B79 The Application of Optical Trapping to Obtain Single-Source Short Tandem Repeat (STR) Profiles From Forensically Relevant Body Fluid Mixtures With Modified DNA Analysis Workflow

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Learning Overview: The goal of this presentation is to demonstrate the utility of optical trapping for separation of a diverse assortment of cells, such as spermatozoa and leukocytes, from forensically relevant mixed samples. Optical trapping is a method used to mechanically manipulate microscopic objects, such as cells. The major operating principle of optical trapping is to direct the momentum of light to impact an object of interest. A laser can be tightly focused through a 100x immersion objective into a point roughly 1µm in diameter. This laser will exert a gradient force on an object, drawing it closer toward the focal point until the gradient force is entirely offset by an on-axis scattering force that traps the object in place. This object can then be moved in three dimensions. Cells caught in the trap and moved on the order of 500µm/s will not be damaged.

Impact on the Forensic Science Community: This presentation will impact the forensic science community by demonstrating a method for deconvolution of DNA mixtures at the beginning of the DNA workflow through the use of optical trapping. This method can be applied to suspected mixed samples to separate a small population of target cells that can then be analyzed within the traditional forensic DNA workflow. Unlike currently in-practice techniques, such as differential extraction, this method can separate a variety of mixtures, such as blood/saliva. This method has the potential to save time at the end of the workflow by negating the need for lengthy mixture interpretation or probabilistic genotyping by generating a single-source STR profile.

The traditional DNA workflow used for analysis of samples that have been separated with optical trapping remains unchanged with only minor modifications. DNA is extracted, quantified, and amplified. Since optical trapping necessarily produces low-copy number samples, samples are concentrated down with vacuum centrifugation prior to amplification. This allows the entire extract to be amplified.

Previous research performed at Virginia Commonwealth University (VCU) has demonstrated that optical trapping can be used to capture and separate both spermatozoa and leukocytes from a larger mixed cell population. Spermatozoa were tweezed out from samples that contained a 1:1 mixture of semen to vaginal fluid in groups ranging from 8–55 cells. Quantification revealed total DNA quantity closely correlated with theoretical yield calculated based on the qualitative observation of the number of tweezed cells. Each sample produced a single-source STR profile with groups of 50 spermatozoa generating STR profiles with greater than 90% of expected alleles. This research demonstrated that small populations of cells could be successfully separated and analyzed from a larger sample; thus, further research was undertaken into other cell types.

A modified transfer protocol was developed that demonstrated improved results from the previous work. Twenty-one samples of leukocytes ranging from 5 to 22 cells were tweezed from liquid samples and analyzed as previously described. Full STR profiles were obtained from 8 of the samples, with 2 of these samples containing only 10 cells. Of the remaining 13 samples, 6 samples had greater than 90% of expected alleles.

Optical Trapping, DNA Mixtures, Cell Separation

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