Advancing Semen Detection Techniques with Sperm Tracker STK in Real Case Scenarios and Diverse Environments

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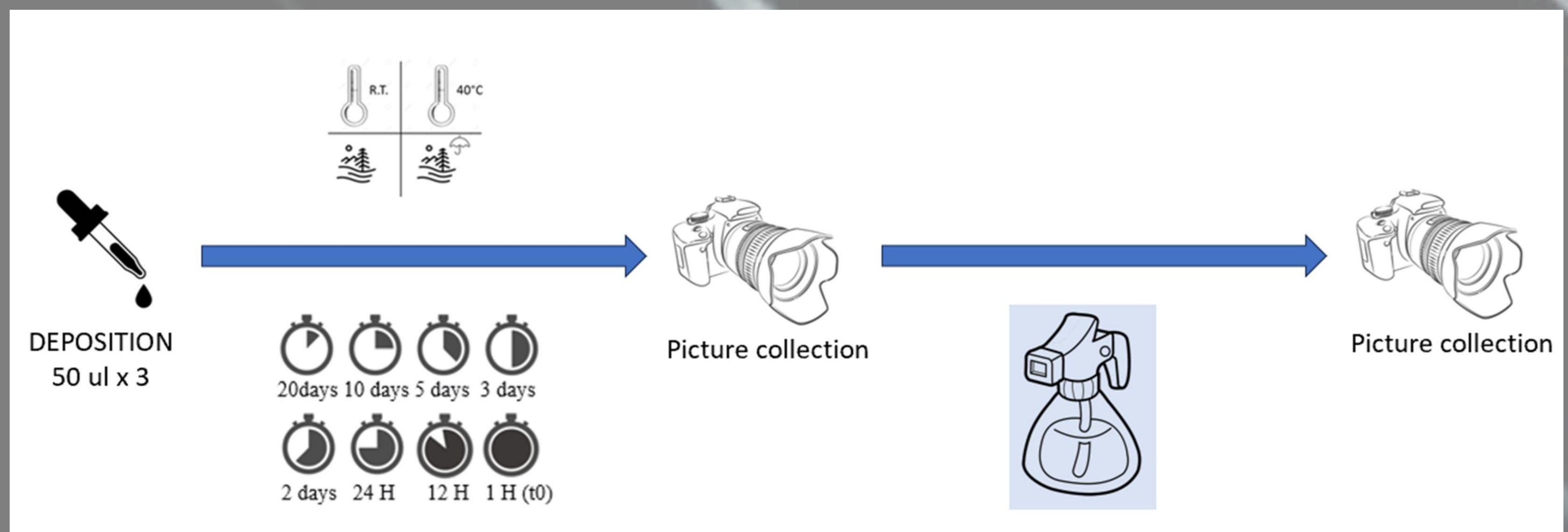
ABSTRACT

There is a pressing need for sensitive, specific, and non-destructive tests before DNA analysis. The study validates the efficacy of the new Sperm Tracker technique, particularly the STK Skin reagent, which preserves DNA integrity and enhances traces effectively. Exploratory analysis using pixel-level and image-level approaches on RGB raw data, along with Principal Component Analysis (PCA), highlights the technique's practicality in identifying sperm traces. Results indicate consistent efficiency across various environmental conditions and time intervals, although color variation over time is observed. However, limitations exist, such as difficulty in detecting traces on certain substrates and potential false positives. Suggestions for improvement include integrating advanced imaging systems, conducting precise segmentation, and studying the specificity of Sperm Tracker products. Additionally, the study considered eight time intervals, with a maximum of 20 days, indicating potential for future research to extend the time window and study the functioning of the reagent over longer periods. Moreover, the application of PCA could be conducted directly at the crime scene or in a Violence Prevention Centres, enhancing the forensic analysis process.

MATERIALS AND METHODS

The aim of this study is focused on how STK Skin may improve the evidence collection phase, and it was not linked to genetic profiles obtainment. Mock samples were created using pig ears, universally known as one of the most faithfully resembling the human skin, along with human hairs, both natural and bleached. Different deposition time and environmental condition were set. Fresh sperm was collected at each deposition time from at least 3 different donors to minimize interindividual variability, and a 50 ul sample was laid down in triplicate on each surface. At the end of each deposition time an inspection occurred as real casework samples, and the VILBER VL-6.L UV lamp (365nm) was used. Pictures were taken with a Nikon digital camera D7100 with a DX AF-S NIKKOR 10-24mm 1 :3.5-4.5G ED camera lens and a yellow filter adopted. To standardize images, the camera was positioned on a tripod, 15 cm far from samples, the VILBER lamp was at a distance of 20 cm, and the aperture of the camera was set to 4.5, with 1 second capture time. A blue fluorescent signal is visible if the presumptive test is positive, while no signal is detected in negative conditions.

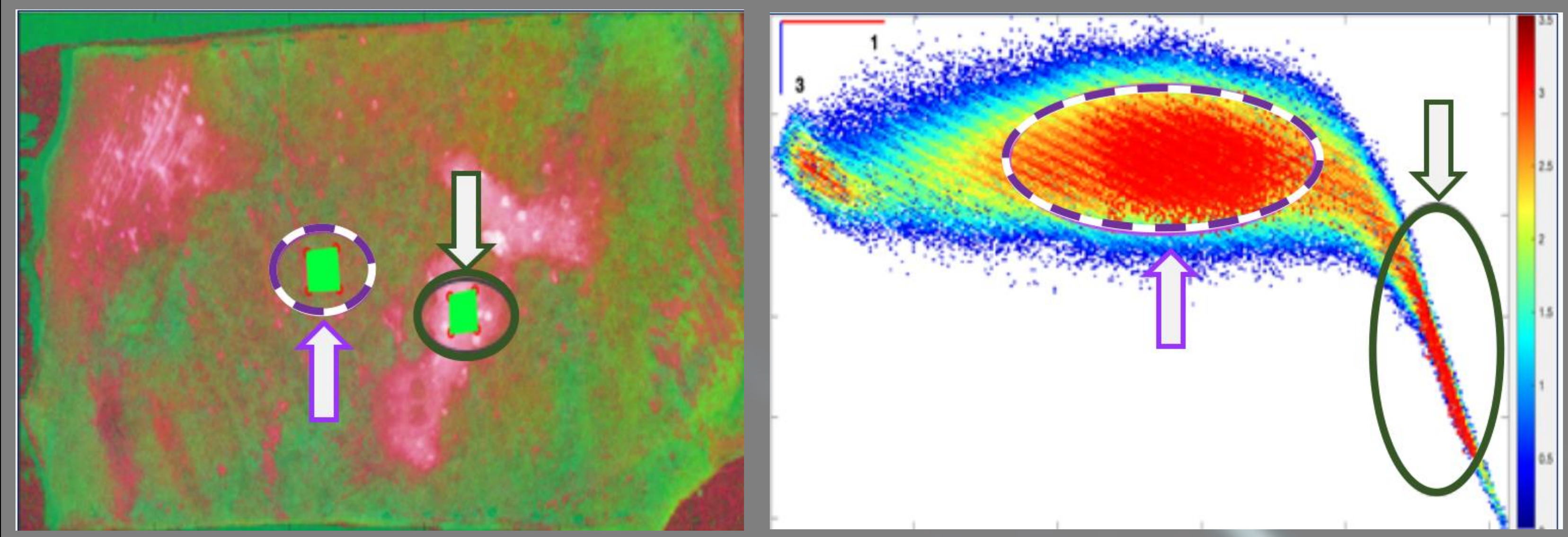


Images captured contain valuable chemical information: in particular, each pixel in a RGB image represents a color intensity value ranging from zero to 255, where the contributions of red (R), green (G), and blue (B) can be distinguished. By separating the image into matrices for each color, chemometric methods can be applied: PCA condenses pixel data into main components for easier visualization. Unfolding matrices from an RGB image enables PCA modeling to produce score values colored by pixel density for visualization. Recoloring the original image based on this density, termed refolding, allows efficient distinction of image portions using PCA-derived scores.

Because of the heterogeneity of the subject, a data reduction method was applied to RGB images to obtain a one-dimensional signal called colorgram: each colorgram integrate frequency distribution curves of R, G, and B channels, along with additional color parameters like lightness and saturation. The resulting colorgram matrix can then be analyzed using multivariate statistical methods such as PCA, facilitating dataset structure study, anomaly detection, and cluster identification.

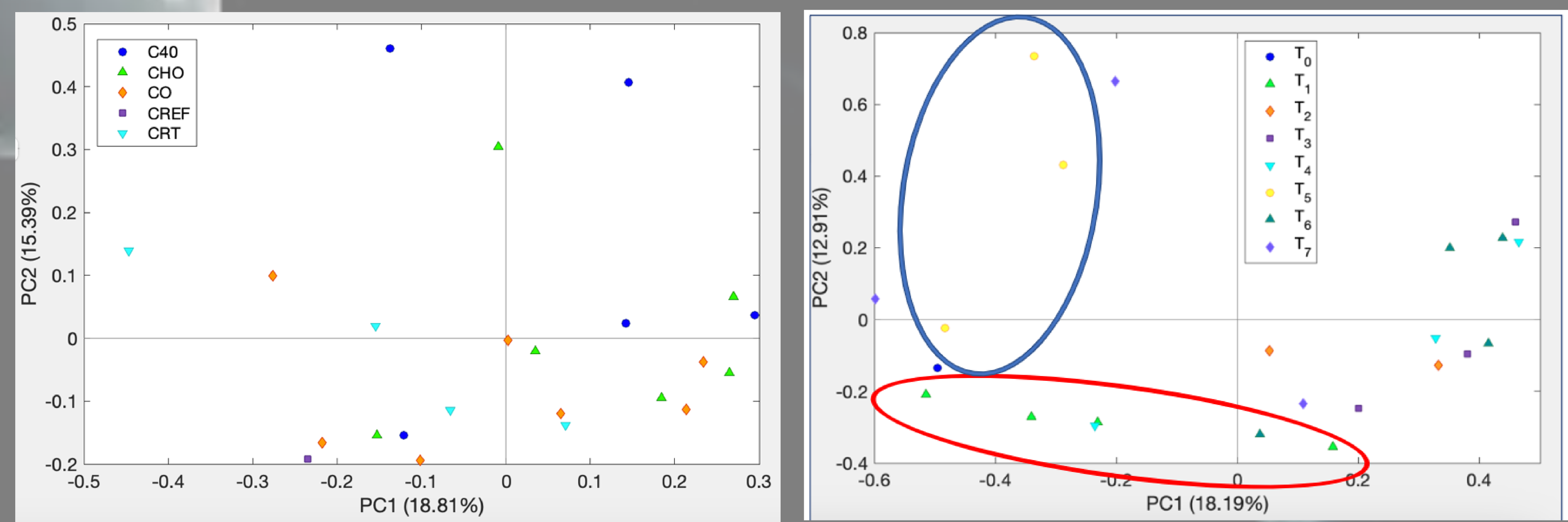
A new MATLAB-based graphical user interface, Colourgrams GUI, processes colorgrams by converting images and analyzing the resulting matrix using PCA or PLS for regression and prediction purposes.

RESULTS: THE CHEMOMETRIC APPROACH

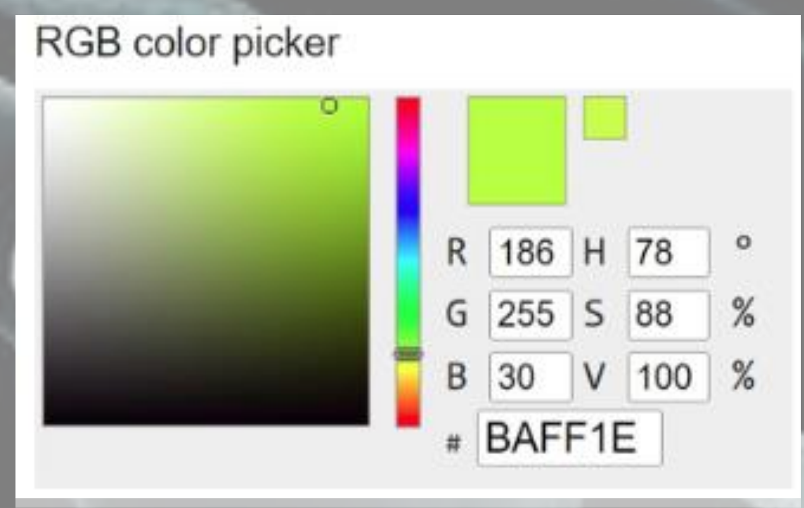


The PCA model was based on three principal components (PCs). In the score graphs near the image, pixel points are colored to indicate density: red for high density and blue for low density. This allows for selecting areas to observe corresponding scores, aiding in spectroscopic correlation and investigating pixel localization differences. The analysis aims to differentiate trace-related areas from irrelevant sample areas. Ideally, non-overlapping red clusters on the score plots indicate effective discrimination. Rightmost points (circled in black) represent trace-related pixels, while those circled in purple represent skin without sperm traces.

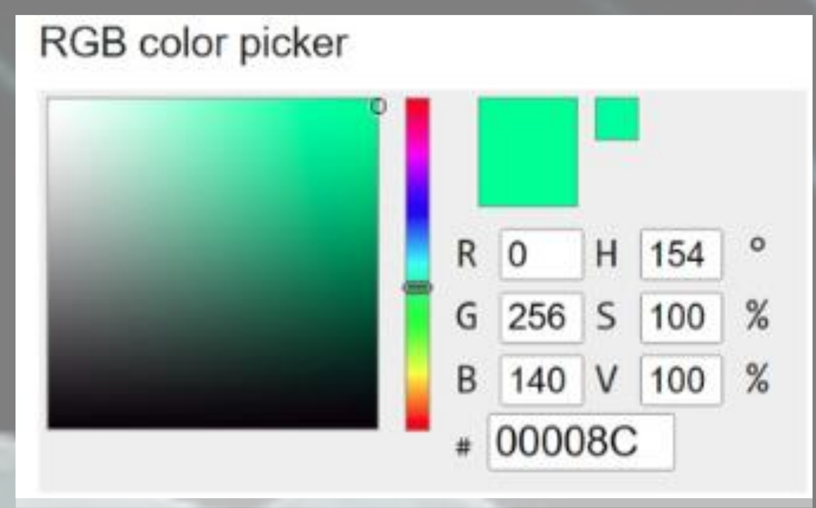
When analyzing multiple images, an efficient platform is crucial. Using MATLAB, the Colourgrams_GUI tool was used to process images into colorgrams. Uploaded in .jpg format, the images were cropped and renamed beforehand. Class vectors from an Excel file were imported to colorize colorgrams based on various sample conditions.



The study aimed to confirm STK reagent efficacy under different environmental conditions. Initial analysis involved PCA on colorgrams, focusing on the first two principal components.



Trace's color for long timescales
t = 5 days

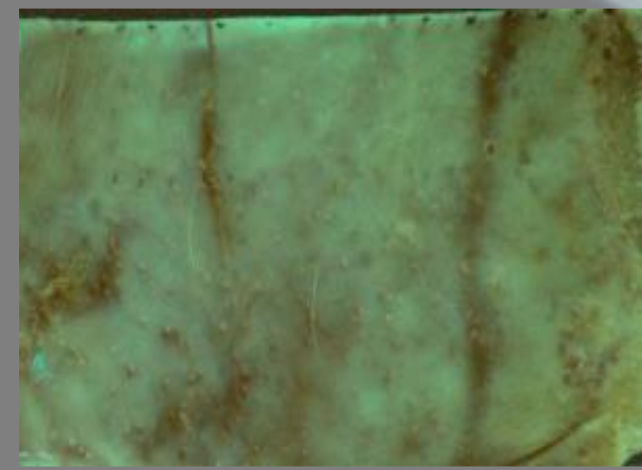


Trace's color for short timescales
t = 12 h

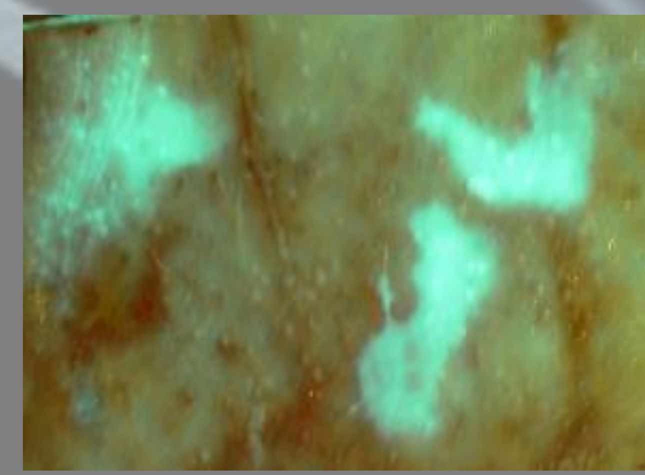
The colors resulting from the color indices appear to be in green/blue shades. The trace color will vary mainly due to red and blue. If the trace detection is carried out in a short time, its color will be bluer. If the trace was deposited earlier, it will be fainter, with a higher red tone.

RESULTS: THE FORENSIC SCIENTIST POINT OF VIEW

In the images below the impact of STK tool has been proposed:



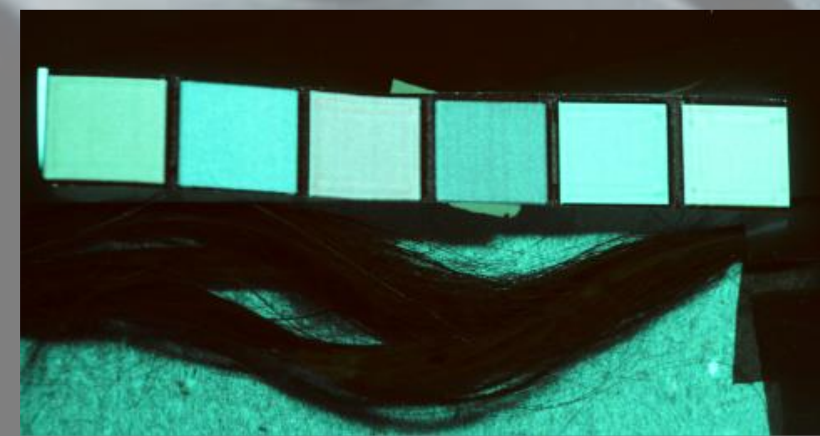
Skin sample at t_0, pre-STK



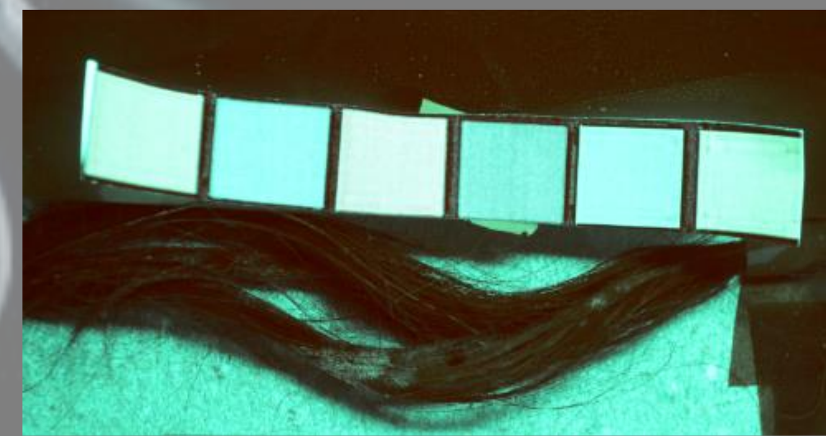
Skin sample at t_0, post-STK

The difference that occurs before and after the application of STK Skin is evident. While in the first condition, even though little time has passed since the deposition, the forensic scientist has not any idea about the location of a sample collection, even if appropriate ALS have been used. In contrast, after the STK application the areas of interest are well defined.

The same approach is being study for hair samples: this kind of surface, even more heterogeneous than the skin, will require a more in-dept application, in particular for the ability to distinguish the variability between bleached and un-bleached.



Hair sample at t = 20 days, pre-STK



Hair sample at t = 20 days, post-STK

CONCLUSION

Rapid and easy-to-use presumptive tests have been used for decades to identify the body fluid of interest at a crime scene, however the main challenge for forensic scientist is the evidence collection: the usefulness of a sample is inevitably linked to its ability to identify, and using of a tool like Sperm Tracker STK could make all the difference.

- The Sperm Tracker (STK) technique shows promise, with its STK Skin reagent preserving DNA and enhancing traces without re-nebulization.
- Exploratory analysis used pixel and image-level approaches, revealing STK's consistent efficiency across varying environmental conditions.
- Time-dependent trace color changes were noted, suggesting potential aging indicators.
- Limitations exist with RGB imaging, as trace colors may blend with the sample, warranting advanced imaging techniques like near-infrared spectrometry.

Future studies should focus on refining specificity, integrating precise segmentation, and expanding datasets. Implementing PCA directly at crime scenes or in Violence Prevention Centers could enhance trace detection. Additionally, future research should extend time intervals to study reagent functionality over longer periods

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