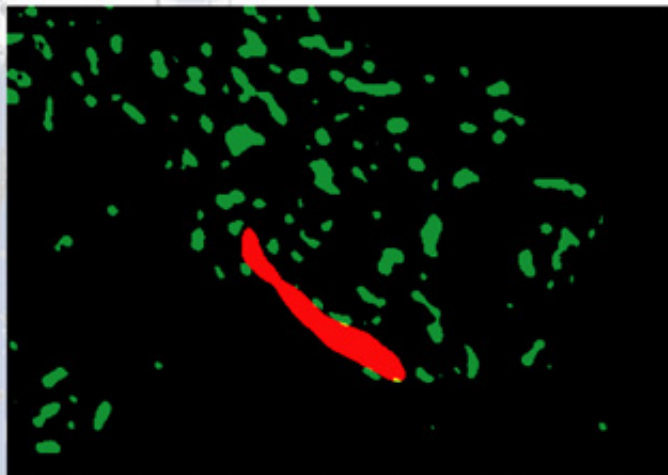
A computer-controlled fluorescence microscope is used to visualize and CCD image the samples for subsequent computer-assisted, semi-automated digital image processing, cell counting & classification

### **FISH Data / Reporting**

Reports are provided in Excel or pdf format with data for each target group reported in units of cells/mL and % of DAPI counts

Each FISH assay provides a minimum of 2 to 4 "data points" per sample -- total cell counts (DAPI staining) along with the cell counts and relative percentages of DAPI counts for each target population

Several "mFISH" assays are available that provide simultaneous data for 2-4 target populations plus total cell counts (DAPI)



Dimorphic yeast (Red) among bacterial cells (Green) showing through biofilm EPS (Black - removed); MP3 assay, gasoline plume

## Examples of FISH Assays / Parameters

### High-Level Taxonomic Groups

Total bacteria; alpha-, beta-, gamma- and delta-proteobacteria; CFB (cytophaga-flexibacter-bacteroides); high and low GC gram+ bacteria

Total Archaea; Crenarcheota; Euarcheota; Total yeast / fungi

### Targeted Groups - Family / Genus / Species Levels

Important subgroups of proteobacteria

*Pseudomonas* spp., *Bacteroides* spp., *Bifidobacterium* spp.

*Dehalococcoides ethenogenes* (DHE)

### Multi-parameter mFISH Assays

MP1 Tot. DAPI; Tot. bacteria; Tot. beta- and gamma-proteobacteria

MP3 Tot. DAPI; Tot. bacteria; total yeast

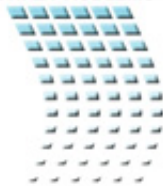
MP5 Tot. DAPI; Tot. CFB; DHE; delta-proteobacteria

MP7 Tot. DAPI; Tot. archaea; Tot. crenarcheota; Tot. euarcheota

MP8 "BST" applications - Tot. DAPI; human-related CFB; ruminant-related *Fibrobacter* spp.; *Campylobacter* spp. (geese)

Many probes and several "MP" multi-color, mFISH probe combinations available. Custom assays available on request.

 **VATION**  
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