B164 Cellular Autofluorescence Signatures for Quantifying Cell Types in Trace Biological Samples and Establishing Age of Evidence

Emily Brocato, Virginia Commonwealth University, Richmond, VA 23284; Kate Philpott, JD, Reston, VA 20191; Christopher J. Ehrhardt, PhD, Virginia Commonwealth University, Richmond, VA 23284*

Learning Overview: After attending this presentation, attendees will learn about cellular autofluorescence profiles and how these signatures can be used to quantitate the abundance of forensically relevant cell types and determine the amount of time between deposition of cell populations and sample collection. Attendees will also learn how flow cytometry and conventional microscopic techniques may be used to rapidly analyze these signatures in biological evidence.

Impact on the Forensic Science Community: This research will impact the forensic science community by introducing a new, non-destructive approach for determining source tissue for cell populations in a biological sample as well as the overall age of the evidence. This may provide probative information for many types of biological samples and complement results from standard genetic profiling techniques.

Characterizing the type of cells present in biological evidence and, therefore, the tissue they originated from within the body, can assist with crime reconstructions and downstream DNA profiling methods. Traditionally, case working methods for determining tissue source are based on microchemical reactions targeted toward proteins within bodily fluids, which have limited sensitivity and/or specificity. In contrast, few forensic techniques have utilized morphological or intrinsic biochemical profiles of cells due to the laborious nature of conventional microscopic characterizations or the need for tissue-specific probes which have limited success on compromised samples. Although autofluorescence signatures have demonstrated applications for clinical diagnostics and separating forensic cell mixtures, they have not been investigated as a method to identify cell types or establish the age of a cell population.

Therefore, the goal of this study was to characterize autofluorescence and morphological signatures for cell populations representing four separate tissue sources: buccal, touch epidermal, vaginal, and blood, and then test whether autofluorescence profiles could be used to rapidly differentiate cell types in aged biological samples. Cell populations were collected from a total of 30 individuals, 10 per each tissue type. Additionally, each sample was aged for between 24 hours and more than two months. Measurements from 45 different variables were collected from individual cells in a high-throughput fashion using flow cytometry and then analyzed using multivariate techniques including Discriminant Function Analysis. Results showed that epidermal cells could be distinguished from vaginal and buccal cells with a high degree of classification accuracy, ~94%. Similarly, blood cells could be differentiated from the three epithelial cell types with an accuracy over 97%. Analysis of variable weights indicated that measurements capturing the circularity, aspect ratio, and autofluorescence between 450-650nm of cells were the largest drivers of multivariate differences between tissue types.

Cellular autofluorescence profiles were also observed to change systematically as the sample aged. Specifically, the median intensity of autofluorescence detected between 350-550nm and between 630-680 nm increased incrementally at 24-hour time points between zero and seven days (e.g., ~4500 RFU at 24 hours; ~7500 RFU at 48 hours, ~9000 RFU at 72 h). Additionally, differences were observed between one week and two months that were less systematic with time. Autofluorescence signatures were then used to construct a multivariate framework to predict the time since deposition in an unknown biological sample. Results showed that cell populations were correctly associated with eight different time points between 24 hours and 2 months with an accuracy of ~85%. Ultimately, cellular measurements such as these, which can be obtained non-destructively, may provide probative information for many types of biological samples and can complement results from standard genetic profiling techniques.

Forensic Biology, Autofluorescence, Serology