Title	PI	Institution	Synopsis
Hyperspectral Light Sheet Raman Imaging of Leaf Metabolism	Keith Lidke	University of New Mexico	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	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	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	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,	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	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	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.
Spatiotemporal Dynamics of Photosynthetic Metabolism in Single-Cells at Sub-Cellular Resolution	Jean Greenberg	The University of Chicago	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, r	University of Colorado-Boulder	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	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	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	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	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.