



The use of secondary ion mass spectrometry in forensic analyses of ultra-small samples

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It is becoming increasingly important in forensic science to perform chemical and isotopic analyses on very small sample sizes. Moreover, in some instances the signature of interest may be incorporated in a vast background making analyses impossible by bulk methods. Recent advances in instrumentation make secondary ion mass spectrometry (SIMS) a powerful tool to apply to these problems. As an introduction, we present three types of forensic analyses in which SIMS may be useful.

The causal organism of anthrax (*Bacillus anthracis*) chelates Ca and other metals during spore formation. Thus, the spores contain a trace element signature related to the growth medium that produced the organisms. Although other techniques have been shown to be useful in analyzing these signatures, the sample size requirements are generally relatively large. We have shown that time of flight SIMS (TOF-SIMS) combined with multivariate analysis, can clearly separate *Bacillus sp.* cultures prepared in different growth media using analytical spot sizes containing approximately one nanogram of spores.

An important emerging field in forensic analysis is that of provenance of fecal pollution. The strategy of choice for these analyses-developing host-specific nucleic acid probes-has met with considerable difficulty due to lack of specificity of the probes. One potentially fruitful strategy is to combine *in situ* nucleic acid probing with high precision isotopic analyses. Bulk analyses of human and bovine fecal bacteria, for example, indicate a relative difference in $d^{13}C$ content of about 4 per mil. We have shown that sample sizes of several nanograms can be analyzed with the IMS 1280 with precisions capable of separating two per mil differences in $d^{13}C$. The NanoSIMS 50 is capable of much better spatial resolution than the IMS 1280, albeit at a cost of analytical precision. Nevertheless we have documented precision capable of separating five per mil differences in $d^{13}C$ using analytical spots containing less than 300 picograms of bacteria.

Perhaps the most successful application of SIMS for forensic purposes to date is in the field of nuclear forensics. An example that has been used by laboratories associated with the International Atomic Energy Agency is the examination of environmental samples for enriched uranium particles indicative of clandestine weapons production activities.. The analytical challenge in these types of measurements is to search complex environmental matrices for U-bearing particles which must then be analyzed for ^{234}U , ^{235}U , and ^{236}U content with high precision and accuracy. Older-generation SIMS instruments were hampered by small geometries that made resolution of significant interferences problematic. In addition, automated particle search software was proprietary and difficult to obtain. With the development of new search software, the IMS 1280 is capable of searching a sample in a matter of hours, flagging U-bearing particles for later analyses, and providing a rough ^{235}U content. Particles of interest can be revisited for high precision analyses, and all U-isotopes can be measured simultaneously in multicollector mode, dramatically improving analysis time and internal precision. Further, the large geometry of the instrument allows complete resolution of isobaric interferences that have traditionally limited SIMS analyses of difficult samples. Examples of analyses of micron-sized standard particles indicate that estimates of ^{235}U enrichment can be obtained with an external relative precision of 0.1% and ^{234}U and ^{236}U contents can be obtained with a relative precision of less than 1%. Analyses of "real" samples show a dramatic improvement in the data quality obtained compared with small-geometry SIMS instruments making SIMS the method of choice for these high-profile samples when accurate, precise, and rapid results are required.