

Video Article

July 2012: This Month in JoVEAaron Kolski-Andreaco¹, Wendy Chao²¹JoVE Content Production²Department of Ophthalmology, Massachusetts Eye and EarURL: <https://www.jove.com/video/5010>DOI: [doi:10.3791/5010](https://doi.org/10.3791/5010)

Keywords: This Month in JoVE, Issue 65

Date Published: 7/1/2012

Citation: Kolski-Andreaco, A., Chao, W. July 2012: This Month in JoVE. *J. Vis. Exp.* (65), e5010, doi:10.3791/5010 (2012).**Abstract**

Historically, JoVE, *The Journal of Visualized Experiments*, has focused primarily on biomedical research and has developed subsections for Bioengineering, Clinical and Translational Medicine, Immunology and Infection, and Neuroscience. This July, JoVE launches its Applied Physics section, which includes a range of content from Plasma Physics to Materials Science. We begin the new section with a notable article from Purdue University, where researchers in the Center for Laser-Based Manufacturing are studying.

Matter exists in three familiar states: solid, liquid, and gas. If a gas is hit with enough energy, atoms can lose electrons, or become ionized, to form a fourth state called plasma. Plasma is the most abundant form of matter; occupying about 99.999% of the visible universe. Using ultrashort laser pulses of 100 femtoseconds, or 100 quadrillionths of a second, our authors demonstrate a technique called pump probe shadowgraphy, which allows the early plasma to be visualized as it evolves from metal surfaces. By constructing a simulation model, these investigators are able to examine early plasma dynamics, enabling a better understanding of how matter becomes ionized.

For the materials science subcategory of applied physics, JoVE materializes at the University of Michigan, where researchers are developing new methods in microfabrication. Our authors demonstrate a method for growing complex, three-dimensional microstructures out of carbon nanotubes, which can be used as master molds to cast replicas out of polymers or biological materials. Scanning electron microscopy reveals that the carbon nanotube master molds are reproduced with high fidelity in microscale shape and nanoscale texture in the polymer replicas made from these molds. Microfabrication technology allows laboratory operations to be performed on small scales – essentially putting a lab on a chip.

Shifting from Applied Physics to cardiac physiology, JoVE visits The George Washington University to capture a modified Langendorff preparation. JoVE has published several articles that demonstrate this physiological prep, which allows the heart to beat in isolation for hours at a time. The approach involves retrograde perfusion of the heart, via the aorta, which shuts the aortic valve and forces oxygenated perfusate through the coronary circulation, in order to sustain cardiac tissue.

Our authors present a modification to this preparation that involves cannulating the left atrial appendage, the inferior vena cava, and the pulmonary artery. In this state all four chambers of the heart are cannulated, thereby providing physiological load pressures to both ventricles, and eliminating the need to retrogradely-perfuse the heart through the aorta. Therefore, perfusion of the heart is in the normal direction, and the heart provides its own pressure for coronary perfusion.

Once this biventricular working heart model is achieved, our authors proceed to set up imaging of nicotinamide adenine dinucleotide, or NADH, fluorescence. This coenzyme, which is found in mitochondria, emits fluorescence in its reduced state and provides a readout for local oxygen concentration, and therefore heart metabolism. These investigators demonstrate measurements of NADH fluorescence during different pacing rates and thereby illustrate the potential of this physiologically-relevant model to provide insight into cardiac pathologies.

In our Bioengineering section, JoVE visits the University of the Pacific for an article dealing with synthetic spider silk production. Back in 2010, JoVE published an article from the Vierra lab, which demonstrated microdissection techniques to isolate the 12 silk producing glands from the Black Widow spider. Isolation of these glands allows for analytical techniques to be performed, in order to identify specific spider silk proteins. Because spiders are venomous and cannibalistic, rearing them for large scale production of spider silk is unrealistic. Therefore, the Vierra lab transforms bacteria with silk protein-containing plasmids and expresses recombinant spider silk proteins in bacteria.

This July in JoVE, the Vierra group takes us through a method for isolating and purifying spider silk protein from these bacteria. They then show us how purified protein is spun into fibers and demonstrate methods for collecting these fibers, as well as assessing their strength. Spider silk is of great interest to biomaterial scientists, because of its biocompatibility and mechanical properties, which make it stronger than tensile steel. The Vierra lab has shown us laboratory-scale production of spider silk that can potentially be extended to a large scale manufacturing process. This brief summary synthesizes four of the fifty articles that JoVE will release this July. Other noteworthy publications include demonstrations of ex vivo electroporation in late stage chicken embryos, production of secreted proteins from human cells, and the ex vivo culturing of fruit fly brains.

Video LinkThe video component of this article can be found at <https://www.jove.com/video/5010/>

Protocol

Investigation of Early Plasma Evolution Induced by Ultrashort Laser Pulses

Wenqian Hu, Yung C. Shin, Galen B. King
Mechanical Engineering, Purdue University

An experimental method to examine the early plasma evolution induced by ultrashort laser pulses is described. Using this method, high quality images of early plasma are obtained with high temporal and spatial resolutions. A novel integrated atomistic model is used to simulate and explain the mechanisms of early plasma.

Fabrication, Densification, and Replica Molding of 3D Carbon Nanotube Microstructures

Davor Copic¹, Sei Jin Park¹, Sameh Tawfik¹, Michael De Volder², A. John Hart¹
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We present methods for fabrication of patterned microstructures of vertically aligned carbon nanotubes (CNTs), and their use as master molds for production of polymer microstructures with organized nanoscale surface texture. The CNT forests are densified by condensation of solvent onto the substrate, which significantly increases their packing density and enables self-directed formation of 3D shapes.

NADH Fluorescence Imaging of Isolated Bi-ventricular Working Rabbit Hearts

Huda Asfour¹, Anastasia M. Wengrowski¹, Rafael Jaimes III¹, Luther M. Swift², Matthew W. Kay¹
¹Electrical and Computer Engineering Department, The George Washington University, ²Pharmacology and Physiology Department, The George Washington University

The objective is to monitor the mitochondrial redox state of isolated hearts within the context of physiologic preload and afterload pressures. A biventricular working rabbit heart model is presented. High spatiotemporal resolution fluorescence imaging of NADH is used to monitor the mitochondrial redox state of epicardial tissue.

Synthetic Spider Silk Production on a Laboratory Scale

Yang Hsia, Eric Gnesa, Ryan Pacheco, Kristin Kohler, Felicia Jeffery, Craig Vierra
Department of Biological Sciences, University of the Pacific

Despite the outstanding mechanical and biochemical properties of spider silks, this material cannot be harvested in large quantities by conventional means. Here we describe an efficient strategy to spin artificial spider silk fibers, which is an important process for investigators studying spider silk production and their use as next-generation biomaterials.

Gene Transfer into Older Chicken Embryos by *ex ovo* Electroporation

Jiankai Luo¹, Xin Yan¹, Juntang Lin², Arndt Rolfs¹
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A method of gene transfer into chicken embryos at later incubation stages (older than Hamburger and Hamilton stage (HH) 22) is described. This method overcomes disadvantages of *in ovo* electroporation applied to older chicken embryos and is a useful technique to study gene function and regulation at older developmental stages.

A Convenient and General Expression Platform for the Production of Secreted Proteins from Human Cells

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In the post-human genomics era, the availability of recombinant proteins in native conformations is crucial to structural, functional and therapeutic research and development. Here, we describe a test- and large-scale protein expression system in human embryonic kidney 293T cells that can be used to produce a variety of recombinant proteins.

Ex vivo Culturing of Whole, Developing *Drosophila* Brains

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This article describes a method by which one can mimic *in vivo* development of the *Drosophila* mushroom body in an *ex vivo* culture system.

Disclosures

No conflicts of interest declared.