

Video Article

October 2011: This Month in JoVE

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Abstract

The October 2011 issue of the Journal of Visualized Experiments (JoVE) presents the journal's first effort to explore oceanographic physics and engineering. Investigators at the Woods Hole Oceanographic Institute demonstrate the use of a self-contained underwater velocimetry apparatus, or SCUVA. Principle components of this instrument include an HD camera and laser, which when aligned with an optical lens, produces a vertical sheet of light. Following alignment and testing for a watertight seal, these researchers seek out inhabitants the water column for analysis. Nightly experimentation is necessary, so that natural light does not interfere with the laser sheet. SCUVA is specifically designed to capture the movement of particles in the water, so that the velocity fields - a vector field that describes the movement of fluid - can be determined around an organism in their natural environment.

In Clinical and Translational Medicine, orthodontic researchers from the University of Graz determine the composition of the oral biofilms - a surface-adhering aggregate of microorganisms - that form on their patient's palatal expanders. These authors use fluorescent *in situ* hybridization combined with confocal microscopy to detect specific bacteria within the biofilm as well as visualize its 3-dimensional structure.

JoVE also visits Graz for Immunology and Infection, where researchers at the University of Graz and the Graz institute of technology use transmission electron microscopy, or TEM, to analyze the ultrastructural changes that take place in the plant leaf as a result of viral infection. Small sections of leaves are cut from the plant and subjected to a microwave-assisted fixation procedure, which drastically reduces sample preparation time for TEM. Following embedding and sectioning, samples are loaded into the electron microscope, and imaged. Aided by image analysis software, these authors can calculate the dimensions of virus particles as well as assess the ultrastructural changes in the plant leaf, which is critical for identifying the infecting virus.

In Neuroscience, researchers from the University of California, Irvine, present a series of behavioral paradigms aimed to measure the degree of cognitive impairment in rodents treated with cranial radiotherapy. These authors demonstrate a novel place recognition task (for spatial recognition memory), an elevated plus maze task (for measurement of anxiety), a fear-conditioning task (learning and memory), and the Morris Water Maze (for spatial learning). The cognitive deficits in rodents resemble those manifested in cancers survivors following cranial radiotherapy, and therefore standardization of such cognitive behavioral paradigms is critical to assess therapy. One potential therapy is stem cell transplantation into the hippocampus, which the authors demonstrate in a separate video-article in October.

On the topic of stem cells, researchers from the University of California, Riverside demonstrate methods for genetic manipulation of human induced pluripotent stem cells. Induced pluripotent stem cells, iPSCs, can be derived from somatic cells, like the fibroblasts you see here, following forced expression of certain transcription factors, which cause the cells to be transformed into a pluripotent state. These authors show how iPSCs can be cultured in feeder-free conditions and provide helpful hints for lifting and seeding the cells at concentrations optimal for transfection and nucleofection.

In addition to these featured publications, JoVE presents methods for taking physiological measurements in individuals under psychosocial stress, tracking bacterial lysis following infections with phage, imaging in the olfactory bulb via a cranial window, and dozens more video-articles of complex methods.

Video Link

The video component of this article can be found at <https://www.jove.com/video/3957/>

Protocol

Transfecting and Nucleofecting Human Induced Pluripotent Stem Cells

Papri Chatterjee, Yuri Cheung, Chee Liew

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Despite recent advancements in genetic modification, transfection of human embryonic stem cells (HESCs) remains a capricious process. To our knowledge, systematic and efficient methods to transfect human induced pluripotent stem cells (iPSCs) have not been reported. Here, we describe robust protocols to efficiently transfect and nucleofect human iPSCs.

Microwave Assisted Rapid Diagnosis of Plant Virus Diseases by Transmission Electron Microscopy

Bernd Zechmann^{1,2}, Gerhard Graggaber¹, Günther Zellnig¹

¹Institute of Plant Sciences, University of Graz, ²Institute for Electron Microscopy and Fine Structure Research, Graz University of Technology
This study describes a method that allows the rapid and clear diagnosis of plant virus diseases in about half a day by using a combination of microwave assisted plant sample preparation for transmission electron microscopy and negative staining methods.

Following Cell-Fate in *E. coli* After Infection by Phage Lambda

Lanying Zeng¹, Ido Golding^{1,2,3}

¹Department of Physics, University of Illinois at Urbana-Champaign, ²Center for the Physics of Living Cells, University of Illinois at Urbana-Champaign, ³Verna and Marrs McLean Department of Biochemistry and Molecular Biology, Baylor College of Medicine

This article describes the procedure for preparing a fluorescently-labeled version of bacteriophage lambda, infection of *E. coli* bacteria, following the infection outcome under the microscope, and analysis of infection results.

Stem Cell Transplantation Strategies for the Restoration of Cognitive Dysfunction Caused by Cranial Radiotherapy

Munjal M. Acharya*, Dante E. Roa*, Omar Bosch, Mary L. Lan*, Charles L. Limoli

Department of Radiation Oncology, University of California Irvine

* These authors contributed equally

Brain tumor patients routinely undergo cranial radiotherapy, and while beneficial, this treatment often results in debilitating cognitive dysfunction. This serious unresolved problem has at present, no clinical recourse, and has driven our efforts to devise stem cell based therapies for the recovery of radiation-induced cognitive decrements.

Quantifying Cognitive Decrements Caused by Cranial Radiotherapy

Lori-Ann Christie, Munjal M. Acharya, Charles L. Limoli

Department of Radiation Oncology, University of California Irvine

Cognitive impairment resulting from the radiotherapeutic management of brain tumors represents a clinically intractable condition that adversely impacts quality of life. The capability to critically evaluate potential interventions for ameliorating radiation-induced cognitive decrements ultimately depends on the capability to undertake rigorous quantitative assessments of cognition.

The Trier Social Stress Test Protocol for Inducing Psychological Stress

Melissa A. Birkett

Department of Psychology, Northern Arizona University

This article describes a protocol for inducing psychological stress in participants, which enables researchers to measure psychological, physiological and neuroendocrine responses to stress within single participants or between groups.

Oral Biofilm Analysis of Palatal Expanders by Fluorescence In-Situ Hybridization and Confocal Laser Scanning Microscopy

Barbara Klug^{1,2}, Claudia Rodler¹, Martin Koller³, Gernot Wimmer³, Harald H. Kessler², Martin Grube⁴, Elisabeth Santigli¹

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We present a protocol for structural and compositional analysis of natural oral biofilm from orthodontic appliances with in situ hybridization (FISH) and confocal laser scanning microscopy (CLSM). Oral biofilm samples were collected from palatal expanders, scraping acrylic-resin flakes off their surface and referring them for molecular processing.

Quantitatively Measuring *In situ* Flows using a Self-Contained Underwater Velocimetry Apparatus (SCUVA)

Kakani Katija¹, Sean P. Colin^{2,3}, John H. Costello^{3,4}, John O. Dabiri⁵

¹Applied Ocean Physics and Engineering, Woods Hole Oceanographic Institution, ²Environmental Science and Marine Biology, Roger Williams University, ³Marine Biology Laboratory, Whitman Center, ⁴Department of Biology, Providence College, ⁵Departments of Aeronautics and Bioengineering, California Institute of Technology

This protocol provides instructions on how to use a self-contained underwater velocimetry apparatus (SCUVA), which is designed for quantification of in situ animal-generated flows. In addition, this protocol addresses challenges posed by field conditions, and includes operator motion, predicting position of animals, and orientation of SCUVA.

Imaging Odor-Evoked Activities in the Mouse Olfactory Bulb using Optical Reflectance and Autofluorescence Signals

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This article presents the protocols of intrinsic optical signals and flavoproteins autofluorescence signals imaging to map odor-evoked activities at the surface of the olfactory bulb in mice.