Embedding and Cutting of Eye Tissue at UAB

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ABSTRACT

To describe the procedure for embedding and cutting eye tissue.

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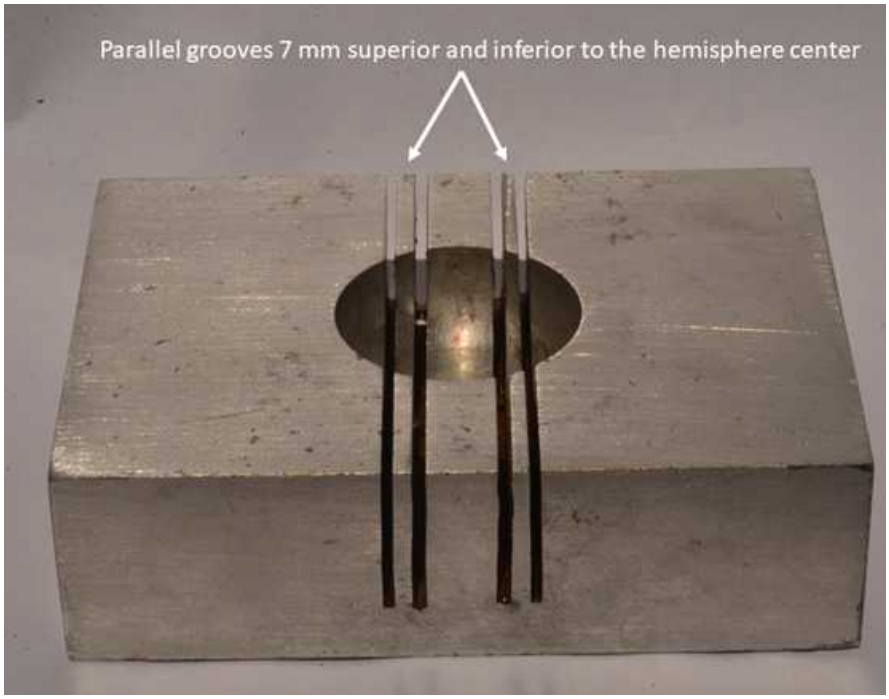
MATERIALS TEXT

1. Custom designed aluminum billet
2. Scissors, Roboz # 500216-G
3. Tissue Slicer, Thomas Sciences #6727C18
4. Carboxymethylcellulose, Sigma C9481
5. Cryomolds, Peel-A-Way 22 x 30 mm molds, Polysciences # 18646B
6. Stereo microscope
7. 10% poly-l-lysine, Sigma Aldrich
8. Oxygen absorbing packets
9. Bitran Freezer Bag
10. Vacuum Sealer

Solutions:
Carboxymethyl cellulose powder is mixed in deionized water, heated to 70°C, stirred until dissolved (30 min -1 hr), and de-gassed before use.

1 Prior to embedding, the posterior pole is trimmed to a 14-mm-wide belt of retina, choroid, and sclera containing major landmarks to help orientation. This includes the optic nerve head, fovea, and horizontal meridian of the visuotopic map and extending anteriorly to pigmented tissue (ora serrata) at the edge of the ciliary body.

2 To stabilize the globe and standardize dissection, posterior poles are placed in an custom-designed, chilled aluminum 3" x 4" x 1" billet with a 30-mm-diameter hemispheric well and a set of parallel grooves 7 mm superior and inferior to the center of the well.



3 Globes are placed facing up in the well and the globe-billet combination oriented so the superior quadrant is positioned to the left and the grooves are vertical, thus making the long edge of the tissue block parallel to the optic nerve head and fovea axis.

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To guide a knife cut, the globe is snipped vertically (10 mm) with scissors (Roboz # 500216-G), guided by the parallel grooves.

A tissue slicer (Thomas Sciences #6727C18) is placed in the two superior scissor cuts. With a guillotine motion, a superior cap is removed from the globe.

While leaving the first blade in place to stabilize the globe, the inferior cap is removed by the same process at the second groove.

The nasal periphery is removed with a slice 2 mm nasal to the optic nerve head (Pang et al., 2015).

4 Tissues are embedded in 2.6% carboxymethyl cellulose (Sigma C9481).

Tissue belts are placed into cryomolds (Peel-A-Way 22 x 30 mm molds, Polysciences # 18646B) using a stereo microscope, oriented so the superior edge is facing up (sectioned first).

Molds are filled with 5 ml of cold carboxymethyl cellulose and frozen at -20°C (NEED TO CHECK THIS FOR ERROR) (Anderson et al., 2020).

5 While cutting, histopathologic evaluation is used to assess location and quality, serial 10 µm cryosections were collected starting at the superior edge of the optic nerve head on pre-labeled 1x3 mm glass slides coated with 10% poly-L-lysine (Sigma Aldrich, St. Louis, MO, USA) and maintained at 37° during sectioning.

Microscopy observations are made throughout the sectioning process.

6 Sections for MALDI IMS, MXiF, Codex, and proteomics are collected at predefined intervals depending on experimental design.

7 Samples are packaged for transport to Vanderbilt using a slide box vacuum-packed with an oxygen-absorbing packet within a Bitran freezer bag to help prevent oxidation and deterioration of lipid signal

Stored at -80°C before transport to Vanderbilt (Anderson et al., 2020)

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