SAND2012-1247P

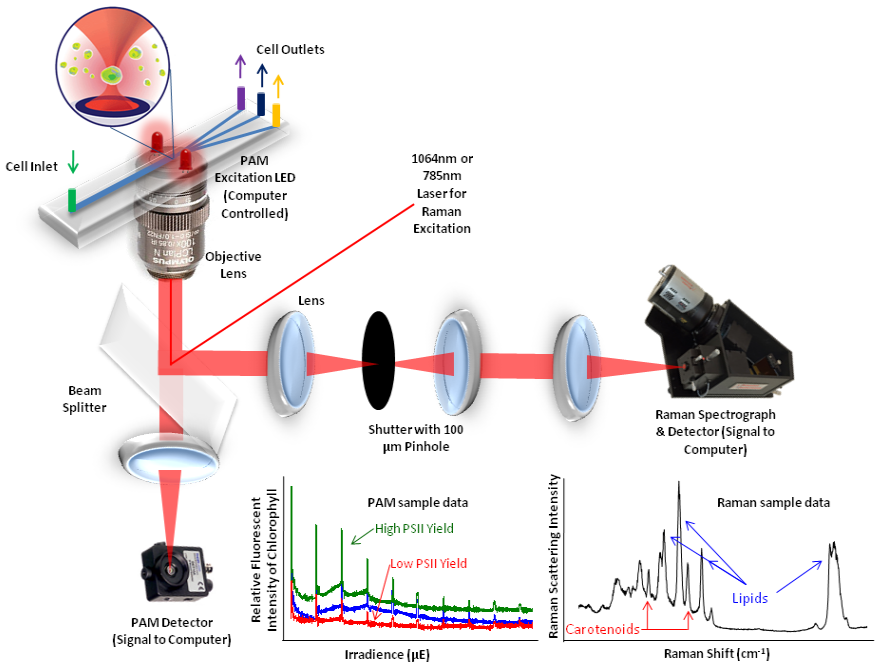
Exceptional service in the national interest

Sandia National Laboratories

Bioprospecting for "Super-Cells"

Multispectral Sorter/Analyzer for Rapid Detection of High Lipid Quality/Quantity Algae for Biofuels

Usually known as the "Algae to Oilgae" Initiative, this LDRD-funded project has soared into some remarkable progress in rendering algae more viable as a source of biodiesel for domestic production of transportation fuels. Such an outcome could seriously (and favorably from the US standpoint) alter the energy horizon, greatly lowering our dependence on Middle Eastern fossil fuels. In addition to research on the growth conditions and factors that appear to be optimal for yielding microalgae that contain large quantities (up to 70% dry cell weight) of the optimal biodiesel precursors, this project has developed an ingenious technology for rapidly screening algal cell populations to identify such super-cells—both on species-, strain-, and individual-cell metabolic levels.



Schematic design of the multispectral microalgal optical sorter with examples of data generated (microscope, computer and connections to computer not shown. Algal cells are optically trapped (at top), above the objective lens of an inverted light microscope where they are interrogated by the laser, producing their Raman signature spectrum and by an LED, producing fluorescence that provides information on photosynthetic potency.

A combination of micro-Raman spectroscopy, laser "tweezers" for single cell manipulation of single microalgal cells, a pulse-amplitude-modulated (PAM) fluorometry system and a microfluidic flow system that rapidly delivers cells into the laser beam, this "bioprospector" carries out analysis of cellular contents in single algal cells in timeframe of just a few seconds enabling hundreds of cells to be interrogated every minute. Such interrogation produces what can be thought of as a "Raman fingerprint" for each cell, the peaks of the spectrogram at given wavelengths, providing information about the types of lipids present in a particular algal cell. Raman spectroscopy, a nondestructive technique delivers photons that interact with the electron clouds of biomolecules within storage compartments (vesicles) of the algal cell—in this case, various lipids that the cell synthesizes via photosynthesis. Overall the method is dubbed, "laser-trapping Raman spectroscopy (LTRS).

Sandia National Laboratories is a multi-program laboratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000. SAND

Such factors as the chain length (number of carbon atoms), degree of unsaturation (number of carbon-carbon double bonds) in the backbone of the fatty acids composing the triacyglycerides (TAG) stored within each cell, and the melting point of these lipids are crucially important to the quality of biodiesel derived from these lipids, and therefore, determine which algal species and which individual cells within that species are the optimal photosynthesizers. While the Raman profile informs researcher about the *types* of lipids (as well as carotenoid pigments, protein and carbohydrates), meanwhile, the LED-based fluorometry system interrogates the cells as to their absolute photosynthetic productivity, detecting their content of chlorophylls. Taken together, this information allows the discovery of "super-cells" — the cells containing the biomolecular apparatus to be the optimal photosynthesizers — producing the highest lipid content per photon of sunlight absorbed — and that are the metabolically optimal in producing the most desirable lipid types under given defined growth conditions (while allowing researchers to define what growth conditions were optimal to yield such an outcome). In essence, "optimal" here defines cells that would most readily yield the highest output of high-quality biodiesel with the least possible effort in processing these lipids, once extracted from the algal cells. But it is important to note that the LTRS method itself is nondestructive and nontoxic. Unlike other methods of determining lipid content (lipid extraction and staining, for example), the cell is caught and released, preserving it for later clonal cultivation for example. Plus this method is far more efficient in terms of time, labor, and expense than the aforementioned alternatives.

Beyond this nationally critical application of the technology in biofuels R&D, the bioprospector can also be utilized as a research tool in other areas of biology. Many possibilities reveal themselves, in all cases, the goal to discover an optimal metabolically configured cell within a slightly diverse population. For example, in the immune response, so-called antigen presenting cells (APCs), for example, in the skin, wall of the GI Tract and respiratory systems trigger immune responses by presenting molecular fragments of viral or bacterial proteins (for example) to T-helper lymphocytes (white blood cells). All APCs express on their cell membranes, a protein complex named HLA-D (or MHC-Class II), molecular complexes essential to their ability to trigger T-helper cell immune responses. Suppose in this population, there existed "super APCs" that possessed higher levels of HLA-D and were therefore more potent at initiating protective immune responses. If the Raman method could be sensitized to identify such cells, that would make the bioprospector a key research tool in that arena of immunology. The example illustrates that many ancillary applications for this technology are conceptually possible throughout cellular and molecular biology. Its value in the biofuels arena is adequate, in itself, to appreciate its value, but it may be valuable in life-science R&D far beyond this single application.

Because of Sandia's unique experience with Raman spectroscopy for microalgae, the Laboratories are now partnering with BaySpec, a Bay Area company, to commercialize a pond deployable algal analyzer configured with the operational elements described above. The project received a phase one National Science Foundation Small Business Innovation Research grant to assist in commercialization.

Point of Contact: Seema Singh seesing@sandia.gov , <ssingh@lbl.gov>
Funding: LDRD, NSF