December 2011: This Month in JoVE

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URL: http://www.jove.com/video/4114
DOI: doi:10.3791/4114
Keywords: This Month in JoVE, Issue 58,
Date Published: 12/1/2011
Citation: Kolski-Andreaco, A. December 2011: This Month in JoVE. J. Vis. Exp. (58), e4114, doi:10.3791/4114 (2011).

Abstract

The Journal of Visualized Experiments (JoVE) closes 2011 with a December issue that begins by honoring World's AIDS Day with an article that deals with visualizing the structure of HIV envelope glycoproteins, which are a critical component of the viral infection pathway.

One key method for determining virion envelope structure is cryo-electron tomography. Prior to using this technique, soluble virus must be vitrified on a gold grid - a process that involves adding gold to a viral suspension, transferring that suspension to the grid, and plunging the grid into liquid ethane. The virus particles, present in a thin film of liquid atop the grid, are frozen at a rate greater than 100,000 K per second. Following vitrification, collected samples are loaded into the electron microscope and precisely defined regions on the grid are hit with a focused electron beam, while the grid is tilted. The tilt series that results from these imaging sessions is comprised of hundreds of individual images, which must be aligned and averaged to resolve individual envelope proteins. This computationally intense process often requires computer clusters, capable of remote-access parallel processing, like NIH's Biowulf.

Once completed, 3D models can not only provide insight into the structure of envelope glycoproteins themselves, but can also reveal the manner in which they interact other proteins, like the neutralizing antibody B12, which is information that can greatly influence vaccine design.

This article, from the laboratory of Dr. Sriram Subramaniam involves contributions from twenty authors, including not only graduates students and postdocs, but also middle and highschool students, showing that you are never too young to make an impact in the fight against a disease that has infected over 60 million people, worldwide.

In Clinical and Translational Medicine, JoVE presents an article from the University College London and the University of Oxford that investigates the physiological correlates of nociception, or pain sensation, in infants. Typically, infants respond to noxious stimuli by crying and changing their facial expressions - reactions considered to be autonomic and reliant on subcortical regions of the brain.

In order to understand the involvement of the cerebral cortex and spinal cord in infant nociception our authors first prepare infants for EEG and EMG recordings. They also set up physiological monitoring and video recording, so that the infant's facial expressions can be linked to physiological data. Following assessment of infant responses to neutral tactile stimuli, the baby is subjected to heel lancing - a clinical procedure used to obtain blood samples from infants. After data processing, electrical potentials can be compared between noxious and non-noxious stimuli in these infants, which will hopefully lead to meaningful conclusions, regarding how nociceptive information is interpreted by newborns.

JoVE's Bioengineering section takes a more clinical focus in December with an article from the University of Wisconsin, Madison, which involves the fabrication of a compartmentalized microfluidic device for studying cancer stem cell migration. Following device design, master molds are created by photolithography for the application of poly dimethyl siloxane (PDMS)- an organosilicon compound that is transparent and biologically inert - making it suitable for microfluidics applications with live cells. Specifically, our authors load tumor stem cells derived from multiform glioblastoma, a highly malignant brain cancer, into the compartmentalized devices and then place them in a specialized imaging system that acquires data over a few days.

Time lapse video from these authors show that cancer stem cells are able to regulate their morphology in the size-constrained microfluidic channels much like they would in the interstitial space, and fluidic isolation made possible by chambers like these facilitates drug screening for potential therapeutics that can affect cancer stem cell migration.

Late on in the month, JoVE travels to France, where investigators from the Insitute Pasteur and the Centre National de la Recherche Scientifique, illustrate a method for inducing expression of channel-rhodopsin, a light-activated ion channel, into the olfactory bulb of mice, as well as subsequent neuronal stimulation using a miniature light emitting diode (LED).

Our authors give detailed step-by-step instructions for preparing injection needles, and for precise injection of virus into the rostral migratory stream, a site of migration for adult born neuroblasts. Furthermore, they show how miniature LED stimulation devices are fabricated, calibrated, and implanted atop a cranial window prepared over the bulb. In particular, our authors are interested in the way that adult born neurons integrate into existing olfactory neuronal circuits, and they show that LED stimulation in awake behaving animals, can lead to activation of virally-tranducded neuroblasts in the olfactory bulb, which have migrated from the rostral migratory stream.

These video-articles comprise JoVE's highlights for the month of December. Other noteworthy upcoming articles illustrate methods for patterning conductive inks into microelectrodes, measuring cytosolic calcium in contractile lymphatics, and imaging neuronal responses to pheromones in the vomeronasal organ.
Protocol

Determination of Molecular Structures of HIV Envelope Glycoproteins using Cryo-Electron Tomography and Automated Sub-tomogram Averaging

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The protocol describes a high-throughput approach to determining structures of membrane proteins using cryo-electron tomography and 3D image processing. It covers the details of specimen preparation, data collection, data processing and interpretation, and concludes with the production of a representative target for the approach, the HIV-1 Envelope glycoprotein. These computational procedures are designed in a way that enables researchers and students to work remotely and contribute to data processing and structural analysis.

Electrophysiological Measurements and Analysis of Nociception in Human Infants

L. Fabritz1, A. Worley2, D. Patten1, S. Holdridge1, L. Cornelissen1, J. Meek3, S. Boyd2, R. Slater1,4

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Planar and Three-Dimensional Printing of Conductive Inks

Bok Yeop Ahn1,2, Steven B. Walker1, Scott C. Slimmer1, Analisa Russo1, Ashley Gupta1, Steve Kranz1, Eric B. Duoss1,2, Thomas F. Malkowski1,3, Jennifer A. Lewis1

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Planar and three-dimensional printing of conductive metallic inks is described. Our approach provides new avenues for fabricating printed electronic, optoelectronic, and biomedical devices in unusual layouts at the microscale.

Evaluation of Cancer Stem Cell Migration Using Compartmentalizing Microfluidic Devices and Live Cell Imaging

Yu Huang1,2, Basheal Agrawal3, Paul A. Clark3, Justin C. Williams1,2,3, John S. Kuo1,4

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A compartmentalizing microfluidic device for investigating cancer stem cell migration is described. This novel platform creates a viable cellular microenvironment and enables microscopic visualization of live cell locomotion. Highly motile cancer cells are isolated to study molecular mechanisms of aggressive infiltration, potentially leading to more effective future therapies.

Imaging Pheromone Sensing in a Mouse Vomeronasal Acute Tissue Slice Preparation

Julien Brechbühl1, Gaëlle Luyet1, Fabian Moine1, Ivan Rodriguez2, Marie-Christine Broillet1

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December 2011 | 58 | e4114 | Page 2 of 3
In mice, the ability to detect pheromones is principally mediated by the vomeronasal organ (VNO). Here, an acute tissue slice preparation of VNO for performing calcium imaging is described. This physiological approach allows observations of subpopulations and/or individual neurons in a living tissue and is convenient for receptor-ligand identification.

Selective Viral Transduction of Adult-Born Olfactory Neurons for Chronic In Vivo Optogenetic Stimulation
Gabriel Lepousez, Mariana Alonso, Sebastian Wagner, Benjamin W. Gallarda, Pierre-Marie Lledo

Laboratory for Perception and Memory, Institut Pasteur and Centre National de la Recherche Scientifique (CNRS)

Adult-born neurons of the olfactory bulb can be optogenetically controlled using Channelrhodopsin2-expressing lentiviral injection in the rostral migratory stream and chronic photostimulation with an implanted miniature LED.

Imaging Neuronal Responses in Slice Preparations of Vomeronasal Organ Expressing a Genetically Encoded Calcium Sensor
Limei Ma¹, Sachiko Haga-Yamanaka¹, Qingfeng Elden Yu¹, Qiang Qiu¹, SangSeong Kim¹, C. Ron Yu¹,²

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The vomeronasal organ (VNO) detects intraspecies chemical signals that convey social and reproductive information. We have performed Ca2+ imaging experiments using transgenic mice expressing G-CaMP2 in VNO tissue. This approach allows us to analyze the complicated response patterns of the vomeronasal neurons to large numbers of pheromone stimuli.

Measurement of Cytosolic Ca²⁺ in Isolated Contractile Lymphatics
Flavia M. Souza-Smith, Kristine M. Kurtz, Jerome W. Breslin

Department of Physiology, School of Medicine, Louisiana State University Health Sciences Center

We introduce an approach to evaluate the cytosolic Ca²⁺ concentration in isolated lymphatics to study Ca²⁺-dependent and Ca²⁺-sensitizing mechanisms of lymphatic smooth muscle contraction.