Dr. John E. Rash
Department of Biomedical Sciences
1617 Campus Delivery
Fort Collins, CO 80523
Phone (970) 491 5606
Fax (970) 491 7907

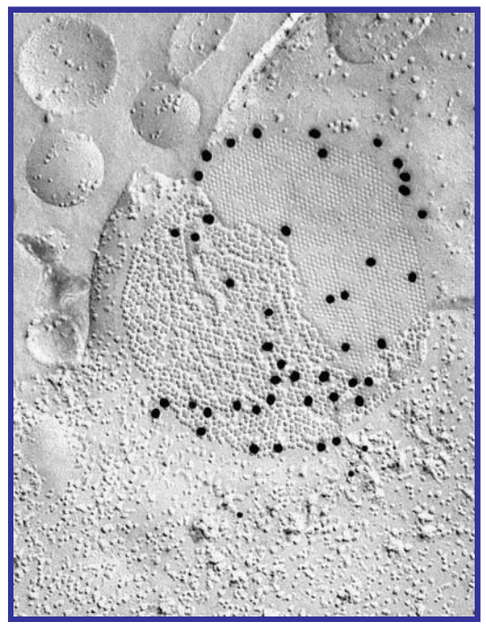
Please visit our website at:
http://www.cvmbs.colostate.edu/rashlab/

Short Course: Freeze-Fracture Replica Immunogold Labeling

July 12-16, 2010

Dept. of Biomedical Sciences

Fort Collins, Colorado



Your FRIL connection

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 topgraphic 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 is an intensive one-week course 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

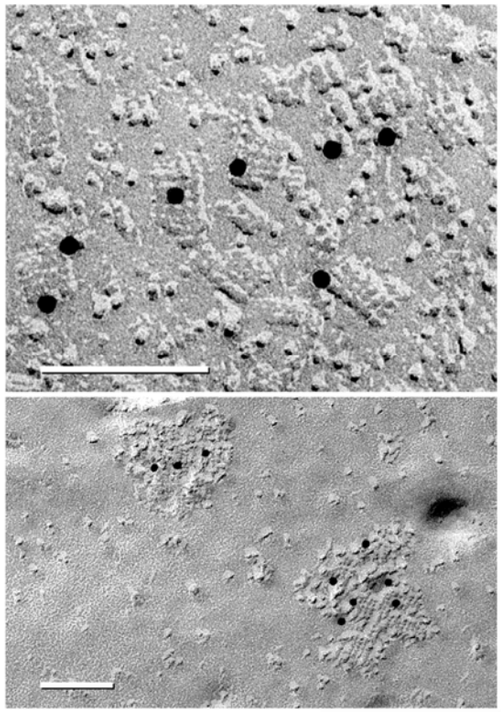
Kimberly Davidson, MS, Department of Biomedical Sciences, Colorado State University

FACTORY REPRESENTATIVES:
William F. Graham, Bibst Labs, Brookline, NH

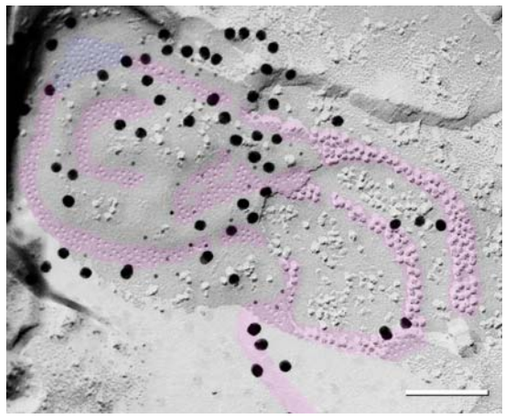
Dave Roberts, RMC/Boeckeler Instruments

Additional Instructors will be added to the course. Please check our website for updates.
http://www.cvmbs.colostate.edu/rashlab/

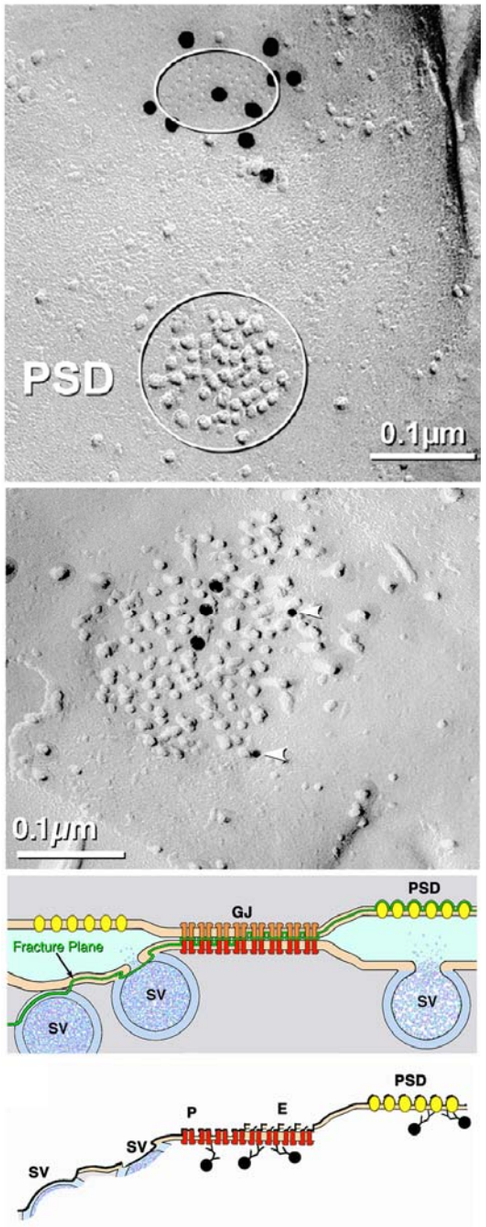
Primary FACILITIES:
JEOL 1400 & 2000 - TEM
JEOL JFDII & 9010c – Freeze Fracture machine
RMC-HPM010- High Pressure Freezer
Other equipment as available.



Square arrays identified by immunogold labeling for aquaporin-4 (AQP4).



"Ribbon" gap junction in rat inner plexiform layer labeled for Cx36 by 6-nm and 18-nm gold beads.



Images of Cx36 immunogold labeled gap junction and NMDA/AMPA double-labeled post-synaptic densities, and drawing of labeled IMP arrays.

REGISTRATION:

Registration fee of $2000 includes all necessary implements and supplies, TEM beam time, TEM negatives, snacks during the day and evening, and lunch each day. Registration will be in order of receipt. Limited to 6 to12 students.

ACCOMODATIONS:

The Hilton Hotel (one block from the laboratory) has single occupancy at $89.00 per nite Upgrade to Concierge Level is available for an additional $20.00 per nite Please book before June 11 for these special rates.

The Best Western University Inn (~ three blocks from the laboratory) has single and double occupancy at $79.00 per nite, which includes a deluxe continental breakfast. Their phone number is (970) 484-1984 or 888-484-BWUI (2984). Reservations will continue to be accepted just prior to the start of the course.

Indicate that you are with the CSU Freeze Fracture Course for the above hotels

INFORMATION:

Information concerning this course, hotel accommodations and travel arrangements may be obtained from:

Karen Solomon or Shazette Tucker
Dept. Biomedical Sciences
Colorado State University
Fort Collins, CO 80523
970-491-5036 or 491-5847

Please provide a brief résumé and statement of research goals pertinent to the course.

FRIL TRAINING COURSE
DR. JOHN E. RASH
COLORADO STATE UNIVERSITY
FORT COLLINS, COLORADO 80523

JULY 12-16, 2010

REGISTRATION FORM

Name:_____

Title:_____

Address:_____

_____

_____

Phone: _____

COURSE FEE: $2000

$250 Deposit Enclosed ____Personal Check
____Purchase Order

Deposit must be received by May 1, 2010.
Full payment must be received by June 1, 2010.

HOTEL RESERVATION

Fort Collins Hilton: _____

University Best Western Motel: _____

# of Nights: _____ # of Adults: _____ Children:____

Arrival Date: _____ Time: _____

Departure Date: _____ Time: _____