Video Article

January 2012: This Month in JoVE

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[1]

URL: https://www.jove.com/video/4194
DOI: doi:10.3791/4194

Keywords: This Month in JoVE, Issue 59,

Date Published: 1/2/2012

Citation: Kolski-Andreaco, A. January 2012: This Month in JoVE. *J. Vis. Exp.* (59), e4194, doi:10.3791/4194 (2012).

Abstract

The Journal of Visualized Experiments (JoVE) begins 2012 by publishing its 1500th article, which focuses on a method for analyzing the free radical composition of cigarette smoke. Although tobacco tar is thought to be the most carcinogenic agent in cigarette smoke, free radicals are well known cancer-causing molecules, and the level of these carcinogens contained in cigarette smoke is not currently known. The method employed in this article combines the use of a single-port smoking device, which simulates cigarette puffing, with electron spin resonance (ESR) spectroscopy, a technique, which measures the spin energy emitted by the unpaired electrons in free radicals. One important modification of the single port smoking device used in this article, is the introduction of a liquid nitrogen trap in front of the spin trap. Liquid nitrogen removes water vapor from cigarette smoke and greatly improves ESR spectra as a result.

In addition to measuring the free radical concentration found in cigarette smoke, plant antioxidants are introduced into modified cigarette filters and the ESR spectra of cigarette smoke passed through these filters is obtained. Grape seed extract and lycopene were observed to have the most significant scavenging effects on free radicals, and if cost effective means of including these molecules into cigarette filters can be developed, a less harmful cigarette could be created.

In Clinical and Translational Medicine, JoVE presents an analytical method for determining the *local Gyrification Index* (*l*GI) - a measurement of the extent of cortical folding. Cortical folds develop during the first few post natal months, and adverse events during this time can lead to abnormalities in the brain's surface anatomy, which may ultimately correlate with neuropsychiatric disorders.

The analysis tool developed for calculating the *local Gyrification Index* is Freesurfer, which can convert T1 weighted MRI scans into 3D reconstructions of the cortex. Separate reconstructions are created for the grey/white mater and grey/pial interfaces, which are manually adjusted before computating the *l*GI. Following a smoothing operation, circular regions of the pial and grey/white surfaces are compared, in order to compute the *l*GI for thousands of points over the entire brain. Freesurfer makes analysis of gyrification straightforward and accessible to clinicians and brain scientists, who are interested in comparing the extent of cortical folding between different individuals in normal and disease states.

JoVE's Immunology and Infection article for the month of January continues the journal's tradition of publishing cutting edge methodology in vector born disease research. Specifically, this article adds to JoVE's comprehensive collection of protocols for malaria research by extending our library to cerebral malaria - a devastating condition with high susceptibility in children. Briefly, this protocol involves exposing a monolayer of human brain endothelial cells, passaged onto petri dishes, to *Plasmodium falciparum*-infected red blood cells. Following washing and enriching steps, high yields of parasites, which carry the potential to infect the brain, can be isolated and further characterized.

In Neuroscience, JoVE showcases a method for studying the migration of neural crest cells. These cells are a transient, multipotent, developmental cell type that can, in addition to becoming neuronal tissue, differentiate into smooth muscle, chromaffin cells, cartiliage, and bone. Chick embryos are carefully prepared, so that the trunk region can be isolated and the neural tube excised for overnight culture on fibronectin-coated coverslips, which allows neural crest cells to emigrate from the neural tube. Following removal of the neural tube, coverslips containing the cultured neural crest cells are transferred to a modified Zigmond chamber - a chamber which enables a concentration gradient of putative chemotactic agent to be established across the culture. Timelapes imaging followed by analysis of cell trajectories, is used to assess the chemotactic potential of molecules loaded into the zigmund chamber, providing insight into the molecular cues that guide migration of this important developmental cell type.

These featured articles comprise four of fifty video-protocols for release in January. Other notable articles include methods for visualizing the cuticle structures of c. elegans, thermal imaging of delayed onset muscle soreness, culturing the ventral midbrain in organotypic slices, and inducing atherosclerosis in mice.

Video Link

The video component of this article can be found at https://www.jove.com/video/4194/

Protocol

Selection of *Plasmodium falciparum* Parasites for Cytoadhesion to Human Brain Endothelial Cells

Antoine Claessens, J. Alexandra Rowe

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An *in vitro* model for cerebral malaria sequestration is described[1]. *Plasmodium falciparum* infected red blood cells are selected for binding to immortalized human brain microvascular endothelial cells. The selected parasites show a distinct phenotype. The selection process can be applied using various *P. falciparum* strains and endothelial cell lines.

Implantation of a Carotid Cuff for Triggering Shear-stress Induced Atherosclerosis in Mice

Michael T. Kuhlmann[1], Simon Cuhlmann[2, 3], Irmgard Hoppe[1], Rob Krams[3], Paul C. Evans[2], Gustav J. Strijkers[4], Klaas Nicolay[4], Sven Hermann[1], Michael Schäfers[1]
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The constricting cuff presented in this article is designed to induce atherosclerosis in the murine common carotid artery. Due to the conical shape of its inner lumen the implanted cuff generates well-defined regions of low, high and oscillatory shear stress triggering the development of atherosclerotic lesions of different inflammatory phenotypes.

Analysis of Trunk Neural Crest Cell Migration using a Modified Zigmond Chamber Assay

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An approach to analyze the migration of explanted cells (trunk neural crest cells) is described. This method is inexpensive, gentle, and capable of distinguishing chemotaxis from both chemokinesis and other influences on migratory polarity such as those derived from cell-cell interactions within the primary trunk neural crest cell culture.

Organotypic Slice Cultures of Embryonic Ventral Midbrain: A System to Study Dopaminergic Neuronal Development *in vitro*

Gabriela Oana Bodea, Sandra Blaess
Institute of Reconstructive Neurobiology, University of Bonn

A method to generate organotypic slices from the E12.5 murine embryonic midbrain is described. The organotypic slice cultures can be used to observe the behavior of dopaminergic neurons or other ventral midbrain neurons.

Visualization of *Caenorhabditis elegans* Cuticle Structures using the Lipophilic Vital Dye, DiI

Robbie D. Schultz[1, 2], Tina L. Gumienny[2]
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We present a method to visualize cuticle in live *C. elegans* using the red fluorescent lipophilic dye DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate), which is commonly used in *C. elegans* to visualize environmentally exposed neurons. With this optimized protocol, alae and annular cuticular structures are stained by DiI and observed using compound microscopy.

A Protocol for Detecting and Scavenging Gas-phase Free Radicals in Mainstream Cigarette Smoke

Long-Xi Yu[1], Boris G. Dzikovski[2], Jack H. Freed[3, 2]
[1]CDCF-AOX Lab, [2]National Biomedical Center for Advanced ESR Technology (ACERT), Department of Chemistry and Chemical Biology, Cornell University, [3]ACERT Research, Center Department of Chemistry and Chemical Biology, Baker Laboratory, Cornell University

Spin-trapping ESR spectroscopy was used to study the effect of plant antioxidants lycopene, pycnogenol and grape seed extract on scavenging gas-phase free radicals in cigarette smoke.

How to Measure Cortical Folding from MR Images: A Step-by-step Tutorial to Compute Local Gyrification Index

Marie Schaer[1], Meritxell Bach Cuadra[2, 3], Nick Schmansky[4], Bruce Fischl[4], Jean-Philippe Thiran[2], Stephan Eliez[1]
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Measuring gyrification (cortical folding) at any age represents a window into early brain development. Hence, we previously developed an algorithm to measure local gyrification at thousands of points over the hemisphere[1]. In this paper, we detail the computation of this local gyrification index.

The Use of Thermal Infra-Red Imaging to Detect Delayed Onset Muscle Soreness

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The purpose of this investigation was to assess whether using an infra-red thermal camera is a valid tool for detecting and quantifying the muscle soreness after exercising.