doi:10.1017/S1431927613003097
Microsc. Microanal. 19 (Suppl 2), 2013
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45 Years of Electron Microscopy and Infectious Diseases, 37 Years at the "Bench" with Negative Stain Processing: What Did I Learn?

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A transition for me has begun. I anticipate and hope for an adjusted pace to the daily laboratory routine of 49 plus years. However, I doubt I will ever lose my interest for microscopy or bench-level/field science. Microscopy has been an avocation that has paid dividends, economic and other. My first hands-on experience was with a vintage 1954 RCA EMU 2C electron microscope (EM) in the Ceramics Engineering Department at Clemson University. Within the first week of working with the microscope, despite its limitations, I was hooked. I did not know then that various EMs would become my mistresses for 45 plus years...a year more than my marriage. Thirty-seven of those years, I have attempted negative stain electron microscopy (NSEM) with varied success.

Understanding of thin-section EM, including immune electron microscopy was nearing maturity in 1967 when I began my graduate studies. Negative Stain EM (NSEM) surprisingly was still somewhat a black art and if one follows the Microscopy Society of America list-server, for many, still is today. Most of my NSEM efforts have been involved with either identification of viruses in the infectious disease settings, or with efforts to control infectious virus diseases. Upon my arrival at CDC in 1983, I learned that answers to my basic questions about how to prepare **consistently** high quality NSEM grids were unsatisfactorily answered. My initial particle-adsorption questions included such things as which side of the grid should one put the specimen, filmed side or opposite? Should the specimen incubate on the grid or should the grid be placed on the specimen? What is the optimal drop-size and adsorption time? Is any advantage gained by adsorbing the specimen onto a substrate (eg. agarose) prior to applying a film or filmed grid? Does specimen ultracentrifugation or purification methods have merit? Can films with different qualities other than formvar, collodion, or carbon be useful? What are the different properties of various negative stains? Additional questions came to mind later. Examples follow. Can better negative stains be developed? Does phosphotungstic acid or fixation really damage viruses? Can quality of stained grids prepared for NSEM be maintained; and for how long?

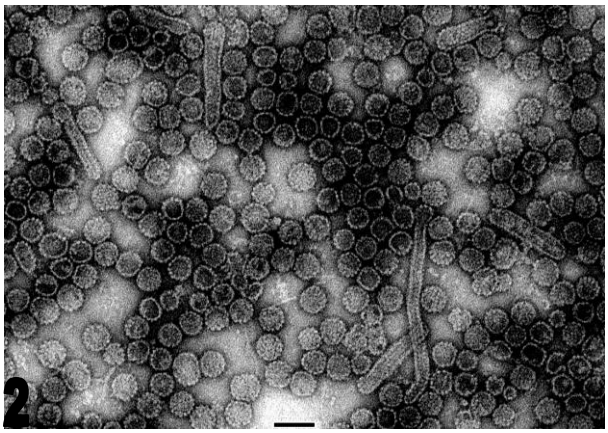
Today, in this presentation, I will share some of what I have learned about the above and other NSEM associated questions including the staining platform used for the last portion of my NSEM experience based on experience of Pirie-Gregory [1] involving adsorption and negative staining of viruses. The final modification of the platform used and images produced are shown in Figs. 1-3.

Reference

[1] R.P.C. Johnson and D.W. Gregory, *Journal of Microscopy*171 (1993) p.125.

https://doi.org/10.1017/S1431927613003097 Published online by Cambridge University Press

FIG. 1. Simple platform used for negative stain electron microscopy specimen preparation.



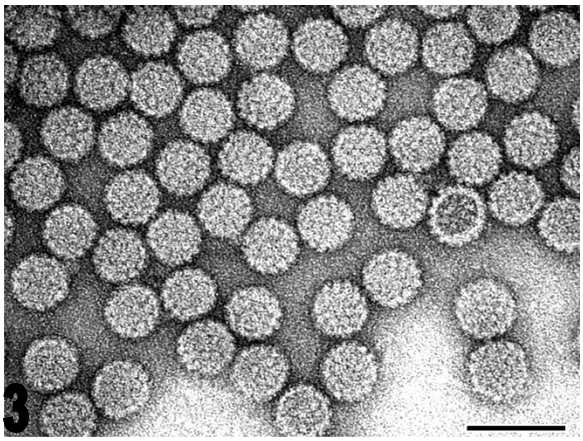


FIG. 2. Human papilloma virus 16.L1 capsid protein. Numerous tubular forms of the capsid protein are interspersed among the typically spherical VLP capsid proteins. Similar tubules are seen in wild-type papilloma and related polyomavirus preparations. Bar represents 100 nm.

FIG. 3. Human papillomavirus 18 virus-like particles of. L1 capsid proteins. Tubule forms are seen less frequently in Papillomavirus type 18 preparations.than in type 16 and other papillpmavirus type L1 capsid proteins. Bar represents 100 nm.

https://doi.org/10.1017/S1431927613003097 Published online by Cambridge University Press