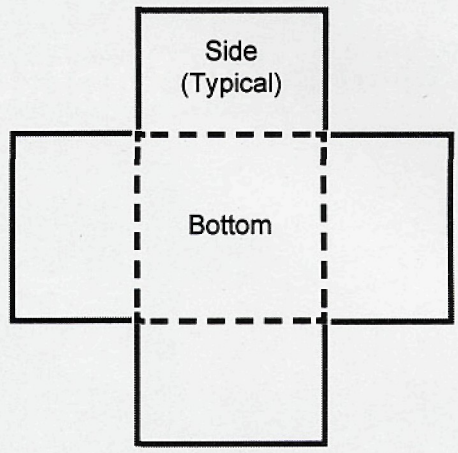
Embedding Brains In Egg Yolk For Cryosectioning

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 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 secfloning with finely crushed dry ice and used a sliding microtome attached to the bench top (with CO_2 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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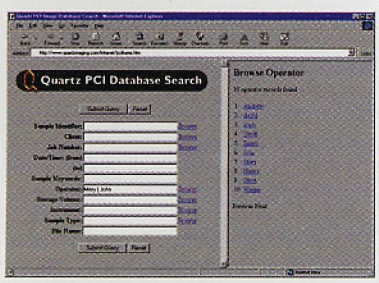

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