

Embedding Brains In Egg Yolk For Cryosectioning

Karen Ayyad

V.A. Medical Center/Presbyterian Hospital, Dallas TX

Fifteen years ago, I learned from a neurologist how to embed perfused, glutaraldehyde-fixed rat brain in egg yolk (from store-bought, separated chicken eggs). The original technical note by Adoff (reference below) for

DIRECTOR/MANAGER OF OPERATIONS OF THE SCANNING AND TRANSMISSION ELECTRON MICROSCOPY RESEARCH FACILITY

GENERAL: The Marine Geosciences Division, Naval Research Laboratory (NRL), Stennis Space Center, MS, is seeking to fill the position of Director/Manager of the division's STEM research facility. The successful candidate will be an accomplished transmission electron microscopist with a Ph.D. degree in a closely related academic field, or possibly a Ph.D. equivalent.

FACILITY MANAGEMENT AND OPERATION: The successful candidate will serve as the director and manager of the facility and will be fully responsible for:

- Competent, efficient, economical and safe operation and maintenance of NRL's 300 kV STEM with Environmental Cell and supporting instrumentation for imaging and analyses - including Scanning Mode Microscopy (SEM); Energy Dispersive X-ray Spectroscopy (EDS); Electron Loss Spectroscopy (EELS); a slow scan, charge-coupled device (CCD) camera and other data acquisition systems, and 100 kV Transmission Electron Microscope with Environmental Cell,.
- Staffing the facility with high quality support personnel.
- Setting up operating procedures and protocol for all aspects of work in the facility.
- Supervision and training of technical personnel.
- Overseeing financial operations of the facility.
- Setting up a cataloging/archiving system for samples, imagery, etc.
- Ensuring that operations conform to all NRL/Navy health and safety regulations and standards.

RESEARCH: As the senior electron microscopy expert, the incumbent conducts forefront basic and applied research directed towards developing state-of-the-art microscopy techniques and applying these to investigations of complex problems in marine geomaterials, geochemistry, microbiology and environmental processes. A primary focus will be directed towards Naval problems particularly as involves understanding fundamental processes associated with formation, deposition, and alteration of cohesive marine sediments and the impact on the geoaoustic and geotechnical properties of the sediments and behavior of these sediments in the marine environment. The inclusion of the environmental cell into the STEM provides NRL with one of the unique capabilities in the world. With the STEM and Environmental Cell, the incumbent will be able to conduct forefront research on gaseous and aqueous chemical processes at the molecular scale.

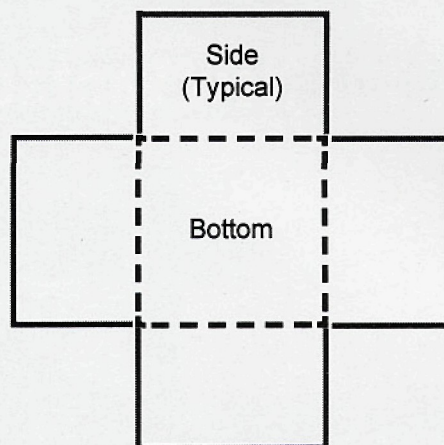
For further information, contact:

Dr. Philip J. Valent (Code 7401)
Naval Research Laboratory
Stennis Space Center, MS 39529-5004
Tel.: 228-688-4650, Fax: 228-688-4093,
eMail: phil.valent@nrlssc.navy.mil

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whole dog brain tissue was used, and we adapted the procedure for rat brain.

- 1) Fix the whole brain. *minus the meninges*, in 10% formalin or glutaraldehyde-sucrose (1% glutaraldehyde + 30% sucrose), then place in a specimen container of 30% sucrose, 10% formalin and 0.9% NaCl. The brain is left in this solution until it sinks (less than a week). This step prevents formation of ice crystals when freezing (The meninges must be removed, or the brain will not adhere to the egg yolk.).
- 2) Make five-sided cubical boats slightly larger than the rat brain (in length, width, and depth) from a pattern drawn on and cut out of index cards. The boats should be large enough to allow a 3mm border of egg yolk around the specimen. Tape the four sides of the boat together with Scotch or similar tape. (See illustration.)



- 3) The yolks should be separated from the whites just before they are needed and stirred for 5 minutes (we used stir bars and stir plates).
- 4) The brain is blotted dry before embedding.
- 5) A thin layer of the stirred egg yolk is poured into the paper boats, the brain is placed in the boats, oriented as desired, and the specimen is covered with the yolk.
- 6) Suspend the boats over 10% formalin in a covered container. Adoff did this for 3 weeks at 4°C. We kept ours at room temperature for much less time, only until the yolks were dry and stiff. A raised platform can be used. The idea is to keep the specimen boat from being submerged in the formalin.
- 7) Remove the paper boat from the jar and carefully pull off the boat from the yolk-embedded brain.
- 8) Mount the yolk-embedded brain on a freezing microtome (a cryostat would be even better) with OCT compound, orienting as desired.

We froze the block for sectioning with finely crushed dry ice and used a sliding microtome attached to the bench top (with CO₂ line attachment). A Vibratome would work, however the freezing helped give the specimen an added stiffness.

We cut very thick sections of 50 μm, which could be floated in water or placed directly into incubation media and later mounted on egg albumin-coated, subbed slides for further staining or processing.

The rationale behind the yolk embedding and frozen sectioning of the fixed tissue was that we could very easily control sectioning to readily obtain either sagittal, transverse, or coronal sections orientation by mounting any of the five flat, solid specimen block surfaces. During sectioning, it was very easy to see the brain architectonics so that we knew at what level we were, how far to trim down, and which sections to save.

Adoff states "The primary function of the egg yolk is support. The egg yolk adheres to and remains with the tissue throughout cutting, mounting, and staining... is especially helpful in handling the sections."

Adoff, L.M. 1981. Egg Yolk Embedding for Frozen Whole Brain Sections, Stain Technology 56(2): 125-126 in the Notes on Technic section. ■

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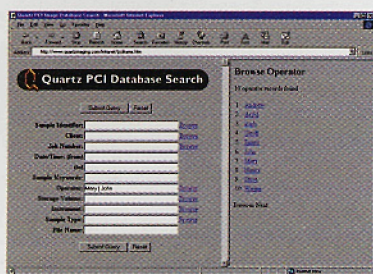
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Nissei Sangyo America, Ltd.

755 Ravendale Drive
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www.nissei.com

25 West Watkins Mill Road
Gaithersburg, MD 20878
(800) 638-4087