Executive Summary of GTL Workshop on Imaging Technologies Held April 16–17, 2002

"Thought is impossible without an image."
Aristotle, 325 B.C.

DOE's Genomes to Life program seeks to understand the composition and function of biochemical networks and pathways that carry out the essential processes of living organisms. Such understanding is critical for DOE to more effectively address its missions—clean energy, carbon management, bioremediation, and mitigation of bioterrorism. Imaging of living organisms links the genome to function and recognizes many of the steps along the way to understanding how cell function changes with time and environmental challenges. Innovations in imaging, coupled with computational advances, will accelerate scientific discovery and enable biological solutions to energy challenges.

GTL has four main goals:
1. Identify and characterize the molecular machines of life—multiprotein complexes that execute cellular functions and govern cell form.
2. Characterize gene regulatory networks.
3. Characterize the functional repertoire of complex microbial communities in their natural environments at the molecular level.
4. Develop computational methods and capabilities to advance understanding of complex biological systems and predict their behavior.

Imaging Requirements
Current imaging techniques provide a wealth of information about eukaryotic (e.g., human) biological systems over a wide range of length and time scales. Imaging of these systems has led to significant advances in understanding cell function and complex cellular systems. Microbial systems with their smaller cells, however, present different challenges. New techniques are needed that will connect genomic information with microbial functions spatially and temporally in model systems and in their natural environments. These new techniques will drive further advances in *all* biological systems.

Imaging and the Molecular Machines of Life (GTL Goal 1)
Imaging will contribute directly to identifying and characterizing the molecular machines of life and understanding their relationships by defining interactions among proteins and other cellular components in the complex networks of living cells. A real-time, molecular-scale description of protein interactions will reveal metabolic relationships that can be engineered to accomplish DOE missions. High-throughput methods (e.g., mass spectrometry) for characterizing protein complexes require validation of the existence and function of these complexes in living cells. Imaging can provide that validation.

New imaging methods will define the states of biological systems in response to differing environmental conditions to enable the functional interpretation of traditional analyses of protein complexes. Imaging provides a direct link between the genomes of microorganisms and the atomic structures of the molecular machines that define their functions. Direct observations of these protein complexes provide an important link between genome knowledge and living cell function. Realizing these potentials will require innovative probes to visualize the structure and dynamics of molecular machines and to locate specific proteins. This will entail substantial innovation in developing spectroscopies that enable measurements of dynamics (function); microscopies with sufficient spatial resolution and sensitivity to image individual proteins; methods to resolve their atomic structures; and computational technologies to acquire, store, access, visualize, and interpret results (see GTL Goal 4).

Imaging to Characterize Gene Regulatory Networks (GTL Goal 2)
Imaging the location of each regulatory protein in vivo to identify its corresponding DNA binding sites is needed to understand the primary step of the complex gene regulatory network. The identities of most of these regulatory proteins are unknown. Methods must be developed that can interrogate DNA-protein bound pairs on virtually instantaneous time scales during the cycles of cell growth and function.
To understand the gene regulatory network's function, the dynamic timing of gene expression is needed as a function of cell cycle and stimulating signals. This requires development of small fluorescent expression tags that can be imaged without delay in vivo. To design computational models of gene regulatory networks in particular, we

must know the temporal path for expression and intracellular distribution of the regulatory proteins themselves. Obtaining knowledge of gene regulatory networks for both microorganisms and eukaryotic systems and imaging each gene product's expression schedule and subsequent distribution provide the basis for understanding the molecular machines of life and their coordinated function in complex microbial communities in their natural environments. Gene regulatory networks actually act as a digital (molecule-by-molecule) computer to specify the target genes' identity and level of expression. Computer models must be developed to enable broad interpretation of experimental results, leading to predictions of biological function (see GTL Goal 4).

Imaging to Characterize Complex Microbial Communities in Model and Natural Environments at the Molecular Level (GTL Goal 3)

The past decade's advances in techniques for imaging living biological material have shown that microbial communities are dynamic, structured assemblages with compartmentalized (e.g., metabolic) activities. Imaging methods enable the interrogation of these spatially and temporally organized features in a multiplexed manner with multiple imaging modalities. Current techniques also have shown that more innovative approaches are needed to understand the rules governing the structure, communication, and metabolic activities of these complex groups. Understanding how community function is distributed among its members in a complex environment and the molecular basis for these different functions is critical in connecting molecular expression data to overall function. To bridge this enormous gap of complexity, relatively simple and well-characterized model systems must capture essential functional aspects of natural communities.

Fig. 1. Confocal laser micrograph of a bacterial microcolony in a river biofilm community. The colony is stained with the electrical-potential-sensitive fluorescent stain JCI; orange regions are areas of high potential.

Scientists must understand what's going on in naturally occurring communities: constituent species, their functions in the community, how they perform these functions, and how the communities change over space and time. This must involve the development of an advanced suite of probes, imaging devices, and computational methods (see Goal 4). Understanding the complexity of natural systems will require their direct study, coupled with research on more controlled model systems.

Imaging and Developing Computational Methods and Capabilities (GTL Goal 4)

New computational methods and capabilities are needed to mine and manage image data, enable visualization, and make possible the quantitative image analysis of biological systems and their components, leading to predictions of function.

Recommended Investment Strategy and Timeline for Accomplishments

Recommendations call for a GTL imaging program that integrates technical approaches and biological needs. This program should draw on capabilities from other disciplines, advance existing methods to address the GTL program's unique requirements, and immediately initiate research toward the most significant imaging challenges, including research with high risk and high potential payoff. The GTL imaging program should include single-investigator projects, multi-investigator and multi-institutional research programs, funds to develop and maintain essential capabilities at DOE national laboratories, and investments in education. Bringing together scientists from different backgrounds is essential for creating innovative, interdisciplinary approaches to critical technical challenges. Accomplishments are expected within the following time frame:

- **2 to 3 years:** Capture, integrate, extend, and apply existing imaging technologies not currently applied to microbes.
- **5 years:** Identify fundamental limitations and bottlenecks to reaching the potential of existing imaging technologies; develop capabilities for converting these potentials to practice; develop a pathway for addressing the most challenging potentials.
- **20 years:** Develop technologies to quantify functions of machines, cells, and communities in real time, in situ with

minimal perturbation to the systems. Apply these technologies to relevant organisms and establish predictive models.

Impact of Imaging on GTL Goals and DOE Missions

Imaging, coupled with computational modeling and the use of genomic, proteomic, and related analytical information, will quantify functions of machines, cells, and communities, which in turn will enable the use of microbial systems to solve problems related to DOE missions. Visualization and quantitative image analysis of biological systems and their components provide a level of understanding that cannot be obtained in any other manner.

Fig. 2. Microbes use organics (in this case toluene, a subsurface contaminant itself) to convert a soluble, carcinogenic form of chrome (chromate CrO_4^{-2}) to its innocuous, insoluble state.

NOTE: Suggested reading list is on the Web (URL).

Organizers:

Steve Colson
steven.colson@pnl.gov
Pacific Northwest National Laboratory

Damir Sudar
DSudar@lbl.gov
Lawrence Berkeley National Laboratory