

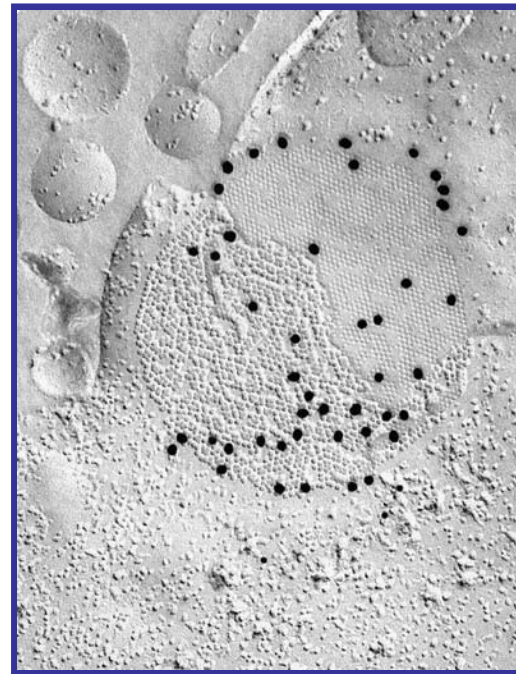
Short Course: Freeze-Fracture Replica Immunogold Labeling

July 18-22, 2011

Dept. of Biomedical Sciences



Fort Collins, Colorado



Your FRIL Connection



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Please visit our website at:
<http://www.cvmb.colostate.edu/rashlab/>

COURSE ORGANIZERS:

The Department of Biomedical Sciences at Colorado State University offers a one-week intensive course in freeze-fracture replica immunogold labeling (FRIL) techniques for research scientists and senior technicians. This course is organized by researchers in Dr. John Rash's laboratory, in collaboration with major instrument manufacturers.

FRIL AND SDS-FRL TECHNIQUES:

Freeze-fracture techniques are based on the mechanical fracture of frozen biological material and the high-resolution metal foil replication of the exposed surfaces. Replication by vapor deposition of platinum and carbon reveals transmembrane proteins as intramembrane particles (IMPs) at ca. 1 nm topographic resolution. In conventional freeze fracture, it was necessary to use harsh inorganic chemical digestion methods to remove all biological remnants from the replica to allow electron beam penetration and image formation in the transmission electron microscope (TEM). In 1995, Dr. Kazushi Fujimoto showed that gentle washing with SDS detergent removed most biological material, leaving only those macromolecules that were coated with Pt/C strongly adsorbed to the Pt/C film. These were then immunogold labeled. He called this method SDS-fracture replica labeling (SDS-FRL). Our laboratory combined SDS-FRL with Lexan stabilization and confocal microscopic "grid mapping", as freeze-fracture replica immunogold labeling (FRIL). FRIL allows TEM and confocal mapping of labeled structures to their corresponding histological and gross anatomical locations. SDS-RL and FRIL permit ultra-structural identification and anatomical mapping of a wide variety of membrane proteins and their strongly bonded cytoplasmic "scaffolding" and accessory proteins, but with the added advantage of 100-fold higher image resolution and 10- to 20-fold higher labeling resolutions than afforded by confocal microscopy. Nowhere is this more important than in the vertebrate central nervous system, where neuronal and glial processes are often much smaller than the limit of resolution of light microscopy. With primary antibodies now made in many different species, and gold labels made in many different but uniform sizes, FRIL now allows simultaneous visualization, identification and mapping of multiple proteins in complex CNS tissues.

COURSE DESCRIPTION:

This intensive one-week course is designed for neuroscientists who are experienced in basic TEM and who have a general understanding of freeze-fracture. Lectures will cover the fundamentals of freeze fracture, preparation of samples for FRIL and SDS-FRL, and search-strategies for FRIL. Participants will use Lexan

to stabilize replicas prior to SDS cleaning and labeling, use confocal microscopy to map replicated tissues, use "labeling blocking buffers" prior to replica cleaning with SDS detergent, perform all immunogold labeling steps, remove the Lexan support film prior to TEM examination, and develop strategies for finding and identifying labeled IMP arrays within the comparatively vast areas of an intact replica. Participants will also learn the importance of stereoscopic analysis of labeled replicas, examine common labeling artifacts, identify sources and methods for minimizing non-specific labeling, and apply methods for increasing the signal-to-noise ratio in labeled replicas. As appropriate, participants will prepare and examine double-replicas. Participants may provide and label their own tissues using primary antibodies supplied by themselves or work on representative samples provided during the course. Participants are also encouraged to supply samples of tissue at least one month before the course so that tissue-specific problems can be identified and at least one FRIL replica obtained before the course begins.

INSTRUCTORS:

John E. Rash, Ph.D., Professor, Department of Biomedical Sciences (Neuroscience Division), Colorado State University

Naomi Kamasawa, Ph.D., National Institute for Physiological Sciences, Okazaki, Japan

Thomas Yasumura, MA, Department of Biomedical Sciences, Colorado State University

Kimberly Davidson, MS, Department of Biomedical Sciences, Colorado State University

FACTORY REPRESENTATIVES:

William F. Graham, Bibst Labs, Brookline, NH

Dave Roberts, RMC/Boeckeler Instruments

Chikako Nakayama, JEOL, Akishima, Japan

Additional instructors will be added to the course. Please check our website for updates.

<http://www.cvmb.colostate.edu/rashlab/>

Primary FACILITIES:

JEOL 1400 & 2000 - TEM

JEOL JFDII & 9010c - Freeze Fracture machine

RMC-HPM010- High Pressure Freezer

Other equipment as available.

