B218 Non-Destructive Separation of Pollen Grain Constituents for Biochemical Analysis

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Learning Overview: After attending this presentation, attendees will understand a comprehensive separation protocol that furthers the use of pollen grain beyond microscopy (i.e., DNA and chemical analysis).

Impact on the Forensic Science Community: This presentation will impact the forensic science community by providing additional avenues for the analysis of pollen grains in forensic laboratories at the state or local levels.

Forensic palynology, the study of pollen grain morphology, has been used in a variety of cases around the globe. Pollen grain evidence provides the ability to determine the location where people or objects have been by the unique assemblage that gets transferred onto them. This deposition can occur from direct contact with a plant or dirt samples and/or by airborne pollen. Current analysis in the field of forensic palynology relies on the use of visual microscopic analysis (optical microscopy or scanning electron microscopy) to determine the plant species a single grain originates from via its morphological features. Pollen grains are a multi-component structure consisting of an external pollen coat over a decay-resistant shell, containing its genetic material. To visualize the unique morphology of pollen grains, the coating and other external material must be removed. The most common technique is acetolysis. It uses a mixture of acetic anhydride and sulfuric acid to dissolve any organic and/or inorganic material from the grain surface. It also dyes the grain a dark brown hue for visual microscopic analysis. The removal of organic material from the pollen also destroys the pollen coat and DNA (via infiltration thought the pollen apertures), leaving only the shell (more explicitly, exine and intine) of the pollen grain for microscopic analysis. This procedure prevents any additional analysis.

In recent studies, it has been known that analytical methods such as Inductively Coupled Plasma/Mass Spectrometry (ICP/MS), Raman, and Infrared (IR) absorption, as well as DNA analysis, can provide additional information for the identification of pollen species. But as mentioned above, current cleaning methods prevent any additional analysis due to the chemical destruction of the coating and the genetic material. Reciprocally, any DNA analysis has been done by crushing the grains, preventing microscopic and chemical analysis.

This presentation will show a simple cleaning method that maintains the pollen grain morphology while also providing material for additional spectroscopic and DNA information. Soxhlet extraction using ethanol as a solvent will be shown to be a method that allows for DNA and coating to be removed without damaging or altering the chemical integrity of both components. This presentation will show the complementary analyses obtained from every constituent of pollen grains from nine different species. Molecular information (from Raman and IR absorption), elemental analysis (from ICP/MS), DNA analysis (via quantitative Polymerase Chain Reaction [qPCR]) and microscopic analysis (via Scanning Electron Microscopy [SEM] and digital microscopy) can be combined for the classification of pollen species.

Such a simple and versatile preparation of pollen grains will indubitably bring the use of pollen as a forensic evidence back to the light and provide forensic examiners with new options for geolocation and association.

Pollen, Palynology, Forensic Botany