this. the resin around otolith is compressed and stronger support can be achieved.

2) Sectioning: (This was done on a Reichert Ultracut E). Use a very low cutting speed, low water level and a diamond knife. Due to the high hardness of otolith, the otolith is actually not sectioned, but micro-broken. The enhanced embedding procedure can prevent otolith from moving and turning, and holds the material of otolith wherever it was in the otolith. You can see a crystal-composed image under TEM. The daily increment rings also can be seen in detail up to 10,000X magnification. Above 10,000X, single crystals will cover the whole screen and the image will be at the level of crystal ultrastructure, not the otolith. The sections are suitable for EDS and EELS analysis as well as element filter mapping.

3) Decalcification: Fish otolith is highly calcitied material with about 90% inorganics. After decalcification, without support from inorganic crystals, the organic residue will be in the form of pieces or dissolved. It can not form a solid structure that represents the structure of the otolith. Young otolith from few-day-old larvae may have more organic materials in otolith. After decalcification, you can see something left, but it is in the form of gel. It is impossible to go through fixation-dehydration and embedding procedure. I have never been successful with decalcified otoliths. You can decalcify otolith on surface of sectioned block, then go through the fixation-dehydration and embedding procedure on top of block. But for otolith research, the information of the location and distribution of the micro-chemical components is much more important than ultrastructure. The number of growth rings and their structure can be better determined by light microscopy with a polarized light source and SEM with SEI or BEI imaging.

This method also can be used on biological hard tissues for TEM studies.

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Suspending An Atomic Force Microscope (AFM) Or Other Instrument By Bungee Cords To Reduce Vibration

The AFM and its optical microscope are placed on a custom-made concrete block, 2 ft. x 2 ft. x 4 in (or, *e.g.,* a granite block), with corner brackets to which bungee cords can be attached. The block is suspended with two strands of 1/4-inch diameter bungee cord from each of the four corners of the block (we used bungees from McMaster Carr Supply Co.). One long strand is looped around at each corner, so that there are 8 strands total extending upward from the corners of the block. Each set of bungee cord ends may be looped through a climbers' carabiner.

The tops of all the bungee cords (or the 4 carabiners at the top) are attached to a loop that is raised and lowered with an electric winch that is bolted to an I-beam suspended near the ceiling of the room.

It is important to have the bungee cords stretched at least one-half meter and preferably 1 meter when the block is suspended. It is also important to be sure that the bungee cords are still elastic and not stretched to the point that the nylon covering is carrying much of the load. If the bungee cords still have some stretch and need to be stretched farther, this can be done by putting weights such as lead bricks on the concrete block.

A correctly suspended block will have a resonant frequency less than one Hertz in all directions.

The resonant frequency is proportional to the stretch (or compression) of the elastic material and is independent of the material, so bungee cords stretched one-half meter or more will isolate to a much lower frequency than inner tubes. etc. But if problem vibrations are of a high enough frequency to be absorbed by an inner tube, they probably will work OK.

Hansma, *et* al., Biophys. J., 68, 1672(1995)

Paul Hansma and Helen Hansma, University of California, Santa Barbara.

A Sucrose Cryo Protectant For Storage Of Frozen Tissue Specimens

Here is the information I have on the sucrose cryoprotectant. This procedure is from Dr. John Olschowka's lab at the University of Rochester and was originally developed by J.S. deOlmos.

Cryoprotective Storage Solution:

Add in order:

500 mL 100 mM sodium phosphate buffer (pH 7.2)
10 gm polyvinylpyrrolidone (PVP-40, Sigma, 1% w/v-optional)
300 gm sucrose (30% w/v)
300 mL ethylene glycol (30% v/v)
Adjust final volume to 1 liter with distilled H_20.

Frozen sections (25-100 µm) should be cut into compartmentalized baskets or plastic trays containing the solution. The solution should be cold (*ca.* 4° C) and the collecting tray kept on ice while the tissue is being cut.

Store tissue in the freezer (-10° to -15° C) overnight or until ready for staining.

To stain:

Wash 3 x 5 minutes in 0.05M phosphate buffer followed by
3 x 5 minutes in 0.05M Tris plus 0.6% NaCl, pH 7.2.

This solution was originally developed for preserving immunoreactivity in brain sections. It will preserve cut sections for at least one to two months, and in some cases actually improves the quality of staining. Also, it can be used for storing blocks of tissue and for preserving general tissue morphology for EM applications.

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Continued on following page