Title	PI	Institution	Location	Synopsis
Biosensor and optogenetics for systems biology of yeast branched-chain alcohol production and tolerance	José L. Avalos	Princeton University	Princeton, NJ	Branched chain alcohols (BCAs), including isobutanol, isopentanol, and 2-methyl-1-butanol, are some of the most promising advanced biofuels in development. These alcohols have better fuel properties than bioethanol. They have higher energy density, their refinement is less expensive and energy intensive, and they have much higher compatibility with our fuel use and distribution infrastructure. The goal of this project is to carry out a comprehensive systems biology study of BCA production and tolerance in yeast. We will leverage a genetically encoded biosensor of BCA production that we recently developed to screen various yeast genomic libraries to measure the effects of different genetic perturbations (gene deletion, overexpression, or mutation) on BCA production or tolerance. We will also screen these collections using different substrates, nutritional requirements, or BCA-induced stress. The proposed approach will allow us to establish a closed-loop control system that we can use to measure transcriptomic changes under well-controlled conditions and develop improved strains to produce BCAs, and help make this very promising class of biofuels more economically competitive.

Systems Biology-Based Optimization of Extremely Thermophilic Lignocellulose Conversion to Bioproducts	Michael W.W. Adams	University of Georgia	Athens, GA	We propose to use systems biology-guided approaches to develop a non-model, microbial metabolic engineering platform based on the most thermophilic lignocellulose-degrading organism known, *Caldicellulosiruptor bescii*, which grows optimally at 78°C. We will apply the latest metabolic reconstruction and modeling approaches to optimize biomass to product conversion using switchgrass as the model plant and acetone and 3-hydroxypropionate as products. Bio-processing above 70°C can have important advantages over near-ambient operations. Highly genetically modified microorganisms usually have a fitness disadvantage and can be easily overtaken in culture when contaminating microbes are present. The high growth temperature of extreme thermophiles precludes growth or survival of virtually any contaminating organism or phage. This reduces operating costs associated with reactor sterilization and maintaining a sterile facility. In addition, at industrial scales, heat production from microbial metabolic activity vastly outweighs heat loss through bioreactor walls such that cooling is required. Extreme thermophiles have the advantage that non-refrigerated cooling water can be used if needed, and heating requirements can be met with low-grade steam typically in excess capacity on plant sites. The over-arching goal is to demonstrate that a non-model microorganism, specifically an extreme thermophile, can be a strategic metabolic engineering platform for industrial biotechnology.

Syntrophic Co-Cultures of *Clostridium* Organisms to Produce Higher Alcohols & Other C6-C8 Metabolites	E. Terry Papoutsakis	University of Delaware	Newark, DE	This project aims to advance the systems biology understanding and predictive modeling of synthetic & syntrophic *Clostridium* microbial consortia, focusing on elucidation of metabolic networks and environmental signals in the consortia. Microbial communities are ubiquitous in nature and have a wide range of applications, including production of biofuels and chemicals. It is now well appreciated that the capabilities of multi-microorganism systems cannot be predicted by the sum of their parts. Rather, synergistic interactions at different levels often result in better overall performance of these systems. The emerging field of co-culture synthetic biology promises the assembly of different metabolic capabilities into functional systems, where the diversity of metabolic pathways and the ability of microorganisms to exchange metabolites and larger molecules dramatically expands the possible metabolic space. *Clostridium* organisms are uniquely capable of utilizing a large variety of biomass-derived carbohydrates, and some of them can also fix CO2 autotrophically, thus enabling maximal substrate-carbon utilization. They possess diverse biosynthetic capabilities for producing a broad spectrum of metabolites, which, together with their derivatives could serve as commodity chemicals, biofuels and biofuel precursors. Significantly, syntrophic clostridial consortia can fix extensive CO2 amounts thus achieving product yields that cannot be achieved by monocultures. The ultimate goal then is to use the knowledge developed from these systems as a basis for future developments of syntrophic systems to produce a broad spectrum of metabolites via *modular syntrophic co-cultures*, involving engineered and non-engineered microorganisms from various genera in addition to the *Clostridium* organisms.

Dissecting the Division of Labor in Microbial Consortia for the Production of Biofuels and Chemicals	Ting Lu	University of Illinois at Urbana-Champaign	Champaign, IL	Microbial metabolic engineering is an attractive strategy for clean and sustainable production of biofuels and chemicals. Over the decades, its canonical paradigm, which involves pathway construction in single strains, has led to many breakthroughs; however, this paradigm has several key limitations including inefficient and slow substrate conversion, heavy burdens in energetics and redox balance, and unexpected accumulation of byproducts. Synthetic microbial consortia have recently emerged as a promising solution to address these challenges by expanding the programmability and enhancing the robustness of desired functionality. In this project, we seek to elucidate the fundamental design principles for the division of labor (DOL) in microbial ecosystems in the context of a *Saccharomyces cerevisiae* and *Lactococcus lactis* consortia that produce 2,3-butanediol, 2-butanol and lactic acid. We hypothesize that the structure of the cellular interaction network in this consortium is essential to ecosystem robustness. The proposed work promises to deliver a quantitative and systematic understanding of the division of labor in microbial ecosystems. It will thus advance the fundamental knowledge of microbial ecology concerning community structure and dynamics. It also provides valuable insights into the design and construction of artificial microbial consortia for the synthesis of bioproducts from cellulosic biomass.

Biosynthesis of bioprivileged, linear molecules via novel carboligase reactions	Keith Tyo	Northwestern University	Evanston, IL	This proposal seeks to characterize and engineer carbon-carbon (C-C) bond forming enzymes to enable novel biosynthetic pathways to a variety of long chain and di-functional fatty acids. More broadly, this proposal will harness enzyme substrate promiscuity to access a broad range of products not found in nature, while mitigating toxic or undesirable side reactions. If successful, we will unlock facile synthesis of new classes of molecules to enable biomanufacturing. The significance of this proposal lies in two areas: enabling synthesis of new molecules difficult to obtain by petrochemical routes and modeling the genome-scale consequences of enzyme promiscuity. First, many useful fuel and chemical molecules, like heptanoic and suberic acid, are difficult to produce by oil refining and petrochemistry. Functionalizing the ends of alkanes is particularly difficult, because interior carbons are more reactive. Biosynthesis of terminally functionalized molecules mediated by enzymatic coupling reactions would allow productions of valuable biochemicals, making scale-up more feasible and lower risk. Secondly, we will develop and utilize tools to predict enzyme promiscuity and mitigate the negative consequences associated with unwanted side reactions. This research and accompanying tool development will help identify deleterious promiscuous reactions and pave the way for other metabolic engineers to readily avoid toxicity and productivity loss due to unwanted side reactions.

Using gene editing and an accumulated bioproduct as a reporter for genotypic tophenotypic heterogeneity in growth-vs-production for *Methylobacterium extorquens*conversion of lignin-derived aromatics to butanol	Christopher J. Marx	University of Idaho	Moscow, ID	Lignin-derived compounds from plant biomass are amongst the most recalcitrant for microbial conversion. Hydrolysates contain a wide variety of aromatic molecules, and a particular issue with these molecules is that many of them are methoxylated: these methoxy groups are released as formaldehyde during degradation, which can overload the detoxification ability of standard heterotrophs. Methylotrophic bacteria, on the other hand, not only rapidly generate internal formaldehyde from oxidation of single-carbon compounds, like methanol, but also can oxidize it fast enough to prevent toxicity. In the course of an earlier DOE project, we discovered that some *Methylobacterium* strains grow exceptionally well on aromatics, and do not release formaldehyde into the medium from the methoxy groups present, unlike classic systems for aromatic degradation (*e.g.*, *Pseudomonas putida*). We have since demonstrated that the pathways for methoxylated aromatic use can be introduced into the emerging model organism, *Methylobacterium extorquens*, and enable it to grow on aromatics. The goal of this project is to develop *M. extorquens* as a catalyst to convert methoxylated aromatics from lignin hydrolysate into a model bioproduct, 1-butanol and develop a novel approach to that combines the advantages of gene editing, deep-sequencing and analysis of phenotypic heterogeneity for both growth and production. These conceptual advances could broadly revolutionize work in DOE-relevant biosystems design.

Rapid Development of Acetogenic clostridia using Highly Multiplexed Genome Engineering for Control of C1 Bioconversion	Howard Salis	Pennsylvania State University	University Park, PA	This project will develop a commercially scalable emerging model organism, called *Clostridium autoethanogenum*, that converts a single carbon (C1) feedstock (carbon dioxide, carbon monoxide, etc. from waste gas emissions) into a short chain fatty acid, 3-hydroxyproprionic acid. 3-hydroxypropionic acid is an ideal biorenewable precursor to industrially important polymers such as acrylates. To do this, we will apply several systems and synthetic biology technologies, coupling together algorithmic design approaches, highly multiplexed genome-scale engineering techniques, and omics measurements, to exert complete control over the metabolism of *Clostridium autoethanogenum*. First, we will employ an integrated computational-experimental approach to engineer optimized biosynthesis pathways for 3-hydroxypropionic acid in *Clostridium autoethanogenum*. Second, to redirect metabolic flows towards 3- hydroxypropionic acid production, we will develop and demonstrate a very highly multiplexed version of CRISPR that utilizes highly non-repetitive genetic parts to up-regulate or down-regulate up to 20 targeted genes simultaneously. Third, we will perform technoeconomic assessments of C1 bioconversion to 3-hydroxypropionic acid and couple those assessments to algorithm-designed genetic modifications, determining genotype-phenotype-cost relationships across several metrics. This project will result in a commercially scalable emerging model organism capable of producing 3-hydroxypropionic acid at economically competitive, high productivities from low-cost C1 feedstock.

Systems analysis of a fast growing N2-fixing cyanobacterium for production of advanced biofuels and nitrogen-containing petrochemical replacement compounds	Himadri B. Pakrasi	Washington University, St. Louis	St. Louis, MO	The overall objective of this project is to use an integrated systems biology approach to develop the filamentous cyanobacterium *Anabaena* sp. PCC 33047 as a model fast-growing, photosynthetic, diazotrophic production platform. Cyanobacteria are photosynthetic prokaryotes with significant potentials as cell factories for sustainable production of biofuels and chemicals by directly using energy from sunlight and CO2. One of the key issues with the current cyanobacterial production strains is the growth rates of these microbes. Compared to other oxygenic photosynthetic organisms such as plants and eukaryotic algae, many cyanobacterial strains have superior growth rates. However, they grow significantly slower than heterotrophic microbes such as *E. coli* and yeast that are commonly used in biofuel research. With our recent discovery of the fast-growing cyanobacterium *Synechococcus elongatus* UTEX 2973, it has become clear that there are strains available whose production potential far exceeds that of the current model systems. Notably, most cyanobacterial production systems require the input of fixed nitrogen, which has been reported as one of the highest operational costs for biofuel production. This taxing requirement can be largely eliminated through the use of N2-fixing cyanobacteria. We have now identified *Anabaena* 33047 that has the remarkable doubling time of 3.8 hours under N2- fixing conditions. Since nitrogen demand is a major cost for photosynthetic bioproduction, the use of this fast-growing diazotrophic strain should significantly improve the cost outlook of target bioproducts. We will pursue a systems approach to develop *Anabaena* 33047 as a versatile photosynthetic CO2- as well as N2-fixing production platform to be used by the bioenergy research community during the coming era.

Employing bacterial microcompartments to create privileged redox pools for biofuel production	Danielle Tullman-Ercek	Northwestern University	Evanston, IL	Metabolic engineering holds great promise for creating efficient, competitive routes for the production of biofuels and biochemicals without the necessity for harsh chemicals and hazardous byproducts. Successes in biochemical production include the production of Dupont's Sorona fibers from 1,3-propanediol from glucose using bacteria and the manufacture of the anti-malarial drug artemisinin from yeast. However, roadblocks to biosynthesis prevent many biochemicals from being produced biologically given current technology. Nature uses compartmentalization (ex. organelles in eukaryotes and in bacterial microcompartments in prokaryotes) to solve issues such as intermediate leakage, toxicity, and byproduct formation. Here we propose to deploy compartmentalization as a strategy to overcome a critical roadblock: the requirement for redox cofactor recycling. In traditional systems, redox cofactors are lost to cellular growth and maintenance needs. By compartmentalizing redox cofactors with the biochemical synthesis enzymes, we anticipate increasing the thermodynamic efficiency and preventing the loss of valuable intermediates and cofactors. If successful, it would be the first direct demonstration of this feature of a bacterial microcompartment, and would provide a tool for improving metabolic pathway performance for all enzymes with redox or other cofactors. If successful, this work would provide insight into the native function of these structures, while also providing a detailed method for selecting and improving biochemical pathway performance. Ultimately, this will lead to the cost efficient production of chemicals that are currently derived from petroleum.

High-throughput chemical imaging for optimizing biofuel synthesis using synthetic biology	Mary Dunlop	Boston University	Boston, MA	Recent advances in the fields of synthetic biology and metabolic engineering have resulted in an unprecedented ability to engineer microbial genomes and design and build gene circuits for improving biofuel production. Here, technology for directly measuring synthesis of biofuels in living cells using a high-throughput platform for chemical imaging of biofuel production will be developed and used to improve *E. coli* fatty acid production. Stimulated Raman scattering (SRS) microscopy will be introduced as a new technology for directly measuring chemical signatures in *in vivo* samples for the engineering and optimization of biofuel production strains. The technology can work on a broad range of cell types (yeast, algae, bacteria beyond *E. coli*) and can detect *in vivo* levels of other biofuels and products (diesels, jet fuels). It is anticipated that SRS imaging will be especially valuable for assessing chemical signatures in strains where tools for genetic manipulation are limited or non-existent.
Rapid flux phenotyping to accelerate metabolic engineering of cyanobacteria	Jamey D. Young	Vanderbilt University	Nashville, TN	The ability to quantify flux alterations in response to targeted genetic manipulations is a key requirement for rational metabolic engineering, but the time needed to complete a comprehensive 13C flux analysis can far exceed the time needed to introduce new genetic modifications to a recombinant host. This project will develop technologies to optimize cyanobacteria and other microbes for producing renewable chemicals at commercially feasible rates and yields by establishing a rapid flux phenotyping platform that can be applied to accelerate metabolic engineering of cyanobacterial hosts. Matching the throughput of 13C flux phenotyping to the rate of strain generation will provide the foundation for a rational “design-build-test-learn” metabolic engineering cycle. It is expected that the findings will be generalizable to a diverse range of biochemical products derived from major metabolic hubs, enabling a systematic strategy for metabolic engineering of cyanobacteria and other microbes.

Establishing the Thermotolerant Yeast *Kluyveromyces marxianus* as a Host for Biobased Fuels and Chemicals Production	Ian Wheeldon	University of California, Riverside	Riverside, CA	This project seeks to develop the thermotolerant yeast *Kluyveromyces marxianus* as a platform host for industrial bioprocessing. A critical area of the US industrial biotechnology sector is the conversion of biomass and other renewable feedstocks to fuels and chemicals. Robust microorganisms that are genetically accessible, can grow rapidly at high temperature and low pH, and that can effectively assimilate a wide range of different sugars, such as *K. marxianus*, are needed to sustain technological and economic growth. New genome-wide CRISPR-based tools for genome editing, genetic screening, and rapid strain development will be developed and applied in systems biology studies to understand industrially-desirable phenotypes and for metabolic engineering. A key aim will be to enhance acetyl-CoA production, a central precursor in the synthesis of many fuels and chemicals. Anticipated outcomes include rapid engineering of *K. marxianus* strains that produce biofuels and chemicals at high titer, rate, and yield, leading to a new, robust platform for low-cost bioprocessing.
Gene regulatory networks enabling fungi to selectively extract sugars from lignocellulose	Johnathan S. Schilling	University of Minnesota	Minneapolis, MN	Fungi dominate the biological decomposition of wood and other lignocellulosic plant tissues in nature through a range of pathways for unlocking the sugars embedded in lignin, offering a proven model for the sustainable production of energy from biomass. Modern approaches to bioenergy production aim to depolymerize polysaccharides to release fermentable sugars (saccharification), saving lignin as a co-product, a good fit for the carbohydrate-selective pathways of brown rot fungi. However, understanding of fungal brown rot metabolism is limited. To address key knowledge gaps brown rot-specific gene regulation patterns will be identified and characterized, enabling *in vivo* manipulations such as CRISPR/Cas9 and metabolomics to map metabolite expression feedback over time, producing an integrated regulatory model for brown rot fungi. This project will enable omics-driven tools for organisms highly relevant to bioenergy, with broader scientific impacts in the fields of ecology, evolution, and biogeochemistry. This project is a collaboration with Clark University, Pacific Northwest National Laboratory and Lawrence Berkeley National Laboratory.

Gene regulatory networks controlling carbohydrate-selective deconstruction pathways in fungi	David Hibbett	Clark University	Worcester, MA	(separately funded collaborator with J.S. Schilling project)
Novel Microbial Routes to Synthesize Industrially Significant Precursor Compounds	F. Robert Tabita	The Ohio State University	Columbus, OH	There is an increasing demand for bioproducts and biofuels from plentiful starting materials such as lignocellulose and carbon dioxide feedstocks. In this project, a combination of systems biology and bioinformatics approaches, along with a unique toolbox of analytical/'omics and molecular/biochemical approaches will be applied to maximize the potential of microorganisms to convert lignocellulose-derived compounds and CO_2 to important synthetic precursor compounds such as ethylene and propylene, the most widely employed organic compounds in industry, used for the synthesis of several multi-billion dollar and industrially significant products. Current chemical processes for precursor synthesis require huge amounts of energy derived from fossil fuels, but recently discovered, efficient anaerobic ethylene synthetic processes offer the potential to significantly impact biological ethylene (and propylene) formation, tenable with plentiful lignocellulose and/or CO2 feedstocks used as starting materials. Overall this project aims to develop an industrially compatible process to synthesize ethylene in high yields using microbial systems. This project is a collaboration with Pacific Northwest National Laboratory.

Creating multifunctional synthetic lichen platforms for sustainable biosynthesis of biofuel precursors	Michael Betenbaugh	The Johns Hopkins University	Baltimore, MD	Lichens are communities of microbes that collect sunlight and carbon dioxide and apply it to power the group's activities, allowing the autotrophic member to optimize photosynthesis and metabolite generation while their heterotrophic fungal partners produce biochemical compounds for the community. Additional members may provide key functions such as nitrogen fixation. While lichens can thrive in the harshest environments on earth, they also represent a novel biotechnology platform that can transform CO_2 and sunlight into valuable energy-related biochemicals. Unfortunately, natural lichens have exceedingly slow growth rates, making them impractical for most industrial applications. In this project, genetic manipulation techniques will be used to enhance the exchange of metabolites between autotrophs and heterotrophs, creating superior synthetic lichens able to generate useful products of interest to the energy and chemical industries. Key metabolite excretion bottlenecks will be identified in cyanobacteria, then engineered to share particular metabolic intermediates with their heterotrophic partners for channeling into natural or engineered metabolic pathways, thus generating energy-related precursors of biochemicals or biofuels with high commercial value. This project is a collaboration with Pacific Northwest National Laboratory and the National Renewable Energy Laboratory.

Understanding and Harnessing the Robustness of Undomesticated *Yarrowia lipolytica* Strains for Biosynthesis of Designer Bioesters	Cong Trinh	University of Tennessee	Knoxville, TN	The goal of this study is to harness the potential of robust undomesticated Yarrowia lipolytica isolates to produce designer bioesters from undetoxified biomass hydrolysates. These isolates will be derived from genetic and phenotypic screening approaches using a rigorous microbe selection platform. Genomic and molecular characterization will be leveraged to elucidate and characterize the underlying mechanisms of how these new strains yield desirable bioesters and other bioproducts. Specifically, we will detail how these Y. lipolytica 1) tolerate and effectively assimilate inhibitory biomass hydrolysates for superior lipid accumulation under hypoxic compared with oxygen sufficient conditions, 2) tolerate organic solvents that are required to produce biofuels and bioproducts in a two-phase fermentation system, and 3) endogenously degrade lipids to produce targeted esters with potential as fuels, solvents, flavors, and fragrances. The results will provide the needed tools to allow in situ production and integrated recovery of custom esters, as well as the insight necessary for engineering Yarrowia strains for production of a wide variety of biofuels and bioproducts from lignocellulosic biomass.This project is a collaboration with Pacific Northwest National Laboratory and the USDA Agricultural Research Station in Peoria, IL.

Harnessing photoautotroph-methanotroph interactions for biogas conversion to fuels and chemicals using binary consortia	Jin Wang	Auburn University	Auburn University, AL	Biogas from conversion of organic waste steams has immense potential to be used as a feedstock for producing high-density fuels and commodity chemicals. However, the utilization of biogas represents a significant challenge due to its low pressure and presence of contaminants such as H2S, ammonia, and volatile organic carbon compounds. To tap into this immense potential, effective biotechnologies that co-utilize both CO_2 and CH_4 are needed. In this project, a model coculture of photoautotroph-methanotroph, *Synechococcus* sp. PCC 7002 - *Methylomicrobium alcaliphilum* 20ZR, will be used to develop experimental and computational tools to gain qualitative and quantitative understandings on the interactions and dynamics of the coculture at both systems and molecular levels. Fundamental understanding of the photoautotroph-methanotroph interaction will lay the foundation for the design and optimization of synthetic binary consortia for production of fuels and chemicals from biogas. Knowledge gained from this project may be generally applicable to other cross-feeding binary consortium, and the tools developed can be adapted to study the interactions and dynamics of other multi-organism platforms. This project is a collaboration with Pacific Northwest National Laboratory.
Development of emerging model microorganisms: Megasphaera elsdenii for biomass and organic acid upgrading to fuels and chemicals	Janet Westpheling	University of Georgia	Athens, GA	The native ability to condense acetyl-CoA groups to efficiently generate C4 to C8 compounds makes *Megasphaera elsdenii* a compelling platform for the production of fuels and chemicals from lactate and plant carbohydrates. The overall objective of this project is to develop *M. elsdenii* as a platform for the conversion of lignocellulosic biomass sugars and organic acids into hexanol and other valuable chemicals. Engineering *M. elsdenii* to efficiently produce next-generation, drop-in lignocellulosic fuels such as hexanol at high yield and titer could provide an efficient bioengineering platform. Initially, lignocellulosic sugars will be converted to hexanol and related products; however, because *M. elsdenii* also ferments lactate to organic acids, this project will also lay the foundation for more advanced processing options such as a co-culture or sequential fermentation in which one organism converts sugars to lactate and an engineered *M. elsdenii* converts the lactate to a higher value product. This project is a collaboration with Oak Ridge National Laboratory.

Hyperspectral Light Sheet Raman Imaging of Leaf Metabolism	Keith Lidke	University of New Mexico	Albuquerque, NM	This project will provide major advances for in vivo, dynamic tracking of pathways of carbon fixation in living plants. A major limitation of metabolic flux models is the ability to constrain fluxes between organelles and between cells across small spatial areas (source and sink cells in the leaf). This project will develop and test a new imaging method coupling Raman imaging with light sheet-based illumination of leaf tissues while simultaneously monitoring photosynthesis and respiration. This novel tool will permit localization and quantification of carbon-13-labeled metabolites in living plants at the microscopic level as well as directly monitor conversion of photosynthetic metabolites (low-energy sugars) into higher energy compounds that can be further converted to biofuels and bioproducts. This project is a collaboration with Sandia National Laboratory.
Single-Molecule Imaging of Lignocellulose Deconstruction by SCATTIRSTORM Microscopy	William O. Hancock	Pennsylvania State University	University Park, PA	The goal of this project is to build a multimodal optical microscope to measure the binding, processive motility, and pausing behaviors of cellulases as they interact with and degrade both in vitro assembled and naturally-occurring lignocellulosic composites. To achieve this, the investigators will use high-resolution, single-molecule imaging to track cellulases, while specifically visualizing the cellulose, lignin and hemicellulose that make up their lignocellulose substrate. The microscope will combine Interferometric Scattering (iSCAT), which provides unprecedented spatiotemporal resolution; Total Internal Reflection Fluorescence (TIRF), which provides single-molecule resolution of multiple fluorophore-labeled molecules; and Stochastic Optical Reconstruction Microscopy (STORM), which allows for three-dimensional super-resolution imaging of intact plant cell walls during degradation.

Time-Resolved 3D Multi-Resolution Microscopy for Real-Time Cellulase Actions In Situ	Haw Yang	Princeton University	Princeton, NJ	Cellulose is the most abundant renewable carbon source on earth and thus is of central importance as a bio-fuel feedstock. A major impediment toward converting it to a liquid fuel is its crystallinity and that it is encased by both lignin and hemicellulose. As such, much research effort has been expended in finding the best cellulases to use for saccharification and how to circumvent the lignin barrier. Little is known with respect to the cellulase mechanism of action. Cellulose is insoluble and is spatially highly heterogeneous extending to all three dimensions. In addition, the three individual dynamic steps of cellulases: initiation, processive hydrolysis, and termination cannot be captured by steady state imaging methods. This proposal aims to capture, in real time, these elementary steps in enzymatic cellulose degradation by following the actions of single cellulases. The proposed work has potential to provide new insights to cellulosic cell wall degradation and interfacial enzymatic catalysis in general. This objective will be achieved by developing a novel time-resolved 3D multi-resolution imaging technology, where quantum-dot tagging will allow reliable real-time 3D tracking in high biological background samples. The platform will likely have broad biological imaging applications.
Meta-Optics Enabled Multifunctional Imaging	Paul Bohn	University of Notre Dame du Lac	Notre Dame, IN	The research addresses two over-arching goals of central relevance to the DOE-BER mission, the development of: (1) a new approach to imaging and spectroscopy enabled by the innovative use of metaoptics; and (2) new tools to control the chemical environment of a microbial sample with nanometer-scale precision. The research strategy used here combines meta-optics-based excitation with in situ control over the local chemical (redox) environment, and tests them in a bacterial species, Myxococcus xanthus, which is relevant to the DOE mission due to its capacity to deconstruct lignocellulosic biomass. The creation of this new integrated imaging/chemical stasis platform has the potential to dramatically expand the range and extent of questions that can be addressed in microbial biology.

Multiparametric Optical Label-Free Imaging to Analyze Plant Cell Wall Assembly and Metabolism	Marisa S. Otegui	University of Wisconsin-Madison,	Madison, WI	This proposals aims to develop a label-free, optical microscopy platform for characterizing multiple important cell wall components and stress-related figure prints at subcellular scale resolution. The new multimodal imaging platform will collect optical fingerprints from both emitted and scattered light that can inform on the chemical nature, subcellular distribution, anisotropy, and molecular environment of multiple cell wall components in intact plant tissues. These imaging capabilities will be combined with open-source computational tools that enable correlated registration, integration, and analysis. This fully integrated, multiparametric optical system would be the first of its kind and used to address biological problems connected to cell wall assembly in grasses. As an example of what the new device will be able to accomplish, the research team will analyze patterns of cell wall silicification in maize and sorghum and determine how silicification affects cell wall properties, lignin, cutin, and suberin deposition in other cell types under different stress conditions.
Real-Time Imaging and Quantification of Plant Cell Wall Constituents Using Cavity-Dumped Stimulated Raman Scattering (cdSRS) Microscopy	Shi-You Ding,	Michigan State University	East Lansing, MI	The proposed research focuses on developing new generation stimulated Raman scattering (SRS) techniques for in situ imaging and quantification of the physicochemical properties of plant cell wall constituents, including cellulose and lignin in their native state (in living plants); as well as during processing for biofuels and biomaterials, such as chemical deconstruction and enzymatic hydrolysis. These techniques, once developed, will significantly increase the experimental signal to noise ratio, thus enabling detection/localization of cell wall chemistry found at low molecular concentration in complex structures, such as plant cell walls. It is also proposed to combine highly sensitive chemical imaging with ultra-resolution atomic force microscopy (AFM). These non-destructive techniques will, therefore, enable mapping both chemical and morphological features of cell walls at the molecular and cellular scales. This research will provide critical insights into the mechanistic understanding of plant cell wall architecture; as well as new strategies for efficient biomass deconstruction processes.

Development and implementation of an in situ high-resolution isotopic microscope for measuring metabolic interactions in soil mesocosms	Elizabeth A. Shank	University of North Carolina at Chapel Hill	Chapel Hill, NC	This project aims to create a novel microscope that combines complementary imaging modalities to overcome current challenges in visualizing the metabolic activities of microbes within soil. The proposed instrument will integrate fluorescence microscopy, Raman microspectroscopy, and ultrahigh-resolution mass spectrometry to enable the direct investigation of microbial activities in both model and native soils using fluorescent- and stable isotope-labels. This proposed 'high-resolution isotopic microscope' will provide a new tool for the scientific community to simultaneously visualize the microbial and the molecular fate of environmentally-relevant substrates. It will be developed at EMSL, and thus will ultimately be available to the entire EMSL User Base. It has the potential to be a transformational technology that will dramatically enhance our understanding of carbon degradation processes occurring within soil communities. This project is a collaboration with Pacific Northwest National Laboratory.
Understanding Plant Signaling via Innovations in Probe Delivery and Imaging	Jean Greenberg	The University of Chicago	Chicago, IL	This project will develop new approaches for the introduction and observation of signaling probes in live plants. The approach will provide two key advances, the abilities (1) to deliver labeled non-membrane permeant probes, such as proteins, signaling reporters, and DNA, to plant tissue with single-cell precision and (2) to perform repeated imaging (e.g. fluorescence) of broad tissue areas within intact living plants, including leaves. A major advance will be iterative, non-destructive imaging of peptide signaling responses in plants that are highly relevant to improving traits for energy applications. Aims are to improve the delivery of signaling probes to plants and to develop a robust, robotic imaging platform for whole plants. This project is a collaboration with Oak Ridge National Laboratory.

Spatiotemporal Dynamics of Photosynthetic Metabolism in Single-Cells at Sub-Cellular Resolution	Jeffrey C. Cameron	University of Colorado-Boulder	Boulder, CO	The goal of this proposal is to provide understanding of how metabolism functions in the landscape of the plant and microbial cell, and provide insights into the basic principles of living biological systems and ways to engineer and improve them for the generation of fuels, chemicals, and other useful products for bioenergy. By combining advanced physics with cutting edge synthetic biology, the proposal aims to design and build a multimode nonlinear optical nanoscopy system to generate adaptive three-dimensional (3D) images with high-resolution, real-time, dynamic quantitative label-free chemical imaging of metabolic processes in biological systems. The research will benchmark and calibrate resolution and sensitivity of multimodal imaging for label-free chemical imaging using a compatible model photosynthetic cyanobacterium, and apply multimodal imaging system to generate dynamic spatiotemporal maps of metabolism in photosynthetic cyanobacteria and other DOE-BER relevant plants and microbes. This proposal will take advantage of new technologies including quantum dots for use in calibration of instrument resolution and sensitivity, as highly stable luminescent markers.
Plasmonics-Enhanced Optical Imaging Systems for Bioenergy Research	Tuan Vo-Dinh	Duke University	Durham, NC	The objective of this proposal is to develop innovative and improved imaging instrumentation that can enable visualization and quantitative characterization of molecular and genomic biomarkers and their dynamic role in carrying out cellular processes and function in plant systems related to bioenergy development. One promising alternative energy to fossil fuels is the next generation biofuels made from nonfood biomass, such as lignocellulose (woody parts) in plant wastes or hydrocarbons produced by photosynthesis (e.g., terpenes and fatty acids) in plants and certain microbes. However, current production of cellulosic and hydrocarbon biofuels is far from optimal, and requires further research to improve efficiency and reduce costs. In order to design a new strategy to increase production, it is important to elucidate the regulation of the terpene synthesis pathway. The development of new bioimaging tools will allow us to monitor the abundance and subcellular localization of important regulators in living plants. This project is a collaboration with Argonne National Laboratory.

In planta single-molecule imaging and holographic force spectroscopy to study real-time, multimodal turnover dynamics of polysaccharides and associated carbohydrate metabolites	Sang-Hyuk Lee	Rutgers University	Piscataway, NJ	This research aims at innovation in multimodal single-molecule manipulation/imaging method through integration of holographic optical tweezers, super-resolution fluorescence microscopy, single-particle tracking, and surface-enhanced Raman spectroscopy to investigate key metabolic processes in live plant cells. This novel approach will reveal in vivo plant cell wall polysaccharides synthesis processes in unprecedented molecular detail through the simultaneous characterization of structure, dynamics, and function of single enzyme complexes as well as intracellular sugar metabolites flux. The ambitious undertaking will greatly advance the mechanistic and holistic understanding of in vivo cell wall synthesis and deconstruction, which will accelerate the development of better transgenic crops for bioenergy applications. This pioneering work will also have broader transformative impacts on cell and molecular biology fields by paving the way for multimodal single-molecule studies in native cellular environments. This project is a collaboration with Oak Ridge National Laboratory.

Multi-Modal Imager of Metabolome and Enzyme Dynamics for Co-Optimizing Yields and Titers in Biofuel Producing Microorganisms	Andreas E. Vasdekis	University of Idaho	Moscow, ID	The ultimate goal of this proposal is to develop imaging approaches enabling replacement of petroleum-derived fuels with plant-product derived biofuels via meeting the grand challenge of co-optimizing production titers and yields by metabolic engineering. This challenge emerges because introducing new synthetic pathways or debottlenecking existing ones often leads to non-optimal metabolic trade-offs between growth and production, thus hindering the improvement of production without disrupting growth. While predicting the genome-wide interactions between growth and productivity remains a central theme of systems-biology, another and considerably less understood issue pertains to how the compartmentalization of metabolic pathways can impact metabolic trade-offs. The project will develop a new imaging system that couples holographic imaging (to measure dry weight/mass of subcellular components) with lattice-light sheet-based fluorescence and Raman imaging (in a single instrument) to measure metabolic pathway localization, precursor utilization, and triacylglycerol (a fatty acid derivative) accumulation. The instrument will enable quantifying key performance metrics in biofuel production with single-cell resolution based upon metabolic trade-offs between growth and productivity, compartmental localization of metabolic pathways, and nutrient consumption. This project is a collaboration with Pacific Northwest National Laboratory.

Development of broadband infrared nano-spectroscopy of biological materials in fluid	Tina Jeoh	University of California	Davis, CA	This research aims to solve two major challenges – the mechanisms of cellulose hydrolysis by cellulases and the lack of label-free, nanometer scale and time-resolved imaging technique to study surface reactions in aqueous biological reactions. Elucidating how cellulases hydrolyze cellulosic substrates is a game-changer for the success of cellulosic biofuels and bioproducts. As the enzyme-catalyzed reactions occur at the surface of poorly characterized and complex plant cell wall matrices, the lack of means to map surface chemistry of the substrates in situ has severely hampered research progress. The development of a method that can map surface chemistry at the nanoscale over time in an aqueous reaction is a game changer not only for the study of cellulose hydrolysis reactions, but also for the study of any heterogeneous biological reaction. Thus, the goals of this research project are to overcome technological limitations to conducting detailed studies of surface reactions in aqueous biological systems with high spatial, chemical and time resolution, and to apply this method towards solving the mechanisms of enzymatic hydrolysis of cellulosic biomass. This project is a collaboration with Lawrence Berkeley National Laboratory.