

Video Article

## April 2012: This Month in JoVE

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### Abstract

The April issue of the Journal of Visualized Experiments (JoVE) includes an article that follows a lab from the University of Miami of Ohio to Lake Bonney - a permanently ice-covered saline lake in Antarctica. Few organisms can survive the harsh conditions under the ice, where light, warmth, and carbon sources are in very short supply. Single celled microorganisms called protists are the major class of microbes living beneath Lake Bonney, and are capable of bringing carbon, via photosynthesis, into the circle of life.

After determining the appropriate sampling site, our authors drill and melt through the ice so that they can collect samples from various depths within the lake's chemically stratified water column. Once collected, samples are taken to a field laboratory for filtration and stabilization. After processing, microbes present in the water sample are cultured for further experimentation. For example, our authors show that their enrichment cultures retain photosynthetic activity, and future experiments should help better understand protist physiology, as well as how such organisms can affect global geochemical cycling.

In Clinical and Translational Medicine, JoVE turns its attention to stereotactic body radiotherapy, or SBRT, for the treatment of gynecological tumors. SBRT is a minimally invasive form of cancer treatment that relies on radiation, delivered by a linear accelerator mounted on a robotic arm, to biologically inactivate tumors.

Our authors, from Case Medical Center, explain how gold soft tissue fiducial markers are placed around the cancer target at various depths based on computed tomography imaging. During a treatment planning session, CT and PET images are taken and then co-registered. Guided by these images, both radiation and gynecological oncologists designate the cancer volumes that will be targeted during therapy as well as contour surrounding normal structures that should be left unharmed. At the start of treatment, a target localizing system detects the fiducial markers via cross-plane radiographic imaging and marker location is compared with previously obtained imaging data.

During treatment, light emitting diodes track a patient's movement, in order to correlate their breathing pattern to the location of the fiducial markers, so that radiation can be delivered accurately. Overcoming the complications associated with patient immobilization, our authors show that their procedure for stereotaxic body radiotherapy can precisely deliver radiation to the target volume while limiting dosage to nearby structures.

In our Bioengineering section, JoVE presents an article, which involves making biocompatible substrates from silkworm cocoons. First, cocoons are screened for contamination and processed so that silk extract is produced. Extract is placed atop a PDMS mold, which has been designed to create a pattern in the silk film drying on its surface. Once dried, the silk film is subjected to a process referred to as water annealing, which renders the film transparent and perfect for microscopy. Following cell seeding on the films, our authors show that tracking cell migration on the micro-patterned silk substrate is just as easy as on more conventional tissue culture surfaces. The biocompatibility of silk films as well as their potential to be chemically and structurally modified makes them attractive candidate materials for in vivo applications, like medical device design.

Surprisingly, primary neuronal culture makes its way into our Immunology and Infection section, because this April, JoVE documents an innovative primary sympathetic neuron culture system used to study herpes simplex virus latency and activation.

Peripheral neurons act like reservoirs for the herpes virus that are the source of reactivation events. To establish a peripheral neuron culture, superior cervical ganglia are isolated from embryonic mice and following digestion and dissociation are plated in 96 well plates. At around day 6 in vitro, herpes viruses encoding a viral-GFP fusion protein are added to the cells along with an inhibitor of lytic replication. Upon inhibitor withdrawal, latent virus will reactivate and GFP expression will be observable via fluorescent microscopy. By counting the number of wells exhibiting GFP fluorescence, the ability of small molecules or gene silencing oligonucleotides to either suppress or induce viral reactivation can be tested, making this assay a potential tool to uncover new therapeutics to help manage HSV-1 infection.

This brief summary describes only four of the fifty articles JoVE will release in April. Other notable releases include methods for quantifying obesity in rats using x-ray computed tomography, inducing myocardial infarction in the zebrafish heart, and retrograde labeling of the excised spinal cord. Stay tuned.

### Video Link

The video component of this article can be found at <https://www.jove.com/video/4421/>

## Protocol

### Silk Fibroin Film Culture System for *In vitro* Analysis and Biomaterial Design

**Brian D. Lawrence<sup>1</sup>, Zhi Pan<sup>2,1</sup>, Michael D. Weber<sup>1</sup>, David L. Kaplan<sup>3</sup>, Mark I. Rosenblatt<sup>1</sup>**

<sup>1</sup>Margaret M. Dyson Vision Research Institute, Weill Cornell Medical College, <sup>2</sup>Margaret M. Dyson Vision Research Institute, Weill Cornell Medical College, <sup>3</sup>Department of Biomedical Engineering, Tufts University

Silk films are a novel class of biomaterials readily customizable for an array of biomedical applications. The presented silk film culture system is highly adaptable to a variety of *in vitro* analyses. This system represents a biomaterial design platform offering *in vitro* optimization before direct translation to *in vivo* models.

### Induction of Myocardial Infarction in Adult Zebrafish using Cryoinjury

**Fabian Chablais, Anna Jaźwińska**

Department of Biology, Unit of Zoology, University of Fribourg, Fribourg, Switzerland

Zebrafish represents a valuable model to study the mechanisms of heart regeneration in vertebrates. Here, we present a protocol for induction of a heart infarct in adult zebrafish using cryoinjury. This method results in massive cell death within 20% of the ventricular wall, similar to that observed in mammalian infarcts.

### Segmentation and Measurement of Fat Volumes in Murine Obesity Models Using X-ray Computed Tomography

**Todd A. Sasser<sup>1</sup>, Sarah E. Chapman<sup>2</sup>, Shengting Li<sup>1</sup>, Caroline Hudson<sup>2</sup>, Sean P. Orton<sup>1</sup>, Justin M. Diener<sup>3</sup>, Seth T. Gammon<sup>1</sup>, Carlos Correcher<sup>4</sup>, W. Matthew Leevy<sup>2</sup>**

<sup>1</sup>Carestream Molecular Imaging, <sup>2</sup>Department of Chemistry and Biochemistry, University of Notre Dame, <sup>3</sup>Freimann Life Science Center, University of Notre Dame, <sup>4</sup>Research and Development, Oncovision, GEM-Imaging S.A.

Fat content analysis is routinely conducted in studies utilizing murine obesity models. Emerging methods in small animal CT imaging and analysis are providing for longitudinal detail rich fat content analysis. Here we detail step by step procedures for performing small animal CT imaging, analysis, and visualization.

### Stereotactic Radiosurgery for Gynecologic Cancer

**Charles Kunos<sup>1</sup>, James M. Brindle<sup>1</sup>, Robert Debernardo<sup>2</sup>**

<sup>1</sup>Department of Radiation Oncology, University Hospitals Case Medical Center and Case Western Reserve University School of Medicine, <sup>2</sup>Department of Obstetrics and Gynecology, Division of Gynecologic Oncology, University Hospitals Case Medical Center and Case Western Reserve University School of Medicine

Stereotactic body radiotherapy (SBRT) involves image-guided, ablative radiation delivered to cancer targets refractory to chemotherapy or to conventional radiation treatment. The robotic-armed Cyberknife SBRT system, using sophisticated target localization, delivers hypofractionated radiation doses capable of sterilizing cancer targets. This article will consider new therapeutic roles of SBRT for gynecological cancers.

### Establishment of Microbial Eukaryotic Enrichment Cultures from a Chemically Stratified Antarctic Lake and Assessment of Carbon Fixation Potential

**Jenna M. Dolhi, Nicholas Ketchum, Rachael M. Morgan-Kiss**

Department of Microbiology, Miami University

Microbial eukaryotes are both a source of photosynthetically-derived carbon and top predatory species in permanently ice-covered Antarctic lakes. This report describes an enrichment culture approach to isolate metabolically versatile microbial eukaryotes from the Antarctic lake, Lake Bonney, and assesses inorganic carbon fixation potential using a radioisotope assay for Ribulose-1,5-bisphosphate carboxylase oxygenase (RubisCO) activity.

### Retrograde Loading of Nerves, Tracts, and Spinal Roots with Fluorescent Dyes

**Dvir Blivis, Michael J. O'Donovan**

Developmental Neurobiology Section, National Institute of Neurological Disorders and Stroke, National Institutes of Health

We describe a simple and low cost technique for introducing high concentration of fluorescent and calcium-sensitive dyes into neurons or any neuronal tract using a polyethylene suction pipette.

### A Primary Neuron Culture System For The Study Of Herpes Simplex Virus Latency And Reactivation

**Mariko Kobayashi<sup>1</sup>, Ju-Youn Kim<sup>1</sup>, Vladimir Camarena<sup>2</sup>, Pamela C. Roehm<sup>3</sup>, Moses V. Chao<sup>4,2,5,6,7</sup>, Angus C. Wilson<sup>1</sup>, Ian Mohr<sup>1</sup>**

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The protocol describes an efficient and reproducible model system to study herpes simplex virus type 1 (HSV-1) latency and reactivation. The assay employs homogenous sympathetic neuron cultures and allows for the molecular dissection of virus-neuron interactions using a variety of tools including RNA interference and expression of recombinant proteins.

## Disclosures

No conflicts of interest declared.