Abstract
Molecular methods are still relatively ineffective for monitoring community dynamics during bioremediation, due primarily to the cost, technical difficulty, and reproducibility of the analyses. For nucleic acid analyses to meaningfully contribute to bioremediation efforts, they must not only contribute to the fundamental understanding of microbiological ecology, but also be formatted in such a manner that in-field analysis can be achieved. The objective of this project is therefore to develop an integrated microbial and nucleic acid detection method and prototype system for the characterization and analysis of subsurface sediments, focusing on the molecular detection of metal- and sulfate-reducing bacteria and activity in sediments obtained from the Oak Ridge Field Research Center. We are meeting this objective by combining environmental molecular microbiology with renewable surface techniques, microfluidic systems and microarray-based analytical chemistry. The fluidic systems are used to evaluate hypotheses on the integrated biosensors that is necessary to directly detect 16S rRNA from metal-reducing microbial communities on a suspension microarray, without using the polymerase chain reaction (PCR). These investigations include the use of peptide nucleic acid capture and detection probes and “tunable surface” concepts to increase nucleic acid capture and detection efficiency and mitigate interferences due to co-extracted humic acids. The unified microfluidic sample preparation method and suspension array is then used to characterize the 16S rRNA metal- and sulfate-reducing microbial community in FRC sediments before and after bio-stimulation.

Reseach Questions, Hypotheses, Milestones
- Develop automated, integrated nucleic acid purification and detection protocols for color-coded, 5 µl microarrays and subsurface sediment extracts.
- Investigate the underlying microfluidic surface chemistry required for efficient rRNA recovery and detection in subsurface sediment extracts.
- Quantify the extent of cross-hybridization or fluorescent interference, and quantify the abundance of microorganisms (or RNA) in uncharacterized environmental extracts. In some environmental samples (e.g. FRC sediment), it may also be possible to directly extract sediment, hybridize and analyze the array without additional sample cleanup.
- Perform on-field analysis for the integrated biosensor that is necessary to directly detect 16S rRNA from metal-reducing microbial communities on a suspension microarray, without using the polymerase chain reaction (PCR).
- Utilize these technologies to characterize the microbial community in metal-contaminated sediments from the Field Research Center and associated microcosm experiments, both before and after bio-stimulation.

Fluidic Systems
Integrated Particle Handling Methods for Multiplexed Microbial Identification and Characterization in Sediments and Groundwater

Taking the Array to the Field

Validating a FeRB and SRB array

Research Questions and Uncertainty

4. What is the threshold (in an environmental sample) where signal becomes ecologically important or meaningful?

5. How do we know if these signals are specific or not given an uncharacterized environmental background? The validation arrays access specificity that can be achieved, but how precise do we need to be when making inferences about the environment?

6. What is the critical mass signal for target organisms relative to their abundance in the environmental sample? For example, 300 ng of total RNA (1-27 organisms) on the validation array generated average MFI of 250-400, and non-specific control hybridization signals at ~25-50 MFI. Most of the signals observed in the environmental sample are not much higher than 50 MFI.

7. What types of internal and external controls will be required to determine thresholds, quantify the extent of cross-hybridization of fluorescent interference, and quantify the abundance of microorganisms (or RNA) in uncharacterized environments?

Project Conclusions
1. Tarnable surface chemistry enhances direct RNA detection (limits and hybridization kinetics to microminutes).
2. Chaperone probes and RNA fragmentation are necessary for hybridization specificity, but cross-hybridization cannot be predicted based upon primary nucleic acid sequence alone.
3. Integrated sample preparation and detection chemistries have been automated in a fluidic system that is compatible with in-field, autonomous monitoring applications.
4. The ecological application and interpretation of RNA arrays still require the development of internal and external standards and statistical models to account for unpredictable cross-hybridization in uncharacterized environmental samples.

Acknowledgements
This project was funded by the Department of Energy under DE-FC26-01NT41443 and the Stanford University, Department of Civil and Environmental Engineering.