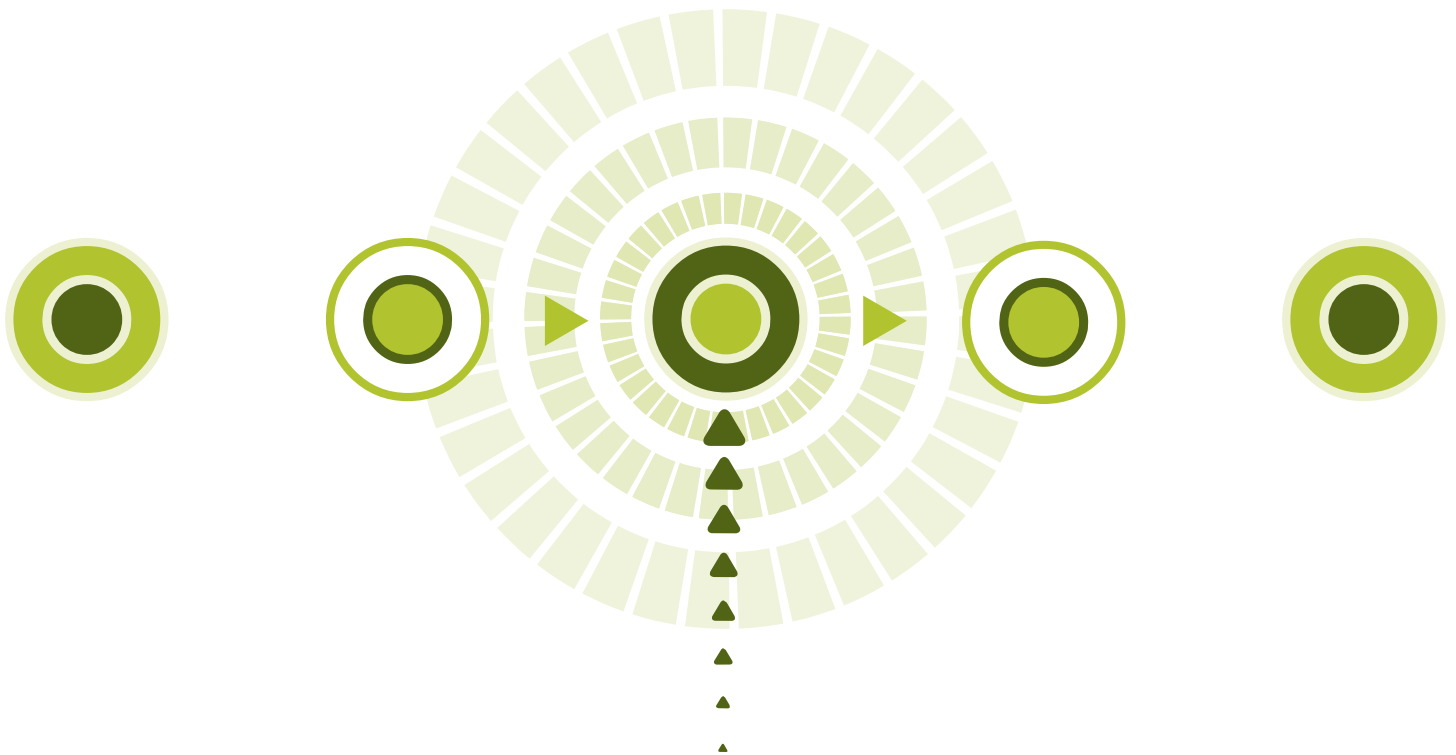




# New Benchtop Flow Cytometers Power the Development of Biofuels

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## Although flow cytometry is routinely used for life science and clinical applications,

the size, complexity, cost, and maintenance requirements of conventional flow cytometry systems have historically confined their use to core facilities and large laboratories with expert users. These factors can limit use of this powerful technology by companies engaged in cutting-edge biofuels research and development as they are unlikely to have access to a central core lab or a flow cytometry expert on staff.

The recent development of benchtop flow cytometry instruments, combined with user friendly software and turnkey assay kits, is now enabling use of this technology right at the lab bench, by both experts and novices alike. New micro-capillary systems require smaller sample volumes, generate significantly less waste, have lower operating costs, enable high sample throughput, and are easier to set up and run than traditional flow cytometers.

With benchtop systems able to deliver data on large quantities of samples with quick turnaround and a minimal learning curve, flow cytometry is now integral to the research and development of biofuels.

Two areas in which benchtop flow cytometry is accelerating progress are the creation of energy from algae and the "biogenic" production of natural gas by microbes in fossil fuel reserves. Lipids extracted from algae can be refined into a variety of fuel types. Benchtop flow cytometry is used to monitor growth and contamination, and assess and select high lipid-producing algae strains. The same technology is helping to define the optimal mix of microbes required to generate natural gas from buried coal beds and oil reservoirs considered by conventional wisdom to be depleted.

## TURNING ALGAE INTO ENERGY

Algae use photosynthesis to combine water with carbon dioxide to create biomass – a mixture of carbohydrates, proteins, and lipids. Although the mechanism of photosynthesis in algae is similar to that of higher plants, algae convert solar energy more efficiently because of their simple cellular structure. In addition, algae grow in aqueous suspension, so they have more efficient access to water, CO<sub>2</sub>, and other nutrients. As a result, algae are capable of producing a significantly higher amount of oil per unit area, compared to terrestrial plants .

Research on the use of algae for biofuel production has continued since the 1950s. From 1978 to 1996, the U.S. Department of Energy (DOE) funded a program to develop biofuels from algae<sup>1</sup>. In 1995, with budgets tightening, the DOE eliminated funding for algae research within the biofuels program.

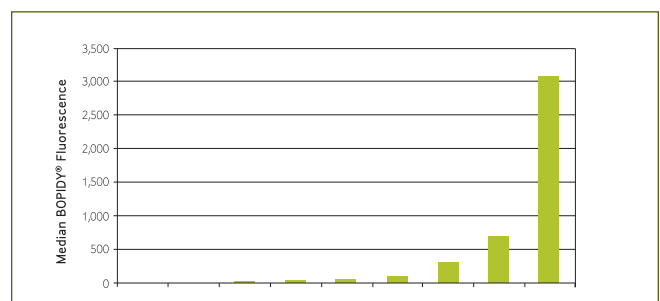
Interest in algae-derived biofuels has regained momentum in recent years due to increasing oil costs and a desire to reduce dependence on foreign sources. In January 2010, the US DOE announced an investment of nearly \$80 million for advanced biofuels research and fueling infrastructure. Over half of this money was earmarked for the National Alliance for Algal Biofuels and Bioproducts (NAABB) for development of the science and technology necessary to significantly increase production of algal biomass and lipids and efficiently harvest and extract algae and algal products.

Thousands of strains of algae exist, but there can be significant variations in lipid content between different strains. Identification of high lipid-producing strains is a prerequisite for the sustainable and economically-viable production of fuel from algae.

Benchtop flow cytometry offers a high-speed, automated, cost-effective method for assessing both lipid content in various algal strains.

"Our Guava flow cytometer is really, really versatile," notes Mark Clark of Solix Biofuels in Fort Collins, CO, a leader in production technology used to grow algae at large scales and a partner in the NAABB. "We use it to monitor for contamination and to get cell counts which tell us the biomass. We also use it for viability testing and to determine the lipid content of cultures using various fluorescent dyes..

Solix has validated flow cytometry assays for triglyceride content deployed on the Guava system using Nile Red. They are currently developing additional flow methods for other algal products. Figure 1 shows the wide range lipid content in nine different strains of algae.



**Figure 1.** Variation in median lipid content in nine different algae strains.

In addition to identifying which algal strains contain the highest lipid levels, the type of chlorophyll present in the strain must also be considered. Algae strains contain different combinations of chlorophyll molecules, designated A, B, and C. Algal strains containing chlorophyll A produce lipids that are best suited for biofuel development. Some algal strains may have high lipid content but the lipid has not been generated via a process involving chlorophyll A. Therefore, the lipid may not be optimal for biofuel production.

Chlorophyll A-positive populations can be identified by flow cytometry by their fluorescence in the red channel (Figure 2a). Applying a gate on chlorophyll A-positive cells allows these cells to then be evaluated directly for lipid content (Figure 2b). Figure 2c shows the range of lipid content in a number of chlorophyll A-positive algae strains. Significant differences in lipid content exist among strains containing chlorophyll A. Both chlorophyll A and lipid content can be evaluated in an algal sample in less than three minutes on the Guava easyCyte system. Samples in 96-well plates can be placed in the Guava system allowing for walk-away automation.

Benchtop flow cytometry is also used for cell counts and to monitor algal cultures for contamination. The forward scatter function of the flow cytometer can distinguish algal cells from bacteria based on size.

The elimination of sheath fluid in benchtop systems – which allows for the small instrument footprint – also provides an additional benefit when working with algae.

“Eliminating sheath fluid greatly minimizes the amount of waste generated,” said Clark. “In addition, most sheath fluids are formulated for mammalian cells, rather than algal cells. Because there is no sheath fluid, we can sample within the matrix in which the cells are growing and not worry about any effect sheath fluid would possibly have on the physiology of the cells before they reach the detector.”

## BIOGENIC METHANE PRODUCTION

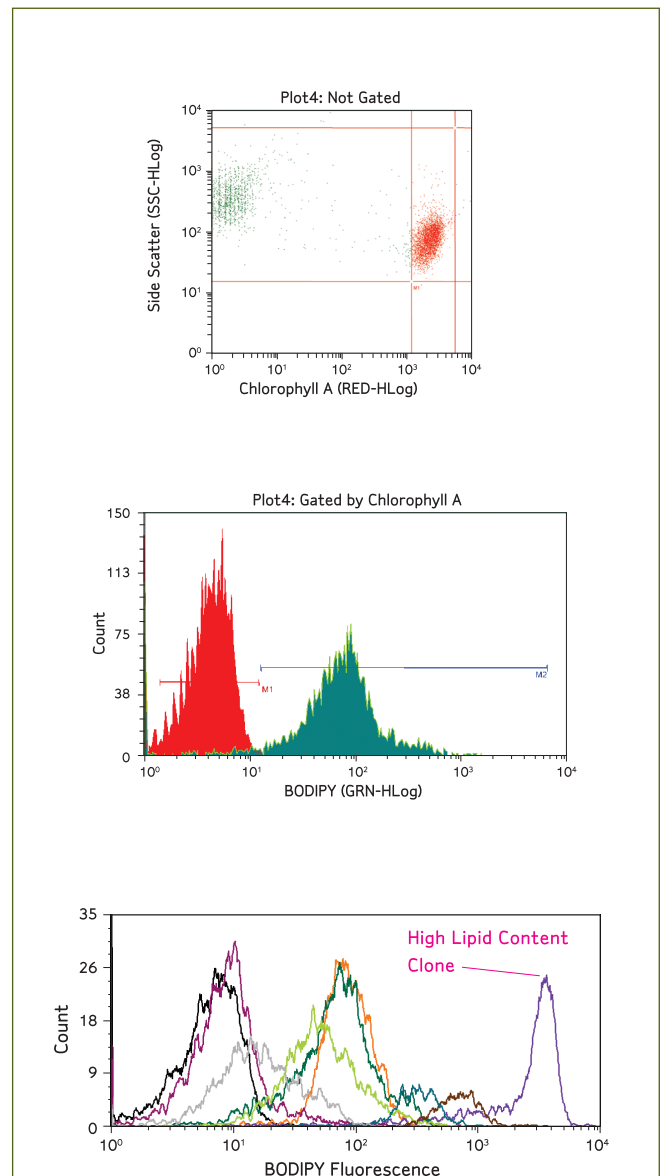
Despite modern extraction methods, existing energy reserves such as buried coal and shale beds and oil reservoirs are often significantly underutilized. An oil reservoir, for example, is considered “dead” or “non-economic” when about 70% of the oil still remains in the subsurface.

The presence of methanogenic microbes in these deposits suggests that production of new methane from existing deposits is possible. This “biogenic” methane production results from indigenous populations of

anaerobic microbes that metabolize large hydrocarbon molecules in coal and oil into smaller hydrocarbons including methane, the least polluting and most energy efficient of all the available hydrocarbon fuels. Studies have indicated that various non-organic nutrients may need to be introduced into deposits to simulate this production.

LUCA Technologies Inc. of Golden, Colorado is studying the wide range of indigenous microorganisms found in these “geobioreactors” and evaluating ways to manipulate them via various treatments in order to maximize methane production.

“We want to understand how to stimulate the growth of these cells as they are integral to methane production,”



**Figure 2.** Identification of algal cells containing chlorophyll A; chlorophyll A fluoresces in the red channel (top). Gate applied to select for chlorophyll A-positive cells (middle). Histograms showing a wide range of lipid content (as evidenced by BODIPY green fluorescence intensity) for a variety of algal strains (bottom).

describes Gary Vanzin, Ph.D., lead scientist at LUCA. "Methane is a byproduct of cellular growth as are many other metabolites. The more growth we can stimulate by our treatments, the more methane generated."

The company has developed thousands of cultures consisting of coal samples collected anaerobically and placed in sealed vessel containing water from the natural environment. Various treatments are then applied to the samples to assess the impact on microorganism growth.

Counting microbes from these cultures in order to monitor growth has presented numerous challenges. The microbes only grow to very low densities, so the abundance of particulates significantly outnumbers the microbes. The particulates are also the same size and density as the microbes and are highly autofluorescent.

Background autofluorescence prevents use of traditional methods to quantify microbes by use of fluorescent tags and physical separation of the two is extremely difficult due to their similarity in size. Total DNA content is also challenging as the isolation protocol is complicated and laborious due to all the hydrocarbons in the sample and the difficulty of lysing the organisms.

Vanzin now uses a Guava benchtop flow cytometer to monitor growth of microbial cultures in the lab, processing about 300 to 500 samples per week.

"We initially evaluated the signal-to-noise of cellular fluorescence versus particulate fluorescence on the Guava system," notes Vanzin. "We get a sufficient signal-to-noise separation so now we have an easy-to-use, high-throughput way to quantitate cell numbers in our samples."

Microfluidic technology in the flow cytometer provides another key advantage. The lab is able to use extremely small sample volumes to get representative cell concentrations – on the order of 10 to 20 microliters.

"With the investment and effort required to create the coal and microorganism cultures, sacrificing only a small amount for routine testing is a significant benefit," said Vanzin.

With an eye towards reducing our dependence on fossil fuels, pollution, and CO<sub>2</sub> emissions, innovative companies like Solix and LUCA are optimizing processes for the development and use of biofuels. Supporting these efforts is flow cytometry – a powerful, high-speed, easy-to-use technology that is now available directly on the lab bench.

#### References

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2. <http://www.arl.arizona.edu/naabb/>.

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