Center for Manufacturing Innovation

Plant-based vaccine factory

In collaboration with Boston University, **Fraunhofer CMI** conducts advanced research and development leading to engineering solutions for a broad range of industries, including biotech/biomedical, photonics, and renewable energy. Engineers, faculty, and students at the Center scale up basic research into advanced technologies that meet the needs of both domestic and global client companies. The primary focus is on the development of next-generation high-precision automation systems, instruments and medical devices.

During 2009, CMI increased its focus on biotech/biomedical systems and was successful in acquiring a number of contracts in this area from government, foundations, and industrial sources. This includes novel methodologies for rapid antibiotic susceptibility testing sponsored by NIH, clinical utility of lab-on-a-chip diagnostic sponsored by the Coulter Foundation, and automated tissue homogenization for sample preparation funded by an industrial client. A major achievement for CMI in this space was the development and deployment a fully automated system for the production of plant-based pharmaceuticals (jointly with Fraunhofer CMB) sponsored by DARPA.

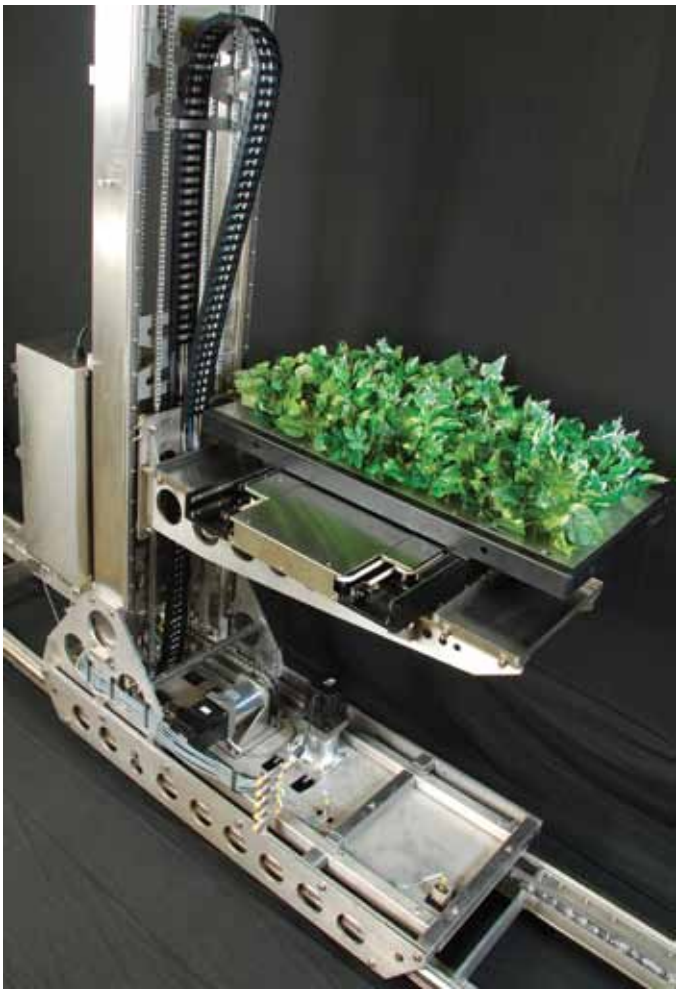
Also during 2009, CMI developed and deployed automation equipment for the production of cymbals, calibration equipment for the production of fuel cells, and novel golf club heads, among other things.

Fully Automated Plant-Based Vaccine Factory

CMI, working jointly with Fraunhofer CMB, has developed a fully automated, scalable "factory" that uses natural (non-genetically-modified) green plants to efficiently produce large quantities of vaccines and therapeutics within weeks. Such a rapid vaccine production facility will play a crucial role in addressing and containing future pandemics and emerging biological threats.

This first-of-a-kind, plant-based vaccine factory takes advantage of plant viral vector technology (developed at CMB) that enables production of specific proteins within the leaves of rapidly growing plant biomass. The factory's robotically tended, custom engineered machines plant seeds, nurture the growing plants, introduce a viral vector that directs the plant to produce a target protein and harvest the biomass once the target has accumulated in the plant tissue (see photos page 28 & 29). Traditional methods of vaccine production can take many months. This plant-based technology enables rapid, large scale production of vaccine material in a cost-effective manner. It has the potential to revolutionize how biological materials are manufactured.

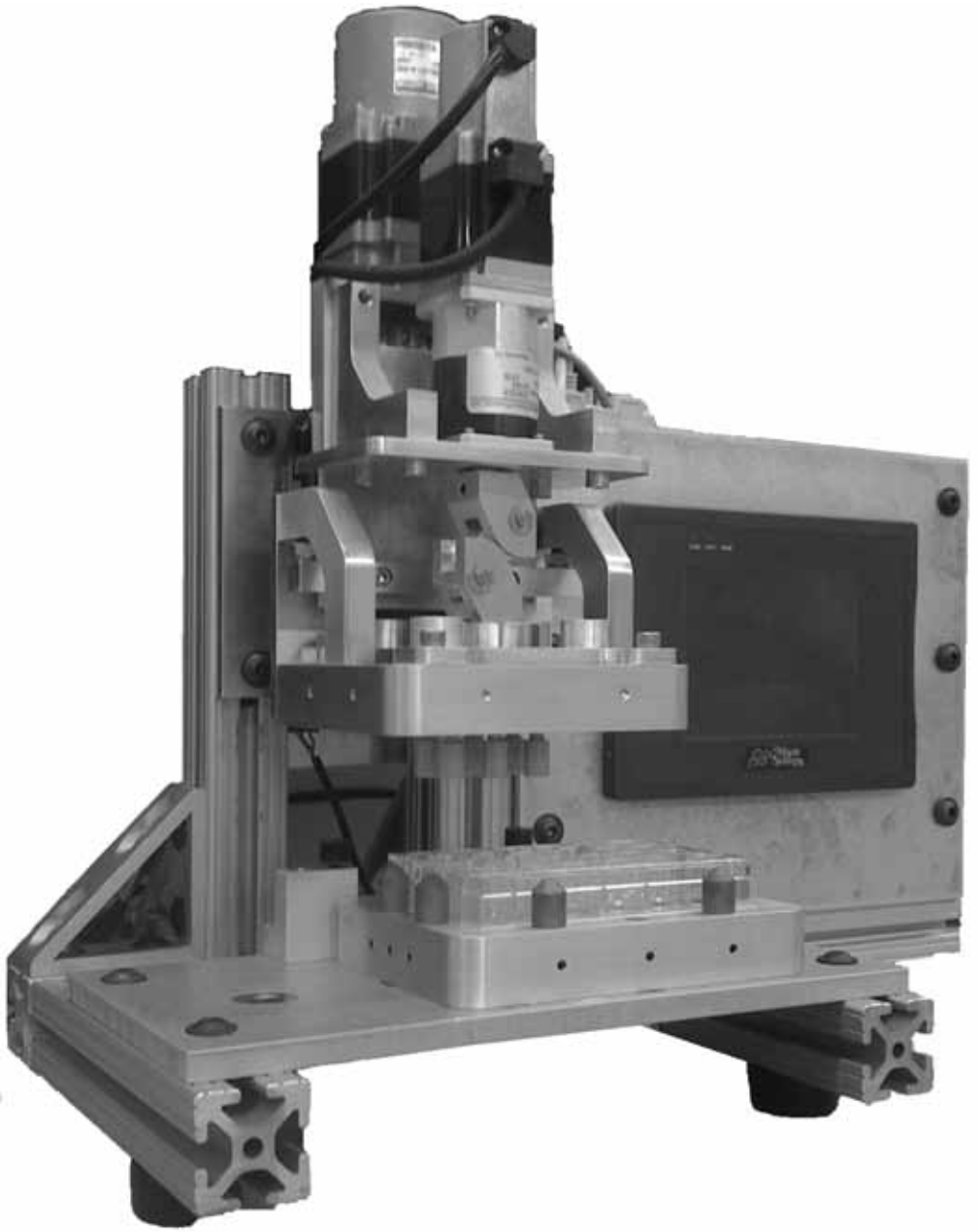
The factory was designed to be time, cost and space efficient. It has the capacity to grow tens of thousands of plants in one batch. The plants are grown in multi-plant trays that are used to handle and transport the plants to the different processing stations. To automate the process, robots glide up and down a track, tending the plants – delivering trays from the lighted, irrigated growth modules to each processing station at the appropriate time. In order to quickly produce large quantities of vaccine material or other protein-based medicines, such as antibodies, in compliance with cGMP, (Current Good Manufacturing Practices), it was necessary to develop a consistent, repeatable process. Even though the process of making vaccines from plants includes many aspects of traditional horticulture such as growing, watering and harvesting, the entire process was automated using techniques found in industrial type processes. This enabled quick, safe, and cost-effective scale-up from a few milligrams in a laboratory setting to the many kilograms that would be required in case of a pandemic. The resulting process is faster, less expensive, safer,



Custom-made robot being tested

and does not require the sophisticated culturing or fermentation necessary in the current vaccine production processes. This will be the first cGMP facility for plant-based protein production.

This unique, plant-based vaccine factory resulted from a three-year collaboration between Fraunhofer and Boston University. "This is a perfect example of coupling engineering expertise and scientific advancement to cost-effectively meet a societal need," remarked Robert Brown, president of Boston University and a chemical engineer. "It is a model for collaboration that we strongly believe in on our campus, as they do at Fraunhofer as well."



CMI's prototype tissue homogenization instrument

10 mg hotdog samples in 100 microliters of water, before and after 60 seconds of homogenization.

Automated Tissue Homogenization

Tissue homogenization is the process of separating and individuating the cells of a tissue sample. It is the first step in many laboratory sample preparation processes in a variety of settings, from the clinical laboratory to food safety testing. Various techniques are regularly used in practice, ranging from large industrial high pressure valve processors that can homogenize liters of tissue at a time, down to the basic mortar and pestle for grinding a few milliliters of sample. When a procedure requires parallel processing of an array of samples, such as in high throughput assays, the options available to researchers are limited. The problem is that although some microplate-based bead beating and ultrasonic products are available, they require skilled users, or are not optimal for solid tissue samples. Furthermore, bead beating requires the careful addition and subsequent removal and sanitizing of the beads, which can be a costly and time-intensive process.

CMI has developed a prototype instrument that is capable of quickly homogenizing an array of unique tissue samples directly in a microtiter plate (See top photo, page 30). The instrument requires no special training to achieve uniform, repeatable results, and is thus adaptable to semi- and fully automated equipment. Additionally, the system is easy to clean and sterilize, has adjustable speed and force to control shear and unwanted heating, and is useful for sample sizes ranging the entire breadth needed for clinical samples (microliters to milliliters).

The homogenization cycle involves the following steps:

1. The instrument is instructed as to which homogenization program to run.
2. Disposable pestles are loaded into pestle holder.
3. A disposable microplate loaded with samples is placed into the machine.
4. The loaded pestle holder with pestles is lowered until the pestle holder reaches its travel stops, at which point the pestles are just contacting the bottom of the microplate wells.
5. The rotary motor drives the pestles around a pre-programmed orbit.
6. When homogenization is complete, the rotary motor stops and the pestle holder retracts.
7. The microplate with the homogenized samples is removed and then proceeds to the next process step.
8. Pestles are removed from pestle holder and are either disposed of, or cleaned and sterilized (e.g. autoclaved) for re-use.

The primary mechanism in the homogenizer is a linkage that transmits torque from the rotary motor to the pestle plate and varies the orbit radius of the pestles through the vertical motion of the linkage. This mechanism serves a few integrated functions. It provides the orbital motion of the pestles, it brings the pestles into contact with the samples in the microplate wells, and it allows entering and retreating from the microplate with the pestles not touching the walls of the wells as they are lowered or raised. This is very important as to not smear sample along the walls of the well, increasing the risk of dripping and cross-contamination. This novel, multi-function, integrated mechanism greatly reduces the complexity and cost of providing an additional, independently actuated degree of freedom for controlling the orbit radius.

To evaluate the ability of the instrument to homogenize tissue, a series of experiments were conducted using a 24-well microplate with small (10mg) pieces of hot dog in water (~100 micro liters). To optimize the homogenization, the pestle design, orbital velocity, pressure, and grinding time were varied. Using a Design of Experiments matrix, the best processing conditions were found. Using this recipe, the tissue was completely ground into a slurry while minimizing the processing time. The homogenate was further characterized by conducting downstream PCR reactions to demonstrate successfully that the procedure was compatible with molecular diagnostic techniques.

This prototype instrument addresses the shortcomings of existing commercially available automated homogenizers, namely not being arrayable at low cost or requiring additional components (beads) which must be subsequently removed from the sample. The design is suitable for homogenizing different types of samples that vary in consistency and size as because the applied shear force, time, and resulting heat generation for homogenization can be controlled by varying the orbital velocity, pestle design, time of applied shear, and pressure. Moreover, the design can easily be scaled to higher throughput (smaller sample sizes and larger sample numbers) in a low cost manner. Additionally, the instrument is compatible with downstream molecular biological analysis.

To Contact Fraunhofer CMI:

Fraunhofer Center for Manufacturing Innovation
Dr. Andre Sharon, Executive Director
Fraunhofer Center for Manufacturing Innovation
15 St. Mary's Street
Brookline, Massachusetts, 02446

Tel: 617 353 8776
asharon@fraunhofer.org