Processing Cochlea For Paraffin Sectioning And SEM

Donna C. Montague
University of Arkansas for Medical Sciences

We have successfully sectioned decalcified paraffin embedded "inner ears" from mice, rats and humans. We have also prepared guinea pig cochlea for SEM. So, briefly:

Paraffin:

1) Immediately after dissection, place the cochlea in 10% neutral buffered formalin (NSF) and let fix with gentle agitation 24 hours per 2 mm thickness at room temperature.

2) Decalcify by immersing in 5% formic acid at least 4X specimen volume.

3) Gently agitate at room temperature and change at least once per 24 hours. Check for completeness of decalcification by decanting 4 mLs of "spent" formic acid into a clean, clear test tube. Add 1 mL of 5% ammonium oxalate in distilled water. Let stand undisturbed for 15 to 20 minutes. White precipitate of calcium oxalate indicates that the sample is not completely decalcified. Change formic acid at least once per 24 hours until the spent formic tests clear.

4) When clear, rinse in gently running tap water for approximately one hour.

5) Dehydrate with serial alcohols, 70% - 95% - 100% X 3.

6) Clear in methyl salicylate (Oil of wintergreen) X 3.

7) Infiltrate with 3 changes of paraffin (We use a 50:50 mixture of Fisher Tissue Prep and Stephens Type 9) at 56 to 60°C at 15 to 18 psi vacuum.

8) Embed in Stephen Type 9 *(not* a mixture).

SEM:

1) After dissection, fix the cochlea by injecting 2% osmium tetroxide in 0.2 M cacodylate buffer (pH 7.0 to 7.2). Make the buffer as in Bancroft and Stevens, and then add the osmium immediately prior to use.

2) Dehydrate in serial alcohols: 40%, 5 minutes; 70%, 10 minutes; 90% ,10 minutes; 100% 3 X 10 minutes then to Critical point dryer "bomb". Store in anhydrous acetone if cochlea cannot be dried immediately. Plus, the use of acetone as a transitional fluid between the alcohol and the CO_2 makes the detection of the endpoint of critical drying easier. When the odor of the acetone is completely gone, the specimen is dry and ready to coat.

We used gold sputter coating.

Note: I found the anhydrous acetone an imperative step given the extremely high humidity in Arkansas.

Cacodylate buffer

Preparation of stock solutions:

Stock A: 0.2 M sodium cacodylate (MW 214).
4.28 g sodium cacodylate into 100 mL distilled water

Stock B: 0.2 M HCl (MW 35.45)
1.7mL HCl into 100 mL distilled water

Composition of Buffer:

25 mL Stock A + x mL Stock B made up to 100 mL with distilled water

pH	x mL Stock B	pH	x mL Stock B
5.0	23.5	6.4	9.2
5.2	22.5	6.6	6.7
5.4	21.5	6.8	4.7
5.6	19.6	7.0	3.2
5.8	17.4	7.2	2.1
6.0	14.8	7.4	1.4
6.2	11.9		

References:

Bancroft, J.D. and A. Stevens. 1996. *Theory and Practice of Histological Techniques* 4th ed. pp 727-728. Churchill Livingstone, New York.

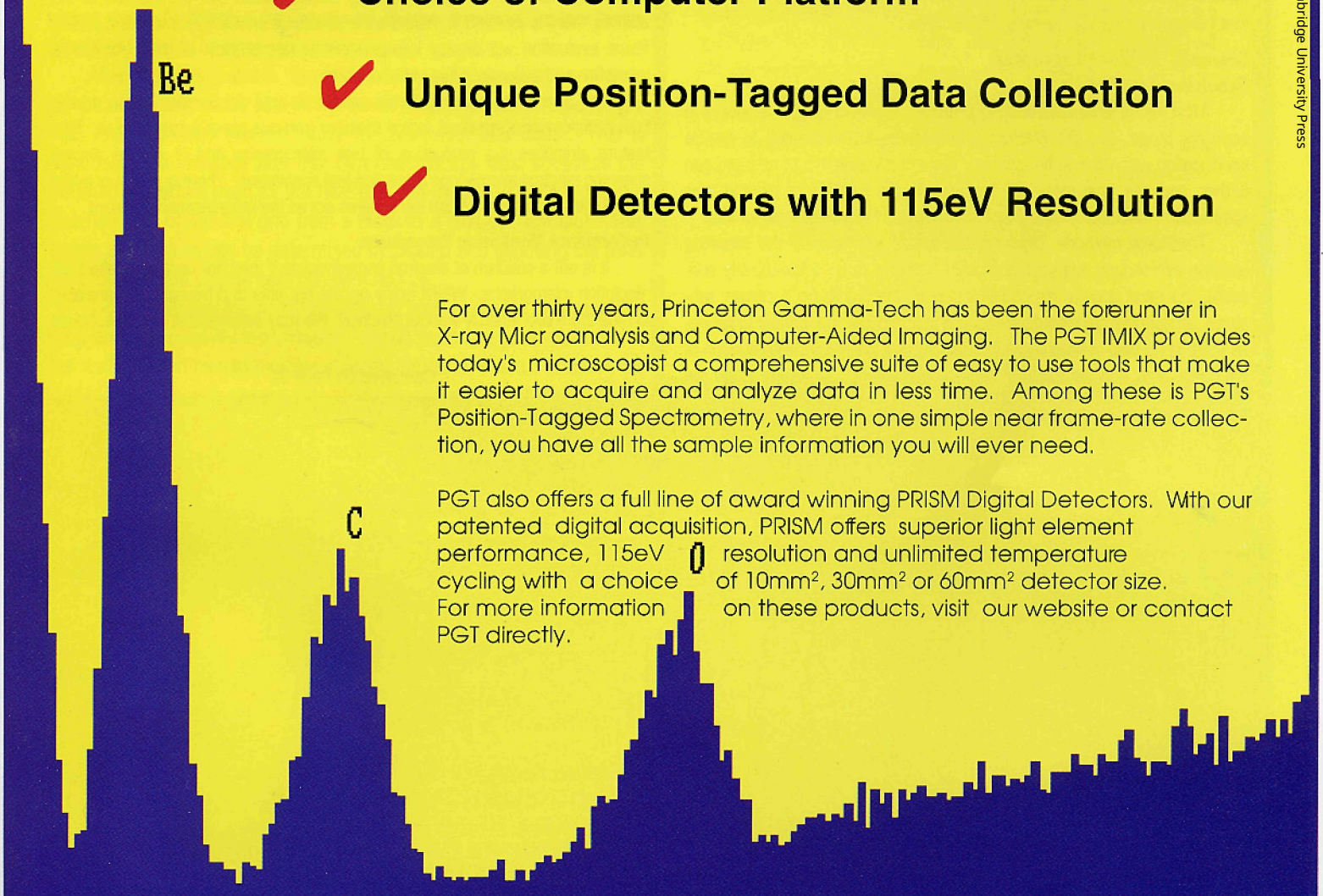
Skinner, R.A. 1986. The Value of Methyl Salicylate as a Clearing Agent. J. Histotech. 9: 27-28.

Skinner, R.A. and R.W. Nicholas. 1991. Preparation of Superior Quality Slides of Orthopaedic Tissues From Fresh Surgical Specimens. Tech Sample, ASCP Press, HT2.

Skinner, R.A., R.W. Nicholas, C.L. Stewart, and C. Vireday. 1993. Processing of Resected Allograft Bone Containing an Expanded Polytetrafluoroethylene Implanted Prosthetic Ligament: Comparison of Paraffin, Glycol Methacrylate and Exakt Grinding System. J. Histotech. 16:129-137.

Skinner, R.A, S.G. Hickmon, C.K. Lumpkin, J. Aronson and R.W. Nicholas. 1997. Decalcified Bone: Twenty Years of Successful Specimen Management. J. Histotech 20(3): 267-277.

A Cut Above the Rest . . .



✔ **Choice of Computer Platform**

✔ **Unique Position-Tagged Data Collection**

✔ **Digital Detectors with 115eV Resolution**

For over thirty years, Princeton Gamma-Tech has been the forerunner in X-ray Micr oanalysis and Computer-Aided Imaging. The PGT IMIX pr ovides today's microscopist a comprehensive suite of easy to use tools that make it easier to acquire and analyze data in less time. Among these is PGT's Position-Tagged Spectrometry, where in one simple near frame-rate collection, you have all the sample information you will ever need.

PGT also offers a full line of award winning PRISM Digital Detectors. With our patented digital acquisition, PRISM offers superior light element performance, 115eV resolution and unlimited temperature cycling with a choice of 10mm², 30mm² or 60mm² detector size. For more information on these products, visit our website or contact PGT directly.

PGT
PRINCETON GAMMA-TECH

Princeton Gamma-Tech, Inc. C/N 863 Princeton, NJ 08542-0863

Telephone: 609•924•7310 • Facsimile: 609•924-1729 • Website: www.pgt.com • e-mail: sales@pgt.com

Circle Reader Inquiry #17