

Diamond™ Nucleic Acid Dye demonstration to visualize DNA on nonporous surfaces of simulated evidence

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Abstract: In recent years, the concept of using DNA staining dyes to visualize latent DNA has expanded rapidly. The use of Diamond™ Nucleic Acid Dye to visualize DNA has been tested on a variety of different surfaces including cell phones, debit or credit cards, and soda cans. However, there has not been much scientific literature regarding the use of this dye on materials that are commonly used to bind or tie up victims in criminal casework. This teaching demonstration shows the effectiveness of Diamond™ Nucleic Acid Dye to enhance visualization of DNA on common binding materials using standard forensic science equipment.

Keywords: Diamond Dye, DNA, human DNA, latent DNA, alternate light source

Introduction

Trace DNA has been studied extensively by examining what it is comprised of, how it persists on surfaces and how it is transferred to other surfaces (1-7). Trace DNA can be single source DNA or mixtures of DNA from many sources. However, trace DNA is latent, and an enhancement reagent would be beneficial to workflow to aid in visualizing areas to collect from on forensic evidence. Since DNA is invisible to the naked eye and microscopic, we describe here a simple method to enhance the visualization of human DNA on nonporous surfaces commonly found as trace or touch evidence in forensic casework using a fluorescent DNA binding dye (8-11).

Diamond™ Nucleic Acid Dye is a nucleic acid binding dye that has been investigated for use as an enhancement reagent for visualizing trace DNA on many evidentiary surfaces (12-26). It was originally a molecular biology reagent sold as an additive to visualize DNA fragments (single and double strands) and RNA (double strands) in agarose and polyacrylamide gels after electrophoresis. The dye is stable for up to 90 days when stored at room temperature. For long-term storage, it is recommended that the dye be stored at -20C. In this study, Diamond™ Nucleic Acid Dye was purchased and stored at -20C, thawed and prepared immediately before use. We use Diamond™ Nucleic Acid Dye as a demonstration to observe latent DNA from fingerprints placed on different nonporous surfaces. This dye also works well on pure DNA in solution added to surfaces and air dried before visualization. Enhancing latent DNA

on surfaces can establish where the DNA accumulates, especially for multifaceted surfaces.

Methods

Prior to the use of Diamond™ Nucleic Acid Dye (Promega Corporation, Madison, WI), the materials were cleaned using DNA/RNA-ExitusPlus™ IF (AppliChem GmbH, Germany) followed by 70% isopropyl alcohol (Thermo Fisher Scientific, Waltham, MA). Then they were individually placed into either paper evidence bags or envelopes and stored at room temperature until DNA deposit and Diamond™ Nucleic Acid Dye application. The DNA used was commercially purchased from Thermo Fisher Scientific as 1Kb Plus DNA ladder with a DNA concentration of 0.5 µg/µl. The DNA (2 µl) was allowed to air dry for 30-60 minutes on each surface. A Diamond™ Nucleic Acid Dye solution diluted in 75% ethanol (Thermo Fisher Scientific) was applied by micropipette to the dried DNA deposits and visualized immediately with a stereomicroscope (20x total magnification). Images were collected after drying again with a hand-held cell-phone camera (Samsung S23+) or Apple iPad A16 camera while viewing through amber filter goggles positioned at and taken through the filter and eyepiece of a stereomicroscope (Fisher Scientific Stereomaster, Waltham, MA) while illuminated with an UltraLite One with UV Magnum (405 nm) alternate light source (Thomas Scientific, Swedesboro, NJ). It was helpful to rest the goggles in place over the eye pieces for capturing images or have a second individual hold the filter in place.

Materials

- Clear plastic zip ties
- Yellow nylon rope
- White plastic-coated electrical cord
- Grey duct tape
- Diamond™ Nucleic Acid Dye in 75% molecular-biology grade ethanol
- DNA/RNA-ExitusPlus™ IF
- 70% isopropyl alcohol
- Stereomicroscope
- UltraLite One alternate light source
- Amber filter goggles
- Scissors
- Cell phone (Samsung S23+) or Apple iPad A16 camera
- Nitrile gloves
- Micropipette with disposable tips
- Kimwipes
- 1 Kb Plus DNA ladder
- Butcher Paper

Hazards and Safety Precautions

Diamond™ Nucleic Acid Dye is harmful if it meets skin. It is also suspected of causing genetic defects. Store in a secure space.

Results

Diamond™ Nucleic Acid Dye is a DNA-binding dye that fluoresces yellow to yellow-green when bound to its substrate. The following demonstration shows a simple imaging technique with common forensic science equipment to use Diamond™ Nucleic Acid Dye for the enhancement of latent DNA. After optimizing this technique, complicating factors that affect documentation and imaging were identified and include the level of magnification required, the reflectance of the surface and the motion of the DNA on the surface as it dries. As is shown in **FIGURE 1A**, the nuclei of cells are small and 20x total magnification was required to visualize and document the DNA from fingerprints. The zip tie was clear and reflective, but the amber goggles were helpful to reduce the glare and increase contrast between the fluorescence and the surface (**FIGURE 1B**). Diamond™ Nucleic Acid Dye is applied as a wet reagent and the fluorescence can be visualized almost immediately even before the solution has fully dried but for imaging, it is better to have the solution dry so that the cells and any debris on the surface are stationary during photography. For **FIGURE 1A**, the cells from the fingerprint were randomly scattered and adherent to the glass surface. For **FIGURE 1B**, the cells from the fingerprint were attached to the upper ridges of the zip tie.

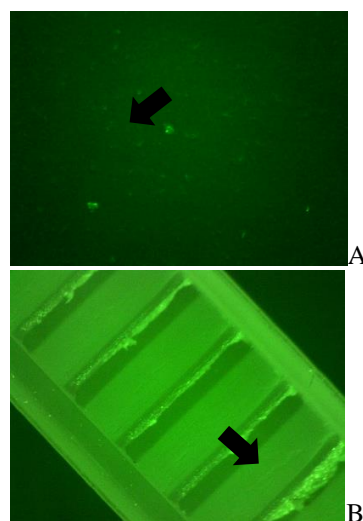


FIGURE 1 A. Glass slide with fingerprint and Diamond™ Nucleic Acid Dye and B. corrugated zip tie surface with fingerprint and Diamond™ Nucleic Acid Dye. Images were taken with an Apple iPad A16 camera through the eyepiece of a stereomicroscope (20x total magnification) illuminated with an UltraLite One with UV Magnum (405 nm) and with an amber goggle filter applied between the eyepiece and the camera lens (images courtesy of Alexa Orlando).

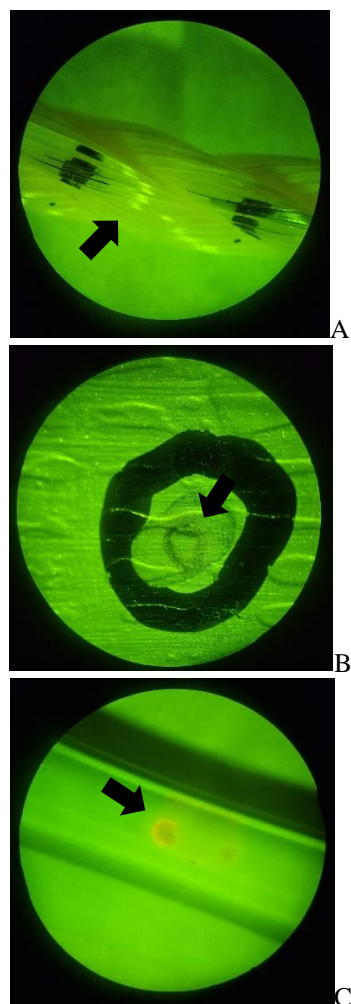


FIGURE 2 Diamond Dye examined on various surfaces using purified DNA (1 Kb Plus DNA Ladder) and a stereomicroscope (20x total magnification) collected with a cell-phone camera (Samsung S23+) through amber filter goggles positioned at the eyepiece (handheld) of a stereomicroscope and illuminated with an UltraLite One with UV Magnum (405 nm) (images courtesy of Jared Duchesne). Purified DNA on A. nylon rope, B. grey duct tape and C. white plastic electrical cord.

DNA in solution when drying can remain stationary or travel on the surface. **FIGURE 2** illustrates the different patterns one might expect to observe on nonporous surfaces that are fiber-like, irregularly smooth and perfectly smooth. **FIGURE 2A**, the twisted nylon rope, shows that the DNA solution after application between the two black markings traveled or wicked along the surface of the rope fibers and fluoresced at the perimeter like many body fluid stains appear on fabrics (e.g. saliva, urine). The duct tape (**FIGURE 2B**) was an irregular but smooth surface and yielded a double ring

image where the center ring is the point of DNA solution application and the outer ring shows the spread of the DNA solution as it dried. The duct tape is grey in color and the yellow color of the dye is reduced in contrast. For **FIGURE 2C**, the white electrical cord is a hard white plastic smooth surface, and the DNA solution remained in place as a bead of liquid and dried in a concentrated orange spot with a light-yellow ring around the spot showing the spread of solution as it dried.

Conclusion

In conclusion, this technique can be useful to demonstrate and discuss the forensic concepts of latent DNA visualization with DNA-binding enhancement dyes, such as Diamond™ Nucleic Acid Dye shown here, on different smooth and multifaceted nonporous surfaces using forensic examination procedures with alternate light sources and filters to discuss concepts such as energy and light excitation, emission, reflectance, absorption and image contrast. Diamond™ Nucleic Acid Dye when excited with ultra-violet light and bound to double stranded DNA has an excitation wavelength of 494 nanometers and an emission wavelength of 558 nanometers. This dye works well to increase contrast and visualize latent DNA on many nonporous surfaces such as described here, but some white, grey and yellow surfaces reflect a lot of light and therefore reduce the image contrast, so it is not universally applicable to all surfaces. This technique also shows that DNA from a fingerprint or in solution may not deposit or dry in a consistent pattern and is highly dependent on the features of the surface.

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