Video Article

September 2012: This Month in JoVE

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Abstract

This September in JoVE, researchers from the School of Medicine at the Free University of Berlin demonstrate a novel method for studying how stroke patients compensate for visual field defects. To do this, our authors make use of a driving simulator complete with brakes, a steering wheel, and turn signals. Using driving simulation software and sophisticated eye tracking, researchers can compare the gaze behavior of stroke patients as they navigate through virtual driving courses with varying degrees of complexity. Though posterior cerebral artery infarction can lead to similar visual deficits in patients, some are able to navigate through the driving courses by developing compensatory eye movements, while others crash into dangerous obstacles, like wild boars. Through the analysis of compensatory gaze behavior employed by patients, our authors see great potential for using driving simulation as a tool to rehabilitate stroke patients trying to overcome the blind spots in their visual fields.

In collaboration with the University of Southern California, researchers in the Department of Ophthalmology at Oregon Health and Science University present a method for measuring total blood flow in the retina using Doppler optical coherence tomography (OCT). The retina contains millions of neurons that capture visual images and convert them into electrical signals, which travel through the optic nerve to the brain. Blood vessels enter the retina at the optic disk, where the optic nerve connects to the retina. These vessels supply oxygen and nutrients, and also remove waste. In some retinal diseases (such as diabetic retinopathy) or glaucoma (which affects the optic nerve), the retinal vasculature may be abnormal. Because these diseases are leading causes of irreversible vision loss, measurements of retinal blood flow can be very useful in clinical practice and research. Unlike traditional optical imaging methods, like laser Doppler and ultrasound color Doppler, laser Doppler OCT can provide absolute measurements of retinal blood flow; these are based on Doppler-shifted light, which is backscattered from red blood cells as they flow through vessels. Our authors demonstrate how to scan the retina and optic disc with Doppler OCT; the scans are then graded and analyzed with DOCTORC software, which our authors developed. This method shows good reproducibility between graders and methods. Furthermore, in eyes with glaucoma, retinal blood flow measurements are highly correlated with vision loss. Thus, Doppler OCT represents a powerful tool that can be used in ophthalmology research and clinical practice.

Coinciding with mosquito season, researchers in the Department of Entomology at Virginia Tech demonstrate a simple and robust technique for chromosome mapping of mosquito genomes. Out of more than 40 mosquito genera containing thousands of species, researchers are particularly interested in the genera *Anopheles*, *Aedes*, and *Culex* because they contain species that transmit harmful human diseases. About 90% of the *Anopheles gambia* genome has been mapped to chromosomal locations; however, it is extremely difficult to prepare suitable chromosome spreads for the *Aedes* genus or the *Culex* genus using cell lines and standard techniques. To overcome this problem, our authors use 4th instar mosquito larvae, which have imaginal discs that produce high-quality chromosomal spreads. The researchers show how to dissect the imaginal discs and prepare suitable chromosome preparations for fluorescence *in situ* hybridization (FISH). Genome mapping is thus possible for mosquitoes in the *Aedes*, Culex, and *Anopheles* genera. This technique paved the way for entomologists to make precise chromosomal maps for not only mosquitoes, but also for other insects.

In the Center for Research at Quebec's University of Laval, researchers demonstrate a method for tracking neuronal migration in the murine forebrain. One important site of neurogenesis in the mammalian brain is the subventricular zone, and newly born neurons migrate away from this area via the rostral migratory stream to the olfactory bulb. The cells are labeled with a stereotaxically injected retrovirus encoding a green fluorescent protein; then, using a combination of acute slice preparation, timelapse imaging, and image analysis, our authors can calculate the migration speed of labeled neuroblasts. Through careful tracking of cell trajectories along blood vessels, this method can help elucidate the different molecular cues and cellular mechanisms that influence cell migration.

This brief summary highlights just a few notable video-articles that will be released this September in JoVE. We also feature methods for tracking cell fate in zebrafish with photoconvertible fluorescent proteins, using micropipettes to test cell stiffness, and imaging the behavior of proteins that respond to DNA damage.

Video Link

The video component of this article can be found at https://www.jove.com/video/5022/

Protocol

Doppler Optical Coherence Tomography of Retinal Circulation

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Total retinal blood flow is measured by Doppler optical coherence tomography and semi-automated grading software.

Micropipette Aspiration of substrate-attached cells to estimate cell stiffness

Myung-Jin Oh[1], Frank Kuhr[1], Fitzroy Byfield[2], Irena Levitan[1]
[1]Section of Respiratory, Critical Care and Sleep Medicine, Department of Medicine, University of Illinois, [2]Institute for Medicine and Engineering, University of Pennsylvania

Here we describe a quick and simple method to measure cell stiffness. The general principle of this approach is to measure membrane deformation in response to well-defined negative pressure applied through a micropipette to the cell surface. This method provides a powerful tool to study biomechanical properties of substrate-attached cells.

Time-lapse imaging of neuroblast migration in the mouse forebrain

Jivan Khlghatyan, Armen Saghatelyan
The Cellular Neurobiology Unit, Centre de Recherche Université Laval Robert-Giffard

We describe a protocol for real-time videoimaging of neuronal migration in the mouse forebrain. The migration of virally-labeled or grafted neuronal precursors was recorded in acute live slices using wide-field fluorescent imaging with a relatively rapid acquisition interval to study the different phases of cell migration, including the durations of the stationary and migration phases and the speed of migration.

Fluorescent *in situ* Hybridization on Mitotic Chromosomes of Mosquitoes

Vladimir A. Timoshevskiy, Atashi Sharma, Igor V. Sharakhov, Maria V. Sharakhova
Department of Entomology, Virginia Tech

Among the three mosquito genera, namely *Anopheles, Aedes*, and *Culex*, physical genome mapping techniques were established only for *Anopheles*, whose members possess readable polytene chromosomes. For the genera of *Aedes* and *Culex*, however, cytogenetic mapping remains challenging because of the poor quality of polytene chromosomes. Here we present a universal protocol for obtaining high-quality preparations of mitotic chromosomes and an optimized FISH protocol for all three genera of mosquitoes.

Two- and Three-Dimensional Live Cell Imaging of DNA Damage Response Proteins

Jason M. Beckta[1, 2], Scott C. Henderson[3], Kristoffer Valerie[1, 2, 4]
[1]Department of Radiation Oncology, Virginia Commonwealth University, [2]Department of Biochemistry & Molecular Biology, Virginia Commonwealth University, [3]Department of Anatomy & Neurobiology, Virginia Commonwealth University, [4]Massey Cancer Center, Virginia Commonwealth University

This protocol describes a method for visualizing a DNA double-strand break signaling protein activated in response to DNA damage as well as its localization during mitosis.

Cell tracking using photoconvertible proteins during zebrafish development

Verónica A. Lombardo, Anje Sporbert, Salim Abdelilah-Seyfried
Max Delbrück Center for Molecular Medicine

Here, we present a method for the photoactivated switch of photoconvertible fluorescent proteins (PCFPs) in the living zebrafish embryo and further tracking of photoconverted protein at specific time points during development. This methodology allows monitoring of cell biological events underlying different developmental processes in a live vertebrate organism.

Driving Simulation in the Clinic: Testing visual exploratory behavior in daily life activities in patients with visual field defects

Johanna Hamel[1, 2], Antje Kraft[1], Sven Ohl[3], Sophie De Beukelaer[1], Heinrich J. Audebert[1, 2], Stephan A. Brandt[1]
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Patients with visual deficits after stroke report about different constraints in daily life most likely due to variable compensatory strategies, which are difficult to differentiate in clinical routine. We present a clinical set-up which allows measurement of different compensatory head- and eye-movement-strategies and evaluating their effects on driving performance.

Disclosures

No conflicts of interest declared.