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The Rapid Manual Method

The "Rapid Manual Method," (RMM) modified by Maat et al. (2001) [3], was used as an alternative to histological preparation for bone. This method was proven to be successful with well-preserved bone samples with high collagen content. The microstructures of tooth enamel, are a low collagen content tissue, but contain valuable information on age, season of death, and weaning time. The aim of this research is to test the (RMM) on teeth. If this method proves successful on *Sus scrofa* teeth, it will provide a more time and cost-effective method for bioarchaeologists or forensic anthropologists to examine tooth microstructure. Success will be determined by the number of dental microstructures that are visible under polarized light microscope.

Histological preparation and analysis can be time consuming, costly, and require training. With the (RMM), the ability to view dental microstructure is extended to a wider scientific audience and cost is cut by ten times.

Key Terms

Birefringence – The double refraction of light in a transparent, ordered material, which is manifested by the existence of orientation-dependent differences in refractive index.

Cementum annulations- a horizontal, linear microstructure in the cementum that is commonly used as an aging technique.

Cementocyte lacunae – cementoblasts that become trapped in the cementum matrix and work to maintain the cementum.

Dentin tubules – linear representations of the odontoblast process.

Granular layer of Tomes – a layer of dark granules that lie parallel to the outer surface of root dentin.

Hunter-Schreger Bands – A light phenomenon in microscopy that reflects the difference in direction of rods and interrods.

Odontoblast branching- the end of the odontoblast process at the dentino-enamel junction.

Striae of Retzius – incremental growth lines in tooth enamel.

Materials

- 3 oz. Dixie cups
- Wooden platform with metal clamp
- Glass slides and slide covers
- Dremel and Diamond blade attachments
- Gap Filling Medium – Cyanoacrylate
- Buehler polisher and grinder with carbomet papers in grit sizes 240, 600, and 1000 and polishing paper.
- Diamond paste.
- Clear Quick-Setting Epoxy
- Perma-mount
- Polarized light microscope
- 26 *Sus scrofa* teeth

Methods



Results

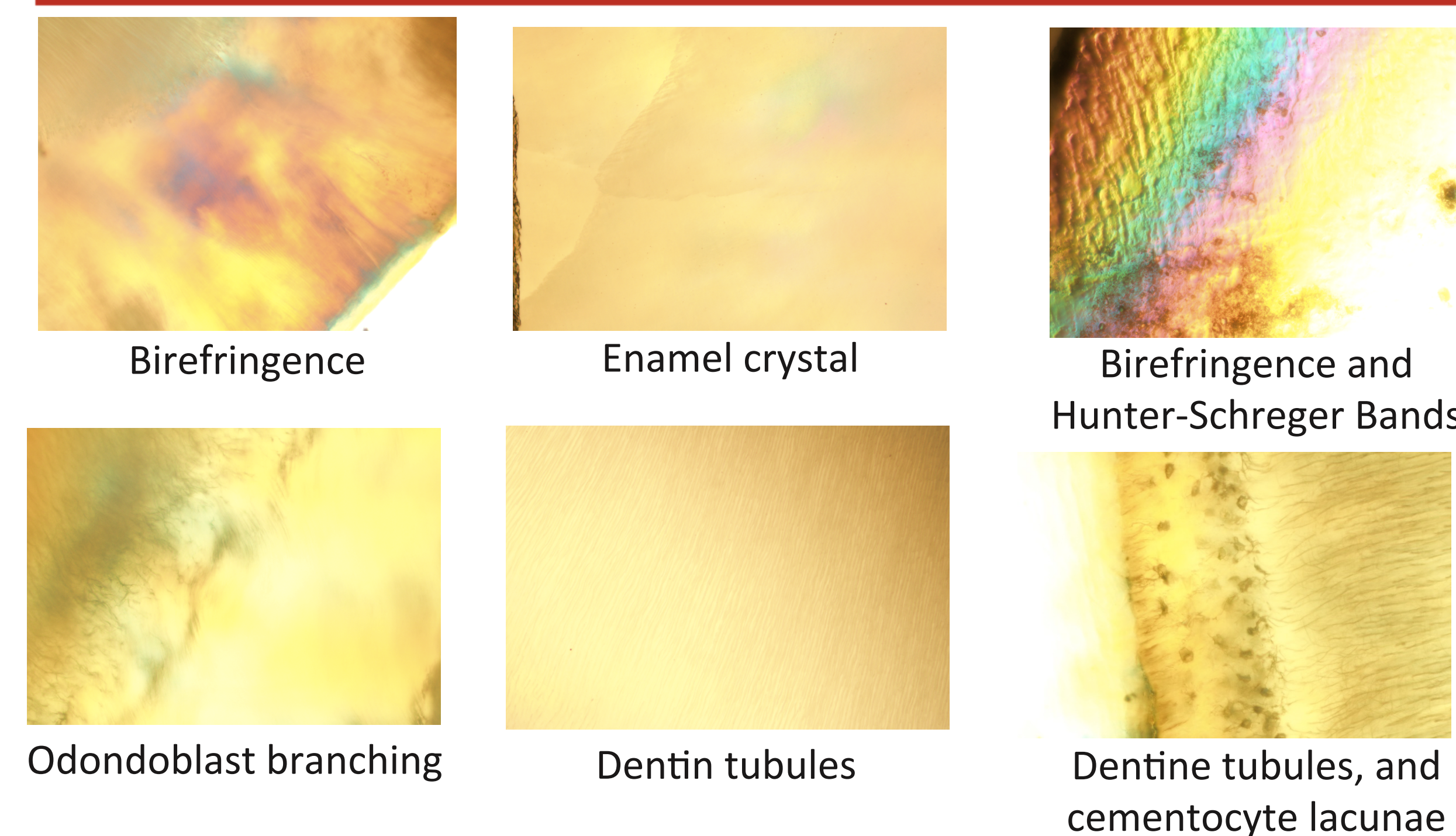
#	SR	HSB	EC	OB	DT	GLT	CA	CL
1	Y	Y		Y	Y			Y
4					Y			
6	Y		Y	Y	Y	Y		Y
7	Y		Y	Y	Y	Y		Y
8	Y		Y	Y	Y	Y		Y
9		Y	Y	Y	Y			Y
10		Y	Y	Y	Y			Y
11		Y	Y	Y	Y			
12	Y	Y		Y	Y			Y
15		Y	Y	Y	Y	Y		Y
17		Y	Y	Y	Y	Y		Y
18		Y	Y		Y	Y		Y
19	Y	Y	Y	Y	Y			Y
23		Y	Y		Y			
25		Y	Y	Y	Y			Y

Table Code

- Sample ID Number
 SR – Striae of Retzius
 HSB – Hunter-Schreger Bands
 EC – Enamel Crystal
 DT – Dentin Tubules

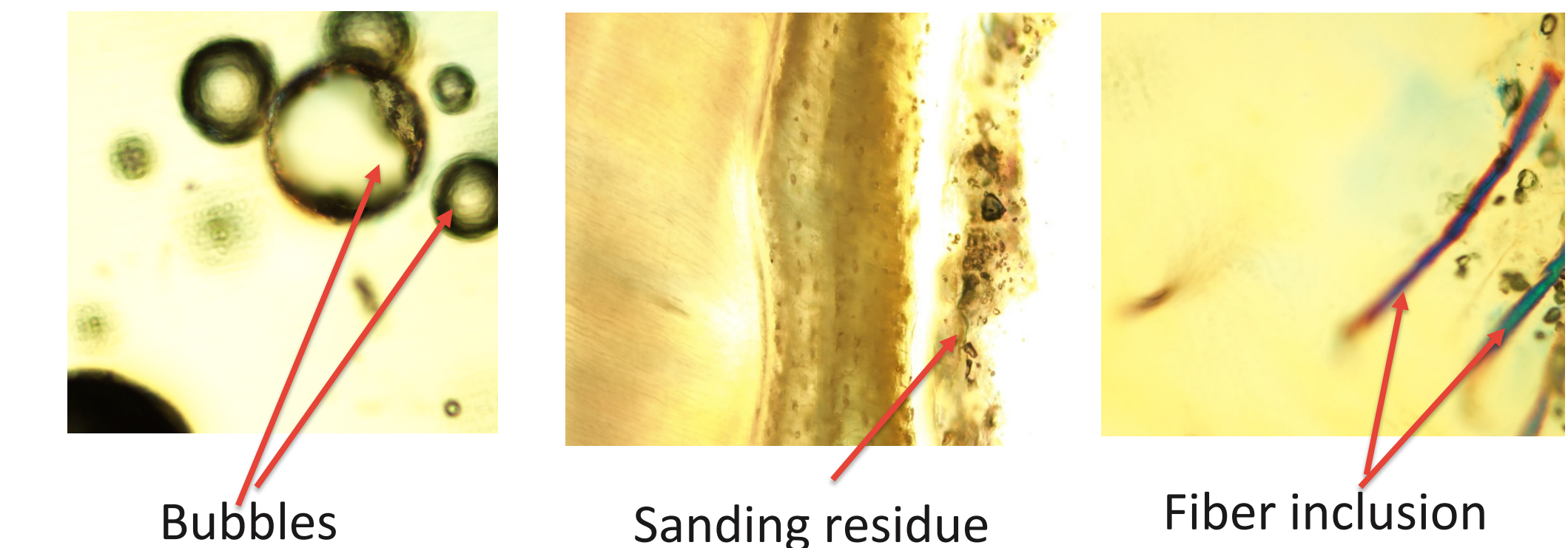
OB – Odontoblast Branching
 GLT – Granular Layer of Tomes
 CA – Cementum Annulations
 CL – Cementocyte Lacunae
 Y – Yes

Image Results



Defects

Common defects that were present under polarized light microscope include bubbles, fiber inclusions, and tooth residue from sanding that moved to the edges of the glue. These defects did not affect the ability to view dental microstructures.



Discussion & Conclusions

After microscopic analysis of *Sus scrofa* molars that were prepared with a few changes to the modified rapid manual method proposed by Maat et al. (2001), I conclude that the method does provide excellent results for fully mineralized molars. The method proved to be inexpensive, relatively fast, easy to follow, and effective. However, only 15 of the 26 samples were viewable. As it stands, this method would not be appropriate for small sample sizes, since the grinding process does not result in a reliable section thickness, which corresponds with findings of two previous tests on bone [1 & 2].

Additional research could be conducted with deciduous teeth to conclude whether the structures that are unique to those teeth, like the neonatal line, would be visible using this method. This test could be repeated to include the use of stains and determine whether staining allows for visibility of additional structures, like cementum annulations.

Acknowledgements

I would like to thank Chris Goforth at the North Carolina State Laboratory for allowing me access to the polarized light microscope that is housed there. I would also like to thank Dr. Jay Levine for providing access to a lab that I could conduct my research in. The guidance of Dr. Chelsey Juarez was instrumental in the completion of this project.

References

- [1] Beauchesne P and Sanders S. 2006. A Test of the Revised Frost's 'Rapid Manual Method' for the Preparation of Bone Thin Sections. *International Journal of Osteoarchaeology*. 16:82-87.
- [2] Hass K and Stora J. 2015. Different Preparation Techniques – Similar Results? On the Quality of Thin-Ground Sections of Archaeological Bone. *International Journal of Osteoarchaeology*. 25:935-945.
- [3] Maat et. al. 2001. Manual Preparation of Ground Sections for the Microscopy of Natural Bone Tissue: Update and Modification of Frost's 'Rapid Manual Method.' *International Journal of Osteoarchaeology*. 11:336-374.
- [4] Nanci, Antonio. 2008a. Structure of Oral Tissues. In: Nanci. 2008. Ten Cate's Oral Histology: Development, Structure, and Function. Mosby Elsevier Publications. St. Louis, MO. p 1-15.
- [5] Nanci, Antonio. 2008b. Enamel: Composition, Formation, and Structure. In: Nanci. 2008. Ten Cate's Oral Histology: Development, Structure, and Function. Mosby Elsevier Publications. St. Louis, MO. p 141-190.

Photographic Comparison

The following black and white images are from Ten Cate's Oral Histology, a textbook by Antonio Nanci (2008) [4 & 5]. These represent the ideal images of dental microstructures.

