Background

There is much need to determine which algal strains and conditions will be most efficient for producing biofuels, and more specifically, which strains offer the highest efficiency of photosynthesis with maximal lipid production. Current methods involve growth of small cultures followed by lipid measurement; these methods require days per sample because a very large number of cells is required for quantification. In addition, bulk measurements are averaged over live and dead cells, and are incapable of resolving heterogeneity within a culture.

Currently, instrumentation for high-throughput (HT) single-cell measurements of algal photosynthesis, growth rates, and biomass does not exist. Some of the individual component technologies are available for measuring ratios of chlorophyll content, but protocols for quantifying lipid production by single cells have not yet been developed. These instruments cannot be configured for photosynthesis measurements. Finally, HT cell-sorters are available, but these cytometers subject algal cells to high shear stresses, which damages them; this limits the ability to grow rare clones in selection experiments.

Technology

A University of Colorado research group led by Ralph Jimenez has built a cytometer that obtains a cell-by-cell resolved profile of growth, extent of light absorption and utilization, and lipid production from samples of one nanoliter at a rate of several thousand cells per minute. This improved screening method profiles individual algal cells quickly and accurately, and is also significantly more compact and less costly than standard flow cytometers.

Dr. Jimenez has also developed protocols for quantifying the lipid content of single diatom and green algal cells employing commercially-available fluorescent probes. This cytometer uses “saturating flash” chlorophyll fluorescence techniques for quantifying photosynthetic performance, allowing it to accomplish what a standard cytometer cannot.

The microfluidic dimensions ensure that only one nanoliter of culture is needed, and also that fluid transport limits shear stress on the cells, thus improving viability for propagating populations. This detection of light-scattering events enables high-speed cell counting and growth-rate measurements, in conjunction with photosynthesis and lipid assays.

Key Documents

“Flow Cytometer Systems and Associated Methods.”
Provisional patent application filed April 1, 2013; available under CDA.

Microfluidic cytometer for high-throughput measurement of photosynthetic characteristics and lipid accumulation in individual algal cells. Lab Chip 2013,13, 2893-2901. PDF available upon request.