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## Nile Red – contrasting colour cyanoacrylate

### Summary

Fingerprints disclosed by cyanoacrylate on non-absorbent substrates, in order to improve readability, require additional contrasting fluorescent dyes. Nile Red is one of them. Its effectiveness was tested under conditions similar to those of daily laboratory practice. The highest Nile Red fluorescence occurred in an excitation light of blue-green and a wavelength of 505nm. Spectral analysis showed that the emission of light oscillates in the wavelength of about 630nm. In order to cut off the light, longpass edge filters of the colours yellow, orange or red can be used. Filter selection depends on the characteristics of the substrate and can be chosen empirically. Nile Red fluorescence does not change over a longer period of time, which allows for the registration of fingerprints to be performed within a time convenient for the testing, without fear of losing their quality. Nile Red may be an alternative to other fluorescent dyes used for visualization in fingerprint testing.

**Keywords** Nile Red, fluorescent dyes, fingerprints, spectrum, CONDOR system

### Introduction

Man has been interested in fingerprints from prehistoric times. The evidence of this are petroglyphs, pictures drawn into rock, preserved until today, showing, among other things, fingerprints. Likely the oldest petroglyph, portraying a hand with a drawing resembling the furrows of flexural lines and the patterns of fingerprints, is a petroglyph found on the shores of lake Kejimikujik in the Canadian province of Nova Scotia (Fig. 1).



Fig. 1. Lake Kejimikujik.

Fingerprint prints can also be found in many ancient documents. Sealing letters with a finger as

a confirmation of the validity of the document most probably had both legal and spiritual dimensions.

On the practical significance of the fingerprint, in the 17th century, the English botanist Nehemiah Grew drew attention, indicating that they improve the grip of the fingers. Grew also described the overall construction of fingerprints. He did not mention, however, the possibility of their use in the identification of individuals.

The origins of modern fingerprinting processes date back to the last decades of the 19th century. Research, among others, by Henry Faulds, William Herschel and Francis Galton established that fingerprints are individual, immutable and indestructible. These three properties became the foundation for identifying persons on the basis of the epidermal ridges of the fingerprints and hands.

Fingerprint traces for the first time were recognized as material evidence in the case against Thomas Jennings in a Chicago court in 1911. Jennings broke in to the house of Clarence Hiller, and Jennings, after encountering the homeowner, fatally shot Hiller. The killer, fleeing through a window, left four fingerprint traces from the fingers of his left hand on a freshly painted window frame.

There has been great improvement since that time in the capabilities of human identification on the basis of a fingerprint. In particular, a big breakthrough came in the possibilities of revealing fingerprints.



As visualization methods, modern forensic science laboratories employ a wide range of chemical, physical and optical techniques as well as technologically advanced equipment.

In practice, methods of revealing fingerprints are focused mainly on highly sensitive colour reactions of print revealing components with chemical substances. One of the most popular and most used in practice is the cyanoacrylate method. This method is used on non-absorbent substrates, e.g. glass, metal, plastic. It involves the polymerization of cyanoacrylate on the print surface. Cyanoacrylate esters of particles under the influence of the water contained in the sweat-fat (print revealing) combine to form a polymer of grey-white colour. Water is the catalyst for the reaction of a merger of smaller ester (monomer) molecules into larger polymer molecules.

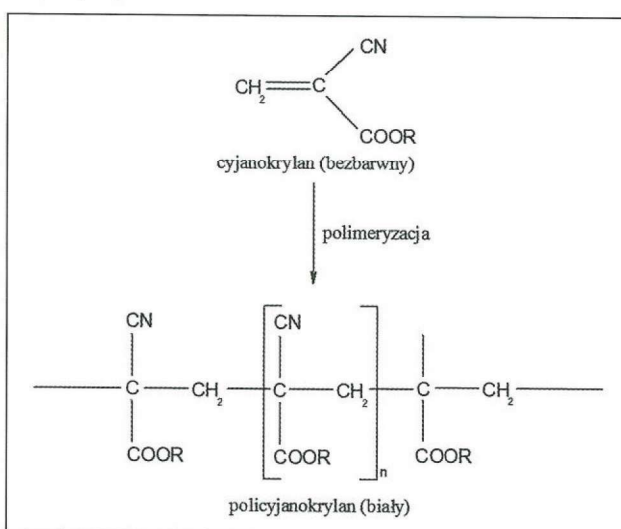


Fig. 2. Cyanoacrylate polymerization reaction scheme.

The cyanoacrylate method was developed by the Criminal Identification Division of the Japanese National Police Agency in 1978. In the 80s, the method was introduced to the practice in the United States and then in European countries. On account of the grey-white colour forming from the cyanoacrylate polymer, the contrast between the detected prints and the substrate is not always sufficient. In order to improve their readability, fluorescent dyes, e.g. Basic Yellow 40, Ardrex, Safranin O, Basic Red 28, are applied to the substrate. The dyes are absorbed by (deposited on) the cyanoacrylate polymer. During illumination of the tested surface with visible or ultraviolet light, the light energy is absorbed (consumed) by the fluorescent dye, whereupon it is emitted. The absorption of light causes the dye molecules to pass on to a higher energy state and a violation of equilibrium. Particles seeking to return to the ground state emit the absorbed energy. The amount of radiant energy is usually lower than the energy absorbed. Part of the absorbed energy is returned nonradiatively. According to Stokes' rule, the wavelength of the fluorescent radiation (energy

conveyed by particles) is greater than the wavelength of the excitation radiation, while lower the frequency. Thanks to Stokes' rule, we have the opportunity, through the use of appropriate filters, to cut away the radiation exciting the fluorescence of dye molecules and of observing only the light energy given back. Prints revealed by the cyanoacrylate, having absorbed a fluorescent dye, are apparent in the form of luminescent lines. Their colour depends on the used fluorescent dye. Prints may take various colours, e.g. yellow, orange, blue. Effectiveness of the fluorescent dyes, and hence an improvement in the readability of the fingerprint, depends on the adsorption of the dye and the intensity of its light. Dyes which to the highest degree full-fill the above criteria are still being sought.

For several years, the Dutch company BVDA International b.v., engaged in the sale of forensic science materials, has offered a fluorescent dye called Nile Red (chemical name: 9-diethylamino-5 h-benzo [a] phenoxazine-5-one, synonym: Nile blue oxazone).

The literature reported that the dye absorbs most of the blue-green light having a wavelength of 450 to 500 nm and emits light having a wavelength of 528 nm, the colour yellow-gold. In some cases, Nile Red absorbs light having a wavelength of 515-560 nm and emits light with a wavelength of 590 nm, the colour red.

The aim of this study was to confirm the possibility of using Nile Red fluorescent dye to contrast fingerprints revealed by cyanoacrylate.

## Research methodology

The research was conducted in accordance with the principles set out below:

### Substrates

Twelve typical non-absorbent substrates were selected, having different structures and colours (Fig. 3):

- Red plastic foil (office cover),
- green plastic foil (office cover),
- bright furniture slat,
- dark furniture slat,
- aluminium foil,
- metal plate covered with navy blue car lacquer,



Fig. 3. Substrates chosen for the preparation of test samples.



- zip lock plastic bag,
- black plastic bag,
- non-sticky side of blue adhesive tape,
- non-sticky side of brown adhesive tape,
- non-sticky side of grey mounting tape,
- plate of glass.

#### Research material – fingerprints

The fingerprints, left by a single donor (the person left fingerprints of good quality) on the non-absorbent substrates mentioned above, were revealed after a period of five days (sample stored at room temperature, humidity approximately 40%). The donor left one fingerprint on each of the prepared substrates. Before leaving the fingerprints on the substrate, the donor washed, carefully rinsed and dried his hands. Over the next forty-five minutes he did not use any hand cosmetics, did not intentionally touch any areas of the face/body, where the sebaceous glands are found, and carried out everyday activities.

#### Revealing the test fingerprints

The fingerprints affixed to the above mentioned substrates were exposed by the polymerization of cyanoacrylates and contrasted with fluorescent dye Nile Red.

Polymerization is carried out in a Foster + Freeman MVC 3000 with the following operating parameters: humidity 80%, temperature of the heating plate 100°C, physical vapour deposition time 5 minutes. After one day, a methanolic solution of Nile Red was applied to the sample, prepared according to the following recipe:

- 100 mg of Nile Red,
- 1000 ml of methanol.

The solution of Nile Red can also be prepared on the basis of organic solvents, such as ethanol, xylene, acetone, n-heptane.

The solution is stable for approximately 6 months.

#### Digital registration of the prints

The digital registrations of the prints were taken using a NIKON D700 camera with a AF Micro-Nikkor 60 mm

f/2.8D using long pass edge filters of the colours yellow, orange and red, cutting out the the fluorescence excitation radiation Nile Red.

#### Hyperspectral registration

The selected samples were recording using the hyperspectral imaging system CONDOR Macroscopic Chemical Imaging System™ equipped with a CCD camera and a LCTF liquid crystal tunable filter (liquid crystal tunable filter).

#### Fluorescence measurement

To measure the intensity of the fluorescence of the selected samples, the CONDOR system was used. The following measurements were made during system operation:

- range from 550 nm to 720 nm,
- fluorescence excitation light – blue green with a wavelength of 505 nm,
- spectral resolution 7 nm,
- CCD camera exposure times of 10 sec.

#### Excitation light source

- forensic illuminator MiniCrimescope, operating in the range of 300–720 nm, set to emit light cyan 505 nm and about 25% of maximum intensity.

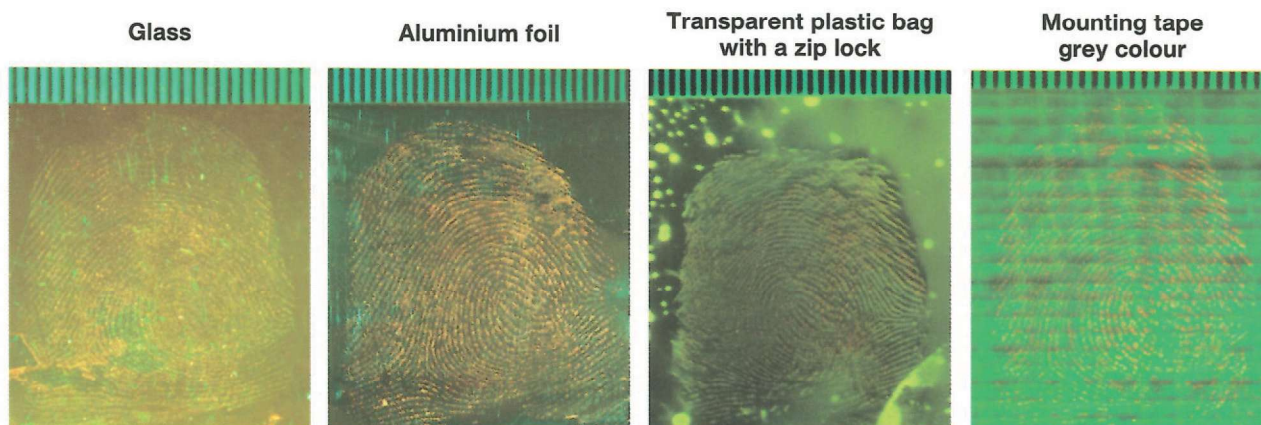
As a result, hyperspectral images of the samples were obtained, each consisting of dozens of snapshots.

#### Collection and verification of data

Preliminary studies were conducted in terms of visual light emission:

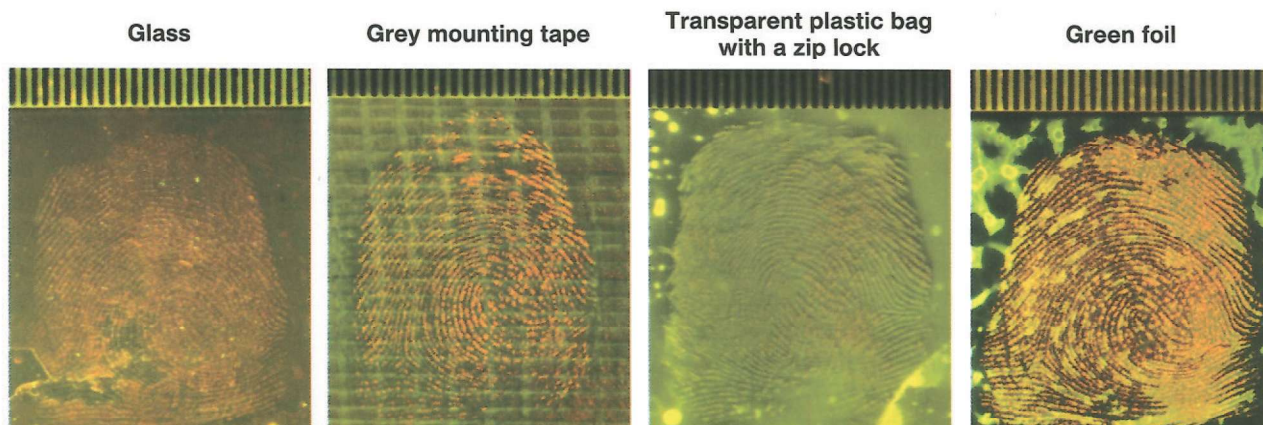
- blue-green with a wavelength of 480–530 nm, using filter: yellow (515 nm), Orange (550 nm) and Red (570 nm)
- red with a wavelength of 590 nm (no filter).

It was found that in the case of all the samples, the greatest intensity of fluorescence occurred during illumination of the prints with blue-green light having a wavelength of 505nm using longpass edge filters: yellow, orange and red. Sample images of the digitally registered fingerprints shown in figs. 4–6.

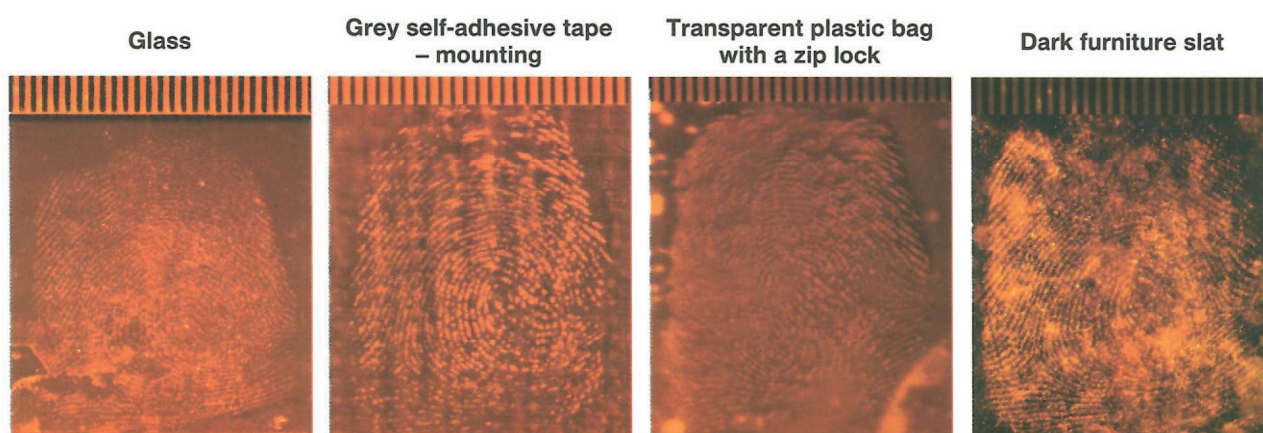


**Fig. 4.** Fingerprints captured photographically in blue-green light exciting fluorescence, using filter yellow.





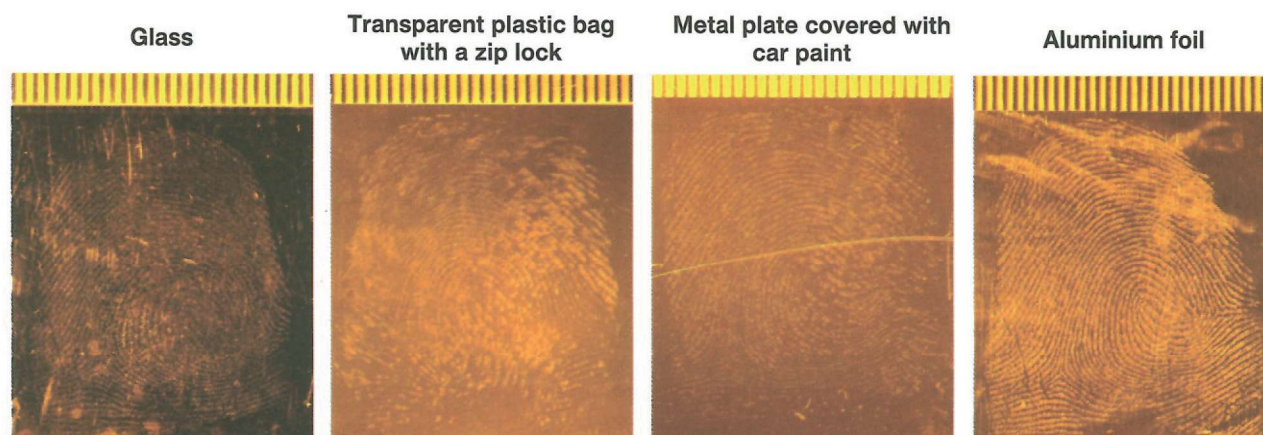
**Fig. 5.** Fingerprints captured photographically in blue-green excitation fluorescence using an orange filter.



**Fig. 6.** Fingerprints captured photographically, in the light blue-green excitation fluorescence using a red filter.

In the case of such substrates as glass, metal plate covered with a layer of paint, adhesive tape, and plastic bags of the colour blue, the reflection of red light (590 nm) has also been observed, allowing for good detection of fingerprints. The sample images of the recorded prints are shown in fig. 7.

The measurement of the fluorescence of the chosen research samples, using the CONDOR system, was conducted selecting the defined area within the limits of the trace and the substrate. The results obtained are shown in figs. 8–16.



**Fig. 7.** Fingerprints captured photographically in red light.



In the spectral image, the value of ROI 1 shows the emission of the chosen substrate point marked with a red plus sign, the value of ROI 2 is a graph of the emission of Nile Red at the selected point in the trace,

marked with a blue plus sign. The horizontal line of the XY system represents the wavelength emitted by the fluorescent dye and the substrate. The vertical line is the intensity of fluorescence expressed in units.

### Aluminium foil



Fig. 8. Print registered photographically.



Fig. 9. Print registered by the CONDOR system.

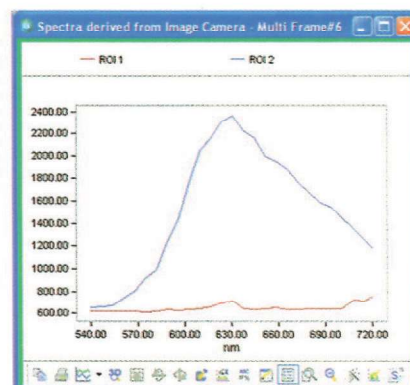


Fig. 10. Fluorescence spectrum of Nile Red.

### Metal plate covered with car lacquer



Fig. 11. Print registered photographically.

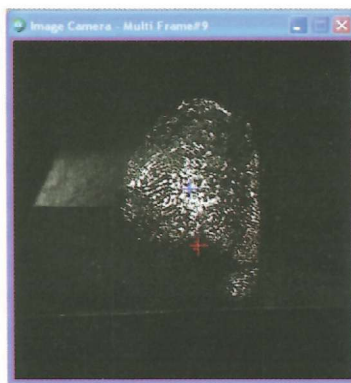


Fig. 12. Print registered by the CONDOR system.

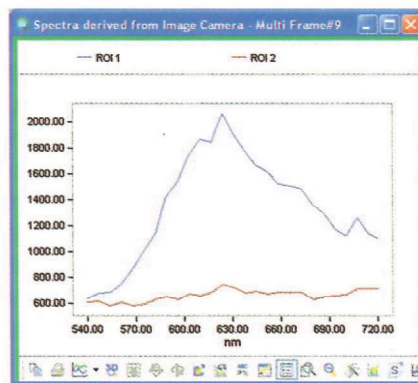


Fig. 13. Fluorescence spectrum of Nile Red.

### Non-adhesive side of brown adhesive tape

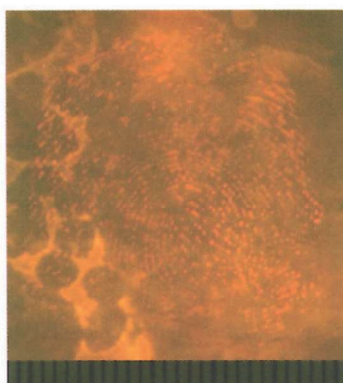


Fig. 14. Print registered photographically.

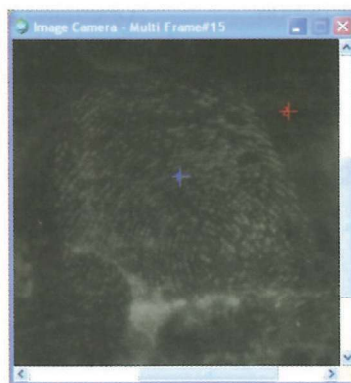


Fig. 15. Print registered by the CONDOR system.

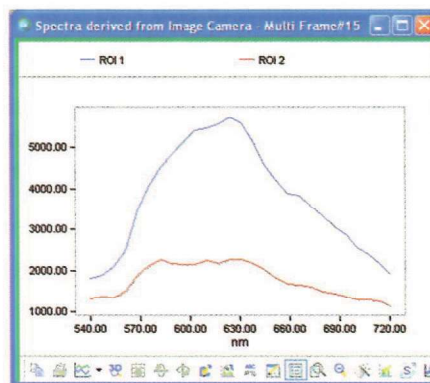


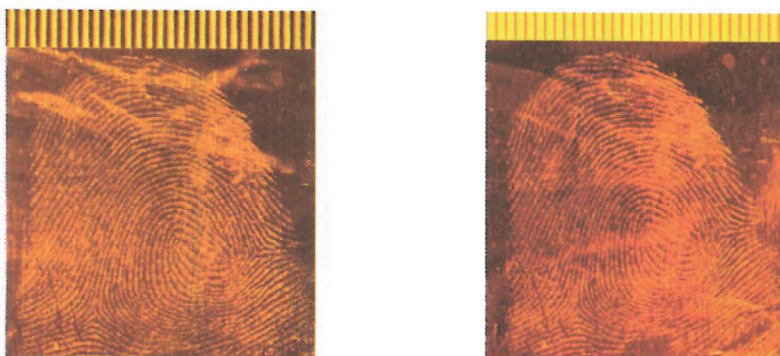
Fig. 16. Fluorescence spectrum of Nile Red.

### The persistence of fluorescence over time

The fingerprints revealed on the non-absorbent substrates by cyanoacrylate and contrasted with the fluorescent dye Nile Red were stored in the laboratory

for a period of three months. After a predetermined time, specimens were subjected to visual examination confirming that the fluorescence intensity remained unchanged (Fig. 17).

### Aluminium foil



**Fig. 17.** Fingerprint contrasted with Nile Red, recorded photographically directly after application of the dye (left) and after three months of storage (right).

### Conclusions

Nile Red fluorescent dye is effective for contrasting fingerprints revealed by cyanoacrylate on non-absorbent substrates. The highest fluorescence excitation occurs at a blue green light having a wavelength of 505 nm. Spectroscopic characteristics do not show major differences for the different non-absorbent substrates. On the basis of spectral analysis it can be concluded that the emission of light oscillates in the wavelength range of about 630 nm.

In order to cut off the light, longpass edge filters of the colours yellow, orange or red can be used. Filter selection depends on the characteristics of the substrate, and it can be chosen experimentally.

In the case of certain substrates, while examining in red light (without the use of inspection filters) quality fingerprint traces can also be obtained.

The fluorescence of Nile Red over a longer period of time does not change, allowing the implementation of the registration of prints at a time convenient for examination, without worrying about losing quality.

Nile Red may be an alternative to other fluorescent dyes used for visualization of fingerprint testing.

### Source

Figs. 1–17: authors

*Translation Ronald Scott Henderson*

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