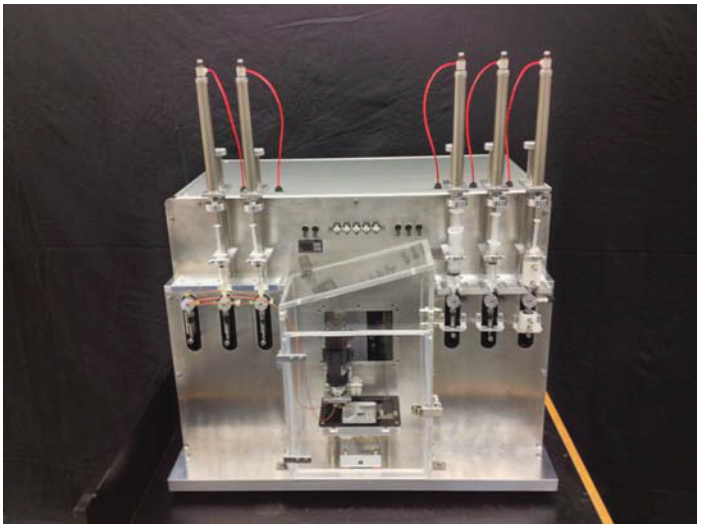


Figure 1. Completed 3-D BioPrinter

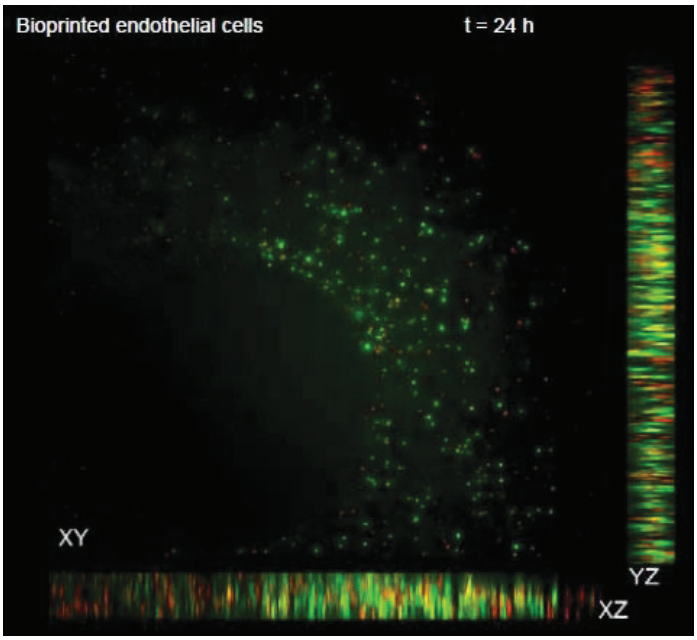


Figure 2. Endothelial cells

The Fraunhofer Center for Manufacturing Innovation (CMI), together with its partners, Boston University and the Fraunhofer Institute for Production Technology, conducts advanced research and development leading to engineering solutions for a broad range of industries, including biotech/biomedical, photonics, and renewable energy. Fraunhofer engineers, faculty and students scale up basic research into advanced technologies for client companies in the U.S. and abroad. CMI's primary focus is on next-generation, high-precision automation systems as well as medical devices and instruments that lie at the intersection of engineering and biology.

During 2012, CMI won its first National Science Foundation research grant entitled "Charge Assisted Protein Sensing." Additionally, CMI participated on the Boston University-led team that was awarded a translational NIH grant for the creation of a Center for Innovation in Point of Care Technologies for the Future of Cancer Care. CMI will serve as the engineering partner for this multi-organization, multi-year program. With on-going NIH grants on Bacterial Drug Susceptibility Identification by Surface Enhance Raman Spectroscopy, CMI is establishing itself as a key player in the biotech/biomedical areas with the U.S. government funding agencies.

On the industrial front, during 2012, CMI has acquired and/or delivered a number of automation systems and instruments to major U.S. corporations. Specific applications ranged from high speed manufacture of disposable products, to highly sophisticated, intelligent automation for genetically engineered products, to novel automated processes for the manufacture of aircraft engine components.

Finally, CMI has further enhanced its reputation in the scientific community with five new journal publications, including one in the prestigious **Science Translational Medicine**.

Representative systems under development at CMI:

3-D BioPrinter

CMI has developed a 3D additive manufacturing system capable of multi-material and multi-scale deposition of biological materials, enabling the next-generation of bottom-up tissue engineering and synthetic organ production. This new area for CMI is an exciting and important scientific endeavor that resides at the interface of manufacturing engineering and

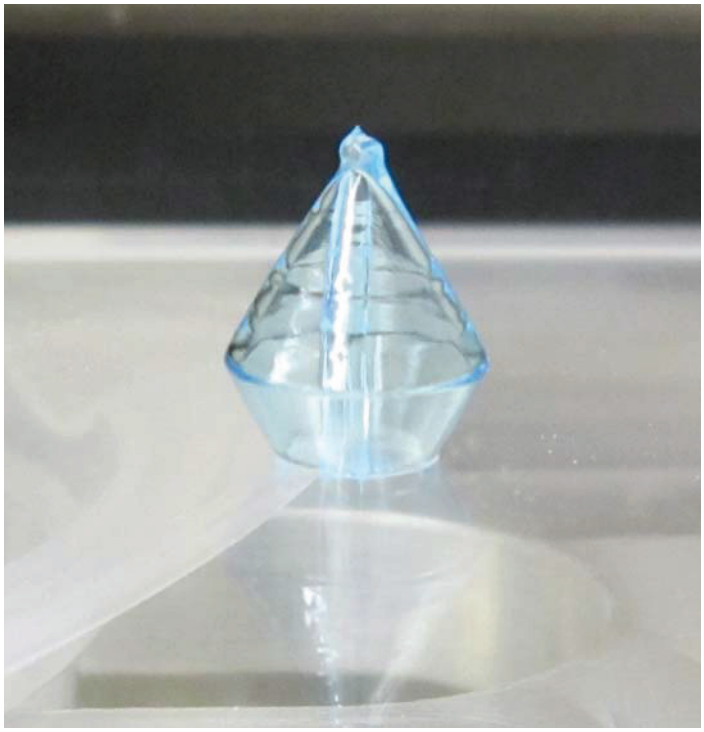


Figure 3. Hollow diamond test structure

life sciences. As such, it entails four diverse elements: a novel hydrogel-based bioink, a 3D bioprinter, software to drive the printer, and mammalian cell culturing.

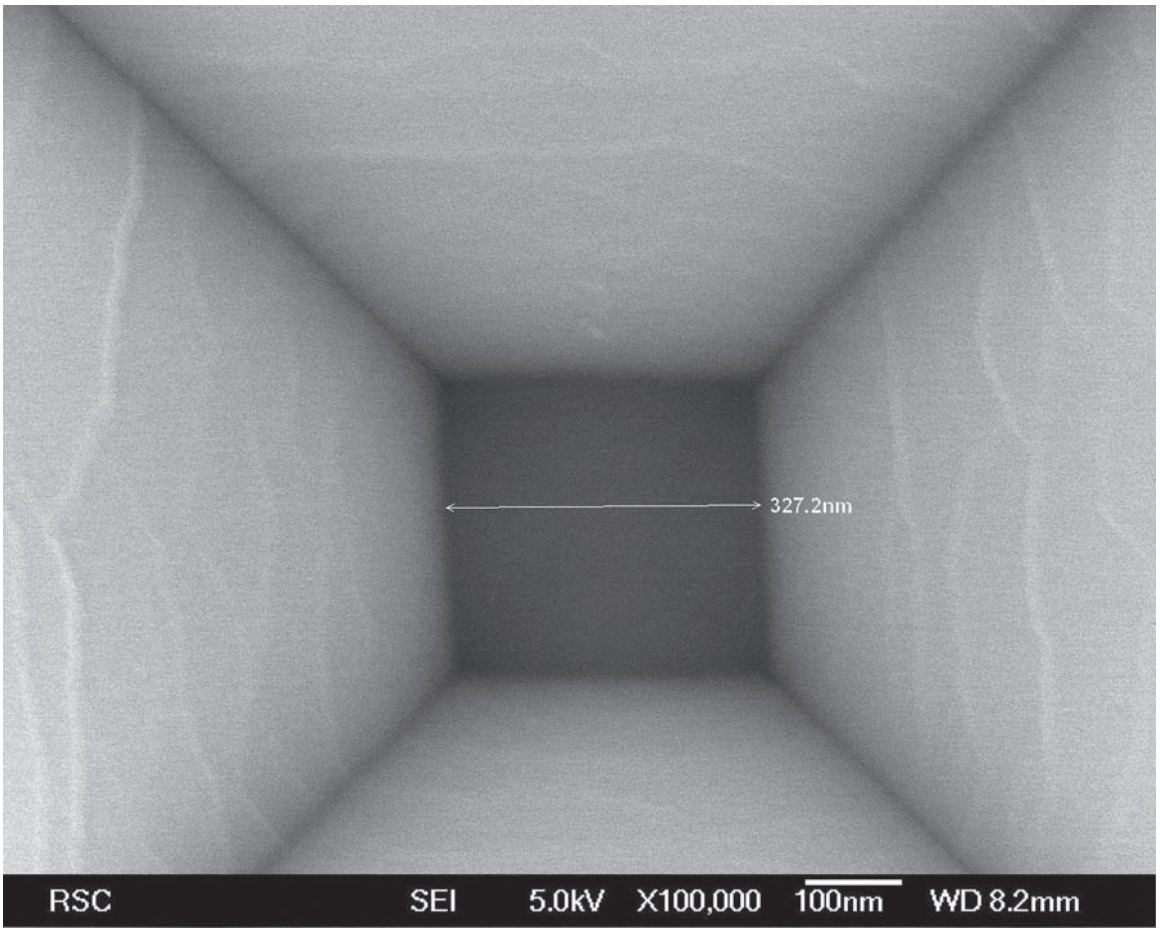
One benefit of a direct writing approach is that one can in principle achieve the multiple length scales found in nature, from that of the microscale (~0.1 mm, e.g. pancreatic islets) to the mesoscale (~10 mm, e.g. large blood vessels) to the macroscale (~100s mm, e.g. length of long bones). Based on concepts from 3D rapid prototyping of non-biological materials, initial forays into 3D printing of biological materials have been met with some initial success. These methods use various types of cell-laden hydrogels that are printed following CAD/CAM procedures. The hydrogels can be photopolymerizable, thermoreversible, or chemically cross-linked. The surrounding material serves two purposes; to suspend the cells in a matrix that has enough viscosity to maintain a three-dimensional shape and to provide the appropriate chemical environment to support cellular viability and in some cases cell differentiation and outgrowth. These purposes can require compromises in the material choice or geometry when the desired tissue construct is as complex as natural tissues. Moreover, often a trade-off exists between the desired resolution and the manufacturing speed, as viscous materials must be printed slowly to maintain a uniform (and large) line width during extrusion. However, there is a time limitation during which the biological material can sustain the printing conditions (humidity, temperature, etc.), thus this can limit the obtainable geometries.

To address these challenges, CMI designed and built a 3D rapid prototype machine that can both achieve the multiple scales required AND simultaneously print various materials (including biologicals) in order to achieve a level of complexity that actually mimics the intricacies of natural tissues. The system has three components uniquely combined into a comprehensive tool: a multi-nozzle head that allows application of different materials simultaneously with varying viscosities and chemistries (and carrying live cells), a high velocity/high precision x-y-z stage to accommodate the most rapid speeds allowable by the materials to be printed, and a methodology that borrows well-developed methods from traditional rapid prototyping to achieve higher complexity 3D bioprinting.

Figure 1 shows the completed 3-D BioPrinter, while Figure 2 shows printed endothelial cells with the bright dots indicating live cells having survived the printing process. Finally, a hollow diamond test structure on its tip was manufactured on the BioPrinter and is shown in Figure 3.

Charge Assisted Protein Sensing

Next-generation sequencing is providing a wealth of information about the human genome. However, not all proteins in the genome are actually expressed, so the importance of understanding the biological interactions of such proteins is becoming increasingly important. Two-dimensional gel electrophoresis and mass spectrometry are the current technologies of choice, but new technologies are needed to allow label-free analysis of proteins in physiological environments. CMI is developing a new technology that takes advantage of extremely well developed silicon technology that now can make arrays of high-gain sensors with dimensions comparable to single biomolecules (5-20 nanometers). Such devices can be configured to be an analysis tool that senses the charge configuration of individual biomolecules while carried in ionic media through small synthetic pores. This technology could potentially serve to deepen our understanding of biochemical interactions and pathways to elucidate mechanisms of normal and disease states.



Scanning electron micrograph of a pore.

The push to use increasingly smaller biological samples for analysis is driving innovation towards high analytical sensitivity; the ultimate sensitivity being at the single molecule level. Single molecule analysis techniques such as atomic force microscopy, optical tweezers, fluorescence resonance energy transfer (FRET), other types of single molecule spectroscopy (e.g. fluorescence anisotropy), and surface plasmon resonance (SPR) have been providing direct access to biomolecular information. These techniques determine characteristics of molecules without statistical ensemble averaging, including their associated distribution functions and possible subpopulations. Techniques such as nuclear magnetic resonance (NMR) and x-ray crystallography have complemented the dynamic techniques' results with structural information. These single molecule technologies have supplied information about concentrations, structure-function relationships, and interaction kinetics of biological molecules; key requirements for modeling cellular organization and temporal dynamics.

Existing single molecule techniques have several shortcomings, including the fact that they do not allow study of the analytes in their native environment, are not high throughput, are expensive, and are labor-intensive. With current techniques, the molecules of interest must be labeled with a probe (e.g. fluorescence), be crystallized (e.g. x-ray crystallography), or be bound to a surface (e.g. AFM, SPR, optical tweezers). Researchers are careful to minimize the effects of these modifications, but an ideal method would allow study of single molecules without modification in solution phase. Although NMR does allow study of unlabeled molecules in solution, it is limited by the size, purity and concentration of the target molecule. Moreover, the methods developed to

study the biophysical properties of single molecules are limited in both their throughput capability and spatial resolution. With the human genome project completed and low cost sequencing on the horizon, a need exists for higher throughput technologies to study the proteome at the single molecule level. This will require parallel methods that are scalable and affordable.

To address these technological shortcomings, CMI is developing a semiconductor nanopore sensor array technology that will enable the high-throughput study of single molecules in solution. It is based on a novel design of integrated FET sensors and microfluidics. Figure 4 shows a scanning electron micrograph image of a pore fabricated for this project. In 2012, CMI received a competitive award from the United States National Science Foundation to develop this technology over three years.

Automated Injection Under Vacuum

Fraunhofer CMI developed a new system for filling and injection of components under vacuum conditions and lower injection pressures. Vacuum can be an important environmental factor for many applications to avoid entrapped air, especially at lower injection pressures required for delicate components. Entrapped air can cause material defects or visible occlusions in the component. The new Fraunhofer system eliminates these issues by enabling filling under vacuum at less than 1 Torr.

The system incorporates an X-Y positioning system to allow an operator to load the machine with a rack of components to be filled. The travel of 200 mm by 180 mm permits a large number of components to be processed in one batch, limited only by component size. After the chamber is pumped down, the system sequentially processes each component. A Z-axis drive engages a fill port on each component and then injects with a positive displacement syringe pump. After all the components are processed, the chamber is vented so another rack can be loaded into the machine and processed in quick succession.

Process parameters, including injection speed, pressure, volume, and dwell are completely customizable to allow a large viscosity range of materials to be processed. Process parameters are stored in recipes which can be recalled by the operator from a menu system to suit different products or components being processed. Data is collected in real-time from each fill and logged for quality control or evaluation.

The system was designed for high reliability in a vacuum environment. All heat generating components are located outside the vacuum chamber. Servomotors for driving the X-Y system, for instance, are located in-air for cooling but coupled inside the chamber with rotary ferromagnetic feedthroughs, while Z axis translation is coupled through a metal bellows seal.

CMI Internship Program

CMI's internship program continues to thrive, providing a global experience to 12 European interns per year. Since its inception, the program has hosted over 150 interns, mostly from Europe. Interns are provided with housing and a stipend, and are encouraged to experience not only the American workplace, but the American culture as well. The program has been tremendously successful, receiving rave reviews from all involved. These students are subsequently highly recruited in Europe, as they bring a global perspective to the job.

For more information on all these technologies, please visit: www.fhcmi.org

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