# Structural-spectroscopic analysis of DFO/PVA films as potential materials used in revealing fingerprints on non-porous surfaces

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## Summary

1,8-diazafluoren-9-one (DFO) is a sensitive luminescent probe targeting α-amino acids. The study described herein exploited its physicochemical properties to form DFO/PVA polymer films. As a result, materials with stable and reactive DFO were obtained. Spectroscopic analysis of the formed films showed that the films with the highest concentration of DFO exhibited the highest reactivity. Novel materials in the form of thin DFO/PVA films with potential applications for revealing fingerprints on non-porous surfaces were obtained.

**Key words:** DFO/PVA polymer film, fluorescence, fingerprints

## Introduction

1,8-diazafluoren-9-one is an aromatic organic compound that forms a complex with α-amino acids to yield a fluorescent reaction product (Petrovskaia et al., 2001). Owing to its properties, it has been used since the 1990s as a fingerprint-revealing reagent for porous surfaces (D'Elia et al., 2015; Friesen, 2015). The DFO solution is prepared based on methanol and acetic acid because they enhance the effect of 1,8-diazafluoren-9-one on the fingerprints (Costa Conn et al., 2001). Very high concentrations of 1,8-diazafluoren-9-one at about 0.25 g/L (i.e., about $10^{-1}$ [mol/dcm$^3$]) are used to make the DFO solution (Bleay et al., 2018). The reagent is applied by spraying the trace or immersing the test surface in it (Ramotowski, 2013), and then heated to approximately 100°C for no more than 20 minutes (Browarny, 2014) – Figure 1.

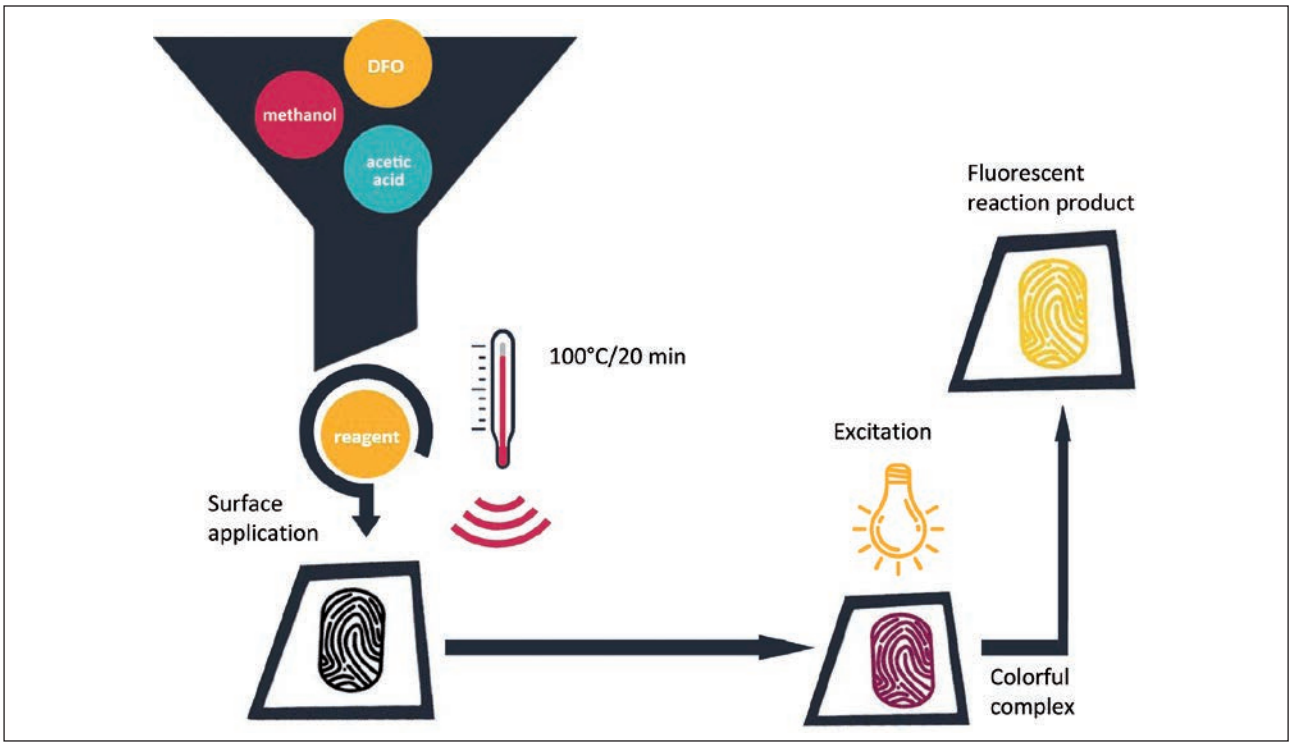



**Fig. 1.** Forensic procedure for revealing fingerprints using DFO.

DFO is a molecule with low fluorescence quantum yield (Lewkowicz et al., 2019). At the same time, this molecule is highly reactive and selective for α-amino acids, resulting in a product with high fluorescence quantum yield, which is the main pursuit of the current forensic procedure used to reveal fingerprints. Using very high concentrations of the main DFO reagent, however, may result in the generation of fluorescence by 1,8-diazafluoren-9-one associations rather than by the DFO-amino acid complexes. This phenomenon has been previously observed for other luminescent molecules, such as Rhodamine 6G (Lewkowicz et al., 2012; Lewkowicz et al., 2014). In the case of aromatic ketones, previous literature findings also indicate the presence of dimers of these molecules, formed as a result of cycloaddition to the C=C bond in the aromatic ring. This phenomenon is considered to be the effect of ketones interacting in the triplet state, for example, the dimerization of cyclohexanone involves a molecule in the $^3(\pi, \pi^*)$ state (Lam, Valentine, Hammond, 1967; Inhülsen, Kopf, Margaretha, 2008; Parthasarathy, Samanta, Ramamurthy, 2013). In this study, the DFO molecule was placed in a different carrier than the standard dactyloscopic solutions. A material that may be a promising matrix for DFO is polyvinyl alcohol (PVA). PVA is an odorless, colorless and non-toxic polymer. This biocompatible and biodegradable material with hygroscopic properties is widely used in medicine (Yang et al., 2021; Liu et al., 2014). Due to their ability to absorb water, PVA-formed films swell, allowing the molecule they contain to be released into the environment (Yang et al., 2021); presumably the same mechanism facilitates the absorption of molecules from the environment into the interior of the polymer.

## Aim of the study

The aim of this study was to obtain polymeric materials with high durability and selectivity towards α-amino acids and with potential application to both porous and non-porous substrates to reveal latent fingerprints.

## Materials and methods

*Equipment, supplies and chemical reagents*

All chemical reagents used in the study were of analytical grade purity. Spectroscopically pure (99% dye content) 1,8-diazafluoren-9-one, polyvinyl alcohol, and glycine were purchased from Aldrich (Sigma-Aldrich Munich, Germany). Deionized water was obtained from HydroLab system.

Raman spectra were obtained with the integrated confocal micro-Raman system from a LabRam Aramis (Horiba Jobin Yvon) 460 mm spectrometer. The excitation source was a diode-pumped semiconductor laser (DPSS) HeNe, emitting red light at 632 nm with a power of 50 mW. Absorption spectra were measured using a Shimadzu UVmini-1240 spectrophotometer. Fluorescence was excited using a UVITEC LF-206. LS LAMP 365/254NM 1X6W 230V EU UV lamp and an illuminator at an excitation wavelength of 465 nm. Emission spectra were obtained using a Horiba Jobin Yvon spectrofluorimeter, model FluoroMax4TCSPC.

*Preparation of PVA film with incorporated DFO*

Figure 2 shows a scheme for obtaining DFO/PVA polymer films. These films were formed by polymerization at room temperature and atmospheric pressure. PVA and DFO were dissolved in demineralized water. The following DFO concentrations in PVA films were obtained: $10^{-5}$, $10^{-4}$, $10^{-3}$, $5 \cdot 10^{-3}$ [mol/dm$^3$].

*Structural analysis*

Figure 3 shows the Raman spectrum for PVA film. The position of the Raman peaks on the spectrum are characteristic of the structure of this polymer, i.e. occurring between 750–1500 cm$^{-1}$ and 2500–3500 cm$^{-1}$ (Publicspectra.com; Thomas, Stuart, 1997).

The absorption spectra of the DFO/PVA films were measured at room temperature and atmospheric pressure. Figure 4 shows the absorption spectrum for the highest concentration of DFO in the PVA film and provides confirmation of the presence of 1,8-diazafluoren-9-one in the PVA matrix – a characteristic absorption band with a maximum at 385 nm. For the highest DFO concentrations ($10^{-3}$ and $5 \cdot 10^{-3}$ [mol/dm$^3$]), a second absorption band was observed with a maximum at 570 nm. The appearance of an additional band confirms the formation of new chemical structures, probably the so-called DFO aggregates.

*Impregnation of DFO/PVA film*

DFO/PVA films were impregnated with two solutions: water (blank) and $10^{-4}$ [mol/dcm$^3$] aqueous glycine solution. Each sample was immersed in the solution

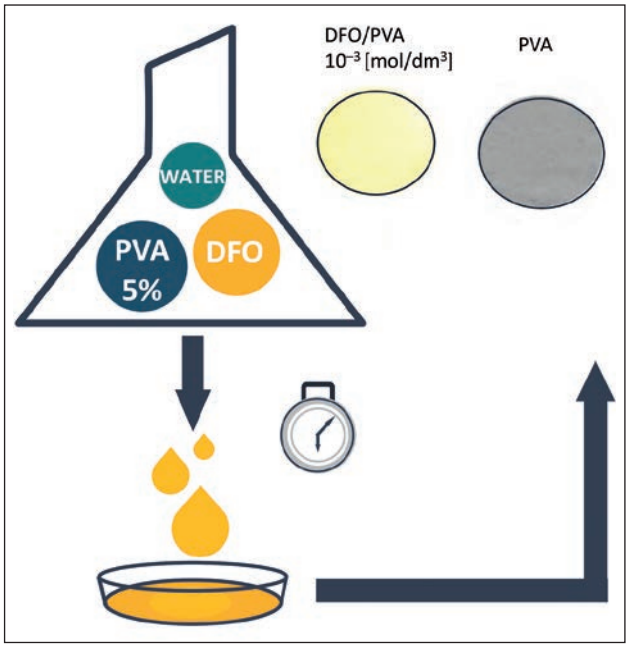



**Fig. 2.** Scheme for obtaining DFO/PVA polymer films.

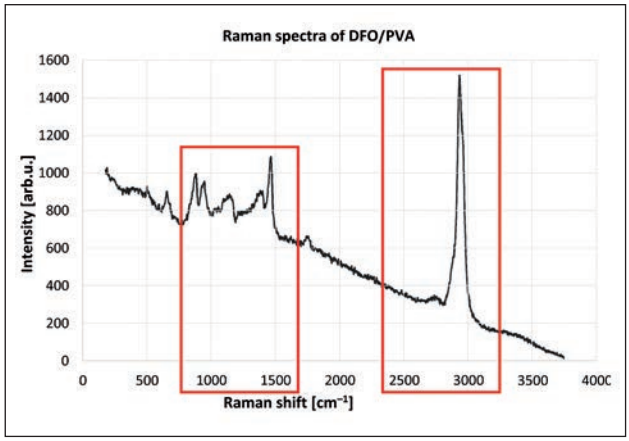



**Fig. 3.** Raman spectrum of DFO/PVA film.

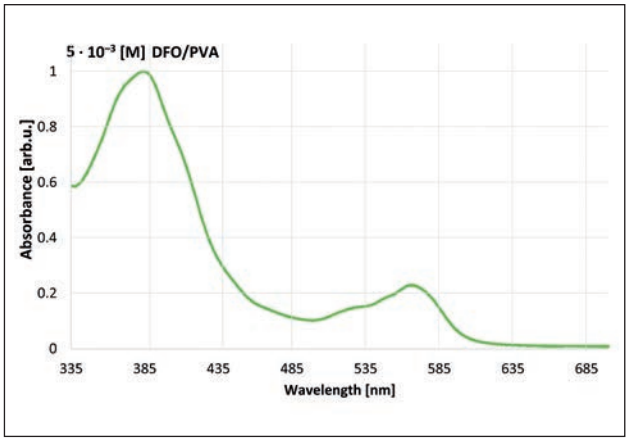



**Fig. 4.** Absorption spectrum of DFO/PVA film for $c_{DFO} = 5 \cdot 10^{-3}$ [mol/dm³].

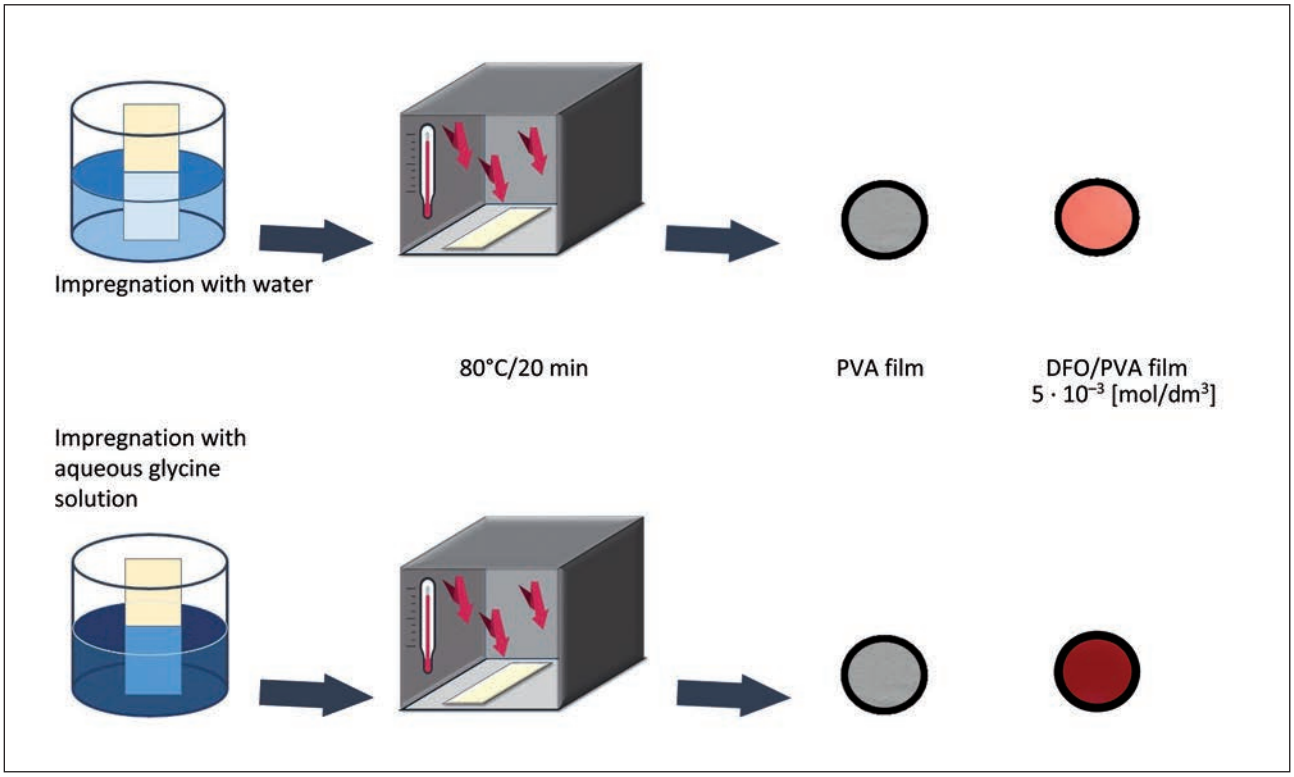



**Fig. 5.** Procedure for impregnation of DFO/PVA polymer films with an aqueous glycine solution.

for 30 seconds and then heated in an oven – Figure 5. DFO/PVA films with high concentrations, i.e. $10^{-3}$ and $5 \cdot 10^{-3}$ [mol/dm³], impregnated with aqueous glycine solution, changed their color to purple.

*Spectroscopic analysis*

After impregnation, the absorption spectra of the DFO/PVA films were measured in the spectral range of 250–700 nm, and next the absorption spectra of the films with the highest concentration of $5 \cdot 10^{-3}$ [mol/dm³] impregnated with water, with $10^{-4}$ [mol/dm³] aqueous glycine solution, and unimpregnated, were compared – Figure 6.

The absorption spectrum again shows two absorption bands for unimpregnated and water-impregnated sample. Three bands are visible in the glycine-impregnated sample. The two extreme absorption bands are common to all films: the first with a maximum at 385 nm and the last with a maximum at about 570 nm. The middle absorption band is characteristic only for the sample impregnated with aqueous glycine solution with a maximum at 470 nm – specific for the DFO-α-amino acids complex.

The samples obtained after impregnation were illuminated by a UV lamp at 365 nm and an illuminator at 465 nm. DFO/PVA films impregnated with aqueous

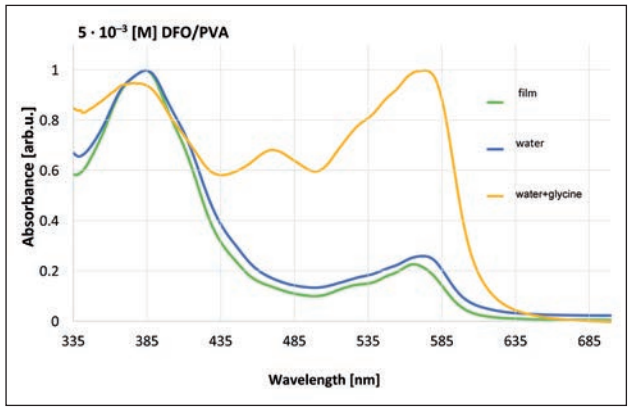



**Fig. 6.** Absorption spectrum of DFO/PVA film for $c_{DFO} = 5 \cdot 10^{-3}$ [mol/dm$^3$].

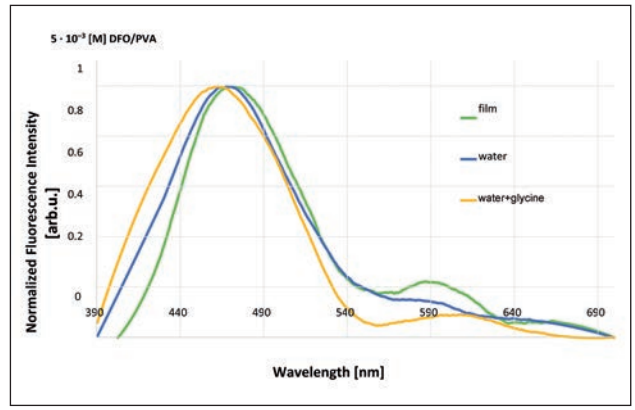



**Fig. 7.** Fluorescence spectrum of DFO/PVA films for the highest concentration of $5 \cdot 10^{-3}$ [mol/dm$^3$] – excitation at 380 nm.

glycine solution showed fluorescence for concentrations of $10^{-4}$, $10^{-3}$ and $5 \cdot 10^{-3}$ [mol/dm$^3$]. Fluorescence was also visible for the concentration of $5 \cdot 10^{-3}$ [mol/dm$^3$] DFO/PVA after impregnating the film with water.

DFO/PVA films impregnated with aqueous glycine solution show an increase in emission intensity with increasing concentration – Tables 1 and 2. At a concentration of $5 \cdot 10^{-3}$ [mol/dm$^3$], all the films exhibit similar emission, regardless of the method of impregnation. The feature that slightly differentiates the films is the color of the emitted light and the emission intensity. A yellow-orange color appears in samples excited at 365 nm as the concentration increases. The $5 \cdot 10^{-3}$ [mol/dm$^3$] DFO/PVA film impregnated with water illuminates yellow while the film impregnated with glycine illuminates orange. Samples with the highest concentration, impregnated with aqueous glycine solution, exhibit more intense color – orange-pink – when excited at 465 nm than when excited at 365 nm.

The fluorescence spectra of $5 \cdot 10^{-3}$ [mol/dm$^3$] DFO/PVA films for each type of impregnation are summarized in Fig. 7. Two bands of fluorescence are visible on the spectrum. The maximum of the first fluorescence band is at 460 nm for impregnation with aqueous glycine solution and it shifts in the long-wave direction to 470 nm for the other types of impregnation. No significant changes were observed in the fluorescence spectral profile of the first band. The second fluorescence band has a maximum at about 590 – 613 nm and it is best visible for unimpregnated DFO/PVA film.

According to the obtained absorption spectrum, the fluorescence of the films was measured at 470 nm excitation. The absorption band with this maximum differentiates the DFO-amino acid complexes and potential aggregates. The fluorescence spectra for each type of impregnation are summarized in Fig. 8. Three fluorescence bands are visible, of which one is best developed for the film impregnated with aqueous glycine solution with the fluorescence maximum at 606 nm. The structure of the remaining fluorescence bands originating from unimpregnated and water-impregnated films is very poorly delineated and comes primarily from DFO with a small contribution on the long-wave side of the fluorescence band coinciding spectrally with the DFO-glycine complex.

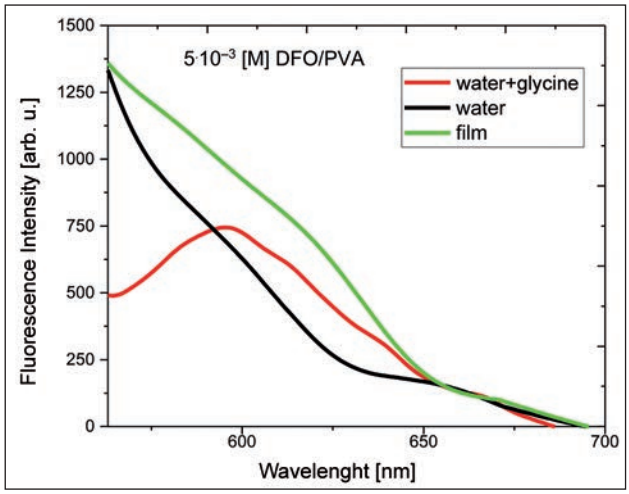
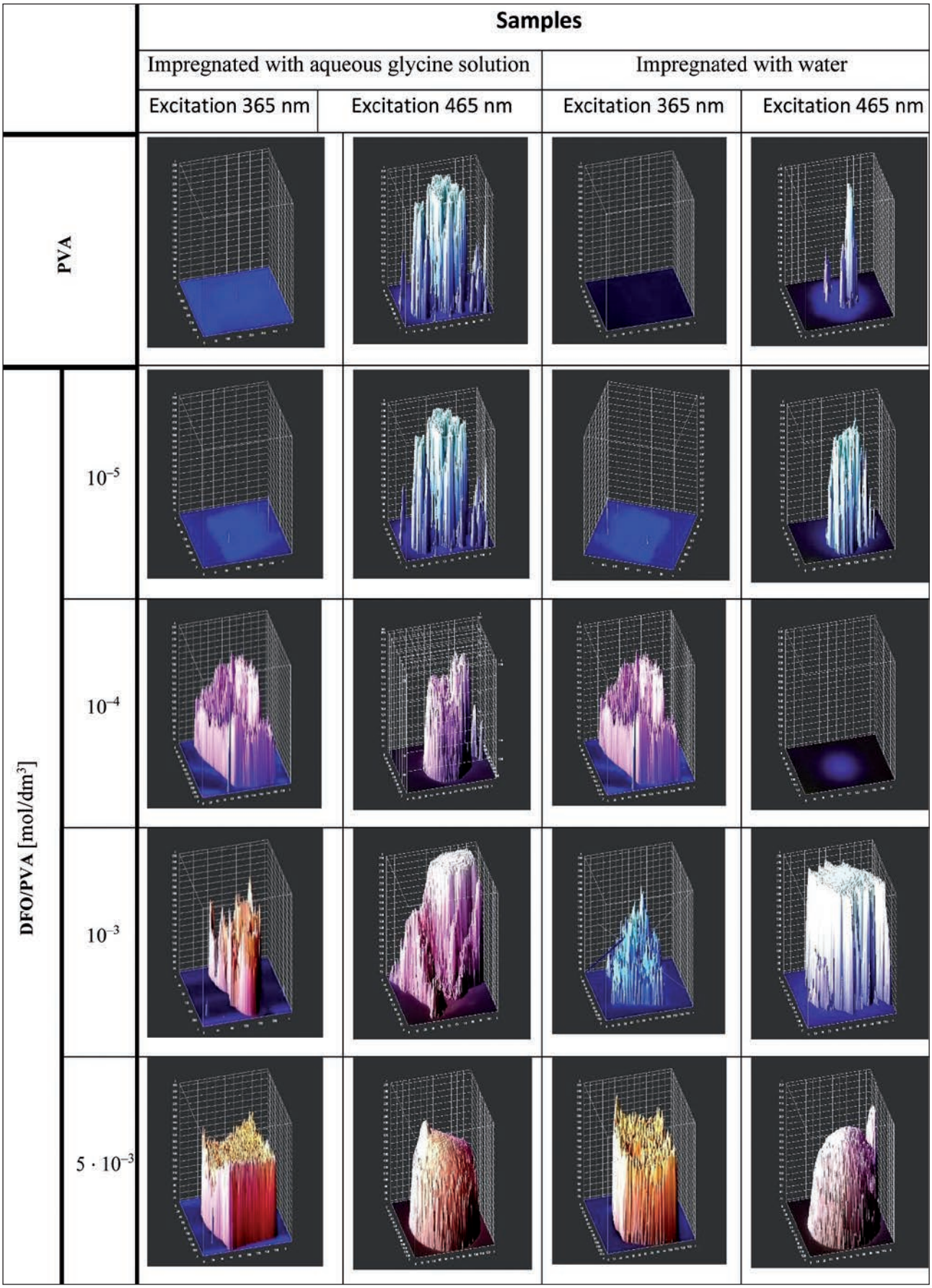


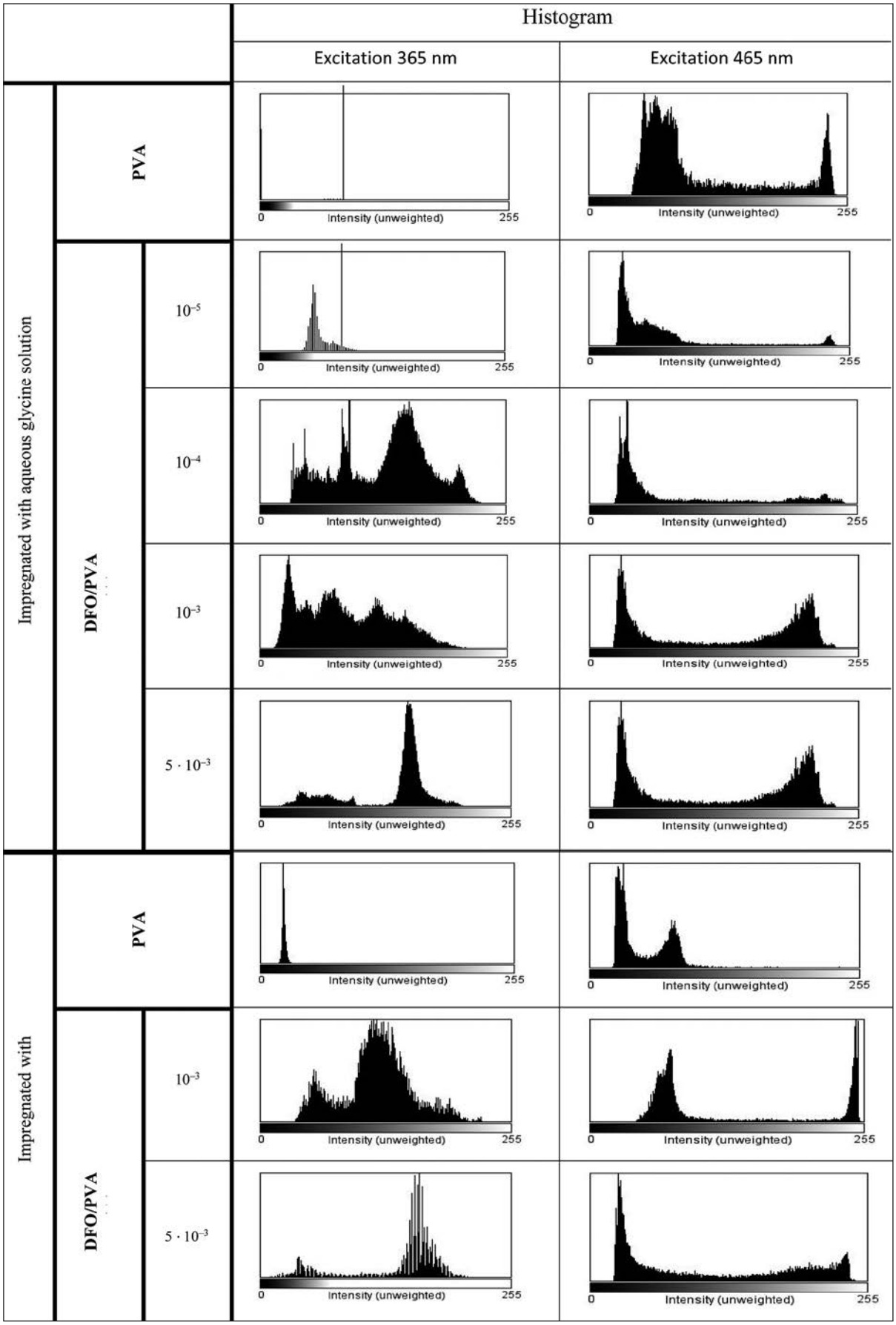

**Fig. 8.** Fluorescence spectrum of DFO/PVA films for the highest concentration of $5 \cdot 10^{-3}$ [mol/dm$^3$] – excitation at 470 nm.

## Summary and conclusions

The color change and fluorescence of DFO/PVA films after impregnation with aqueous glycine solution indicate the formation of DFO-amino acid complexes. These changes were observed in only three samples, and the limiting concentration of DFO/PVA for which the reaction occurred was $10^{-4}$ [mol/dm$^3$]. No reaction was observed in the sample with the lowest concentration of $10^{-5}$ [mol/dm$^3$] DFO/PVA. This has a theoretical representation in the recommendations of forensic procedures that provide for high concentrations of 1,8-diazafluoren-9-one in solution. The highest concentration of DFO used in the study was 0.09 g/L ($5 \cdot 10^{-3}$ [mol/dm$^3$] DFO/PVA). Forensic procedures report an amount of DFO >0.2 g/L ($10^{-1}$ [mol/dm$^3$]) as necessary for the reaction between DFO and amino




**Tab. 1.** A comparison of DFO/PVA samples after impregnation – indicated emission differences for 365 nm and 465 nm excitations.




**Tab. 2.** Histograms of DFO/PVA samples after impregnation – indicated emission differences for 365 nm and 465 nm excitations.

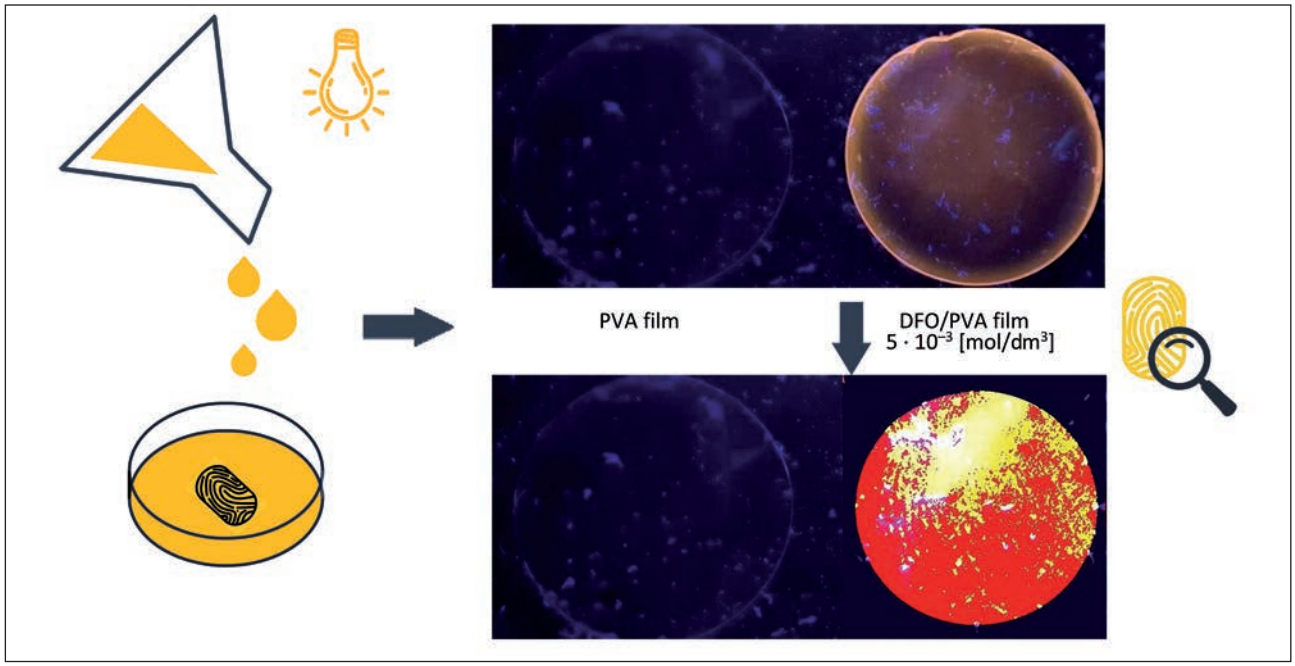



**Fig. 9.** Fingerprints revealed using DFO/PVA film – excitation at 365 nm.

acids to occur. This is almost twice the amount of 1,8-diazafluoren-9-one used in the present study. The lowest concentration at which a fluorescent complexes were observed was approximately 0.0018 g/L ($10^{-4}$ [mol/dm$^3$] DFO/PVA). This means that the concentration needed to form complexes with α-amino acids is much lower than that currently proposed.

During film preparation, a $10^{-3}$ [mol/dm$^3$] DFO/PVA solution was poured over the fingerprints imprinted on the plastic surface of the Petri dish. The polymerization process yielded a film permanently bonded to a legible luminescent fingerprint deposited on a non-absorbent surface. The DFO/PVA film (Fig. 9) confirms the occurrence of reaction between DFO and α-amino acids. The sample fluorescence was measured at 365 nm and 380 nm excitations; these wavelengths should specifically identify DFO fluorescence. Thus, according to theoretical assumptions, all samples containing 1,8-diazafluoren-9-one should illuminate, regardless of the type of impregnation. Under an illuminator with 365 nm excitation wavelength, not all DFO/PVA films showed luminescence. However, this wavelength was sufficient to observe emission for samples containing a DFO-α-amino acid complex (Tables 1 and 2; Figure 9) and for $5 \cdot 10^{-3}$ [mol/dm$^3$] DFO/PVA water-impregnated samples. When using a spectrofluorimeter and 380 nm excitation, fluorescence spectra were recorded for all samples containing 1,8-diazafluoren-9-one, regardless of the type of impregnation.

The films tested in this study were differentiated by the absorption spectrum shown in Fig. 6, in which the characteristic absorption band with a maximum at 470 nm in samples impregnated with aqueous glycine solution indicated the formation of a DFO-α-amino acid complex that was not present in other DFO/PVA films. In addition, all films, regardless of the type of impregnation were characterized by a similar structure of fluorescence spectra when excited at 380 nm (Fig. 6), while at 470 nm excitation a well-developed fluorescence band was observed only for the sample impregnated with aqueous glycine solution – Figure 8. Excitation of the film with a 465 nm illuminator resulted in emissions for DFO/PVA concentrations ranging from $10^{-4}$ [mol/dm$^3$] to $5 \cdot 10^{-3}$ [mol/dm$^3$] and impregnation with aqueous glycine solution. The fluorescent response for the above wavelength was is also seen in $5 \cdot 10^{-3}$ [mol/dm$^3$] DFO/PVA film impregnated with water – Table 1. The illumination of films lacking the DFO-α-amino acid complex, $5 \cdot 10^{-3}$ [mol/dm$^3$] DFO/PVA films impregnated with water, upon excitation at 465 nm (Tables 1 and 2), as well as the formation of additional bands shifted to the long-wave side of the absorption spectrum (Figure 6), suggest the presence of 1,8-diazafluoren-9-one associations in films with high DFO/PVA concentrations. In addition, the formation of DFO aggregates in the PVA matrix was confirmed by the intense fluorescence and similar color of the emitted light in samples with the highest concentration of $5 \cdot 10^{-3}$ [mol/dm$^3$] DFO/PVA, excited at 365 nm. This phenomenon was also confirmed by the identical fluorescence spectra of $5 \cdot 10^{-3}$ [mol/dm$^3$] DFO/PVA films excited at 380 nm, regardless of impregnation type.

1,8-diazafluoren-9-one contained within the matrices formed from PVA is stable and acts as a luminescent probe for α-amino acids. Impregnation of DFO/PVA films with aqueous glycine solution enables the formation of a DFO-glycine complex (Lewkowicz et al., 2020; Wilkinson, 2000; Kołek-Kaczanowska,

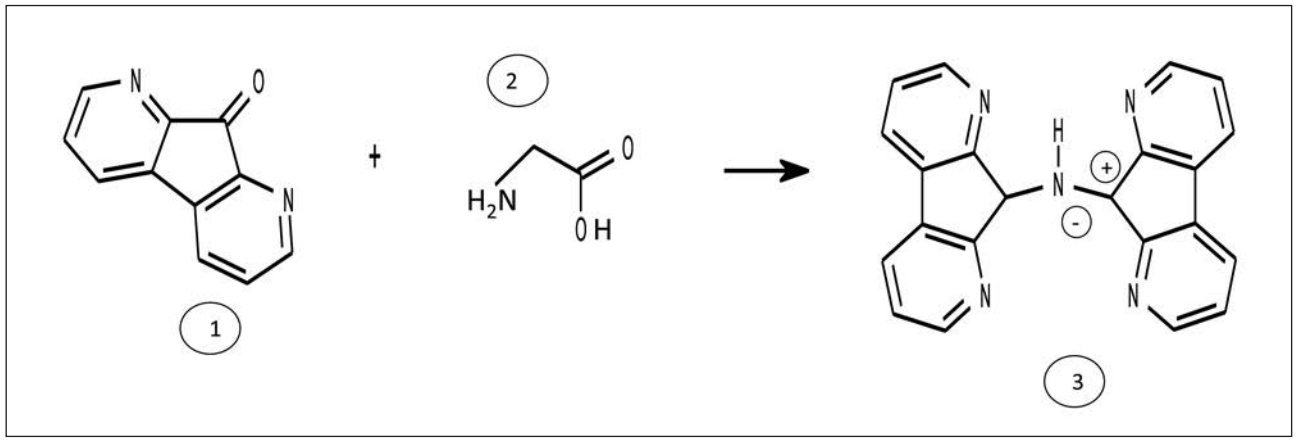



**Fig. 10.** Reaction of DFO (1) with -amino acid (2).

Kreczko, Maćkiewicz, 2014) – Figure 10. It should be emphasized, however, that only PVA films with the highest DFO concentrations exhibit sufficiently high reactivity and support the formation of DFO-α-amino acid complexes to be observed with the unaided eye. The obtained DFO/PVA polymers are α-amino acid probes, and the research findings presented in this article are preliminary with an indication of their potential application in revealing fingerprints.

**Source of Figures and Tables:** authors

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a) **odsyłacze do przywoływanych prac –** w przypadku powoływania się na prace innych autorów zawsze należy podać nazwisko autora/ autorów oraz rok publikacji.

   Przykłady:

   – **jeden autor:**

     Według Malinowskiego (2015)…

     W słowniku języka polskiego (Doroszewski, 1961)…

   – **dwóch autorów:**

     Według Widackiego i Dukały (2015)…

     W badaniach poligraficznych stwierdza się (Widacki, Dukała, 2015)…

   – **od trzech do pięciu autorów** – wszystkie nazwiska podajemy wyłącznie za pierwszym razem powoływania się na daną pracę w tekście, w kolejnych odsyłaczach podajemy wyłącznie nazwisko pierwszego autora oraz skrót „i in.”.

     Jak w swoim artykule wykazali Bajerlein, Wojterska, Grewling i Kokociński (2015)…

     We wspomnianym wyżej artykule Bejrlein i in. (2015) wykazali….

     Jak wykazały badania (Bajerlein i in., 2015)…

   – **sześciu autorów i więcej –** należy podać nazwisko tylko pierwszego autora, dodając skrót „i in.” oraz rok (za każdym razem – zarówno dla pierwszego, jak i następnych odsyłaczy).

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   „……..…” (Kowalski, 2016, s. 31)…

c) **bibliografię należy zredagować alfabetycznie w oparciu o podane przykłady:**

   Arntzen, F. (1989). *Psychologia zeznań świadków*. Warszawa: Państwowe Wydawnictwo Naukowe.

   Buller, D.B., Burgoon, J.K. (1996). Interpersonal Deception Theory. *Communication Theory*, *6*(3), 203–242.DOI: 10.1111/j.1468-2885.1996.tb00127.x

   Sweetser, E.E. (1987). The definition of lie: An examination of the folks models underlying a semantic prototype. W: D. Holland (red.), *Cultural Models in Language and Thought*. New York: Cambridge University Press.

   Widacki, J. (red.). (2012). *Kryminalistyka*. Warszawa: C.H. Beck.

16. Po zakwalifikowaniu pracy do publikacji z Autorem zostaje zawarta umowa o przeniesieniu na Redakcję autorskich praw majątkowych.
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7. Every submitted article is to be accompanied by an abstract (max. 150 words) and 3 to 7 key words.
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a) **references to other works –** in case of referring to works of other authors the name of author/authors and year of publishing should always be given.

   Examples:

   – **one author:**

     According to Malinowski (2015)…

     In Polish Language Dictionary (Doroszewski, 1961)

   – **two authors:**

     According to Widacki and Dukała (2015)…

     It is stated in Polygraph examinations (Widacki, Dukała, 2015)…

   – **three to five authors –** all the names are given only in the first instance of referring to a given work in the text; in subsequent references exclusively the name of first author and an abbreviation "et al."

     As Bajerlein, Wojterska, Grewling and Kokociński (2015) demonstrated in their article…

     In the article mentioned above Bajerlein et al. (2015) demonstrated…

     As research has shown (Bajerlein et al., 2015)…

   – **six and more authors –** the name of the first author followed by the abbreviation "and others" as well as the year should be provided each time for the first and subsequent links.

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   "……..…" (Kowalski, 2016, p. 31)

c) **Bibliography should be made in the alphabetical order basing on the following examples:**

   Arntzen, F. (1989). *Psychologia zeznań świadków*. Warsaw: Państwowe Wydawnictwo Naukowe.

   Buller, D.B., Burgoon, J.K. (1996). Interpersonal Deception Theory. *Communication Theory*, *6*(3), 203–242.DOI: 10.1111/j.1468-2885.1996.tb00127.x

   Sweetser, E.E. (1987). The definition of lie: An examination of the folks models underlying a semantic prototype. W: D. Holland (ed.), *Cultural Models in Language and Thought*. New York: Cambridge University Press.

   Widacki, J. (ed.). (2012). *Kryminalistyka*. Warszawa: C.H. Beck.

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# ZAPRASZAMY DO UDZIAŁU W WYKŁADACH SEMINARYJNYCH CENTRALNEGO LABORATORIUM KRYMINALISTYCZNEGO POLICJI

**Od 2012 roku Centralne Laboratorium Kryminalistyczne Policji prowadzi wykłady seminaryjne, podczas których biegli i specjaliści przezentują ciekawe zagadnienia z zakresu wybranych specjalności kryminalistycznych.**

Z uwagi na utrzymujący się stan zagrożenia epidemicznego podjęliśmy decyzję o zmianie formy organizacji wykładów seminaryjnych, które do odwołania będą odbywać się w ramach wideokonferencji, za pośrednictwem aplikacji Webex Meetings.

Wykłady mają charakter otwarty i prowadzone będą w godzinach 11:00–15:00 w terminach określonych w harmonogramie.
Osoby zainteresowane udziałem w webinariach proszone są o wysłanie zgłoszenia na adres e-mail **clkpw2@policja.gov.pl**.

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## HARMONOGRAM WYKŁADÓW SEMINARYJNYCH W 2021 R.

| L.p. | Temat wystąpienia | Prelegenci | Termin |
|---|---|---|---|
| 1. | Nanocząstki w daktyloskopii | mgr Ewa Rogoża, mgr Katarzyna Drzewiecka | 24 lutego 2021 r. |
| 2. | Wykorzystanie powszechnie dostępnych baz danych w identyfikacji pojazdów | kom. Robert Mróz, podkom. Krzysztof Biskup, mgr inż. Ewa Jędrych | 24 marca 2021 r. |
| 3. | Wirtualizacja systemów operacyjnych w ujęciu informatyki śledczej | st. asp. Marzena Holak, sierż. Rafał Czech | 21 kwietnia 2021 r. |
| 4. | Pilotażowe badania parametrów ruchu hulajnóg elektrycznych w zmiennych warunkach drogowych wraz z omówieniem przykładowego badania uszkodzonej hulajnogi elektrycznej | sierż. szt. Bartłomiej Pelc, st. sierż. Waldemar Pusty, mgr inż. Ewa Jędrych | 19 maja 2021 r. |
| 5. | System do określania stref bezpieczeństwa i zagrożeń związanych z wybuchem materiałów i przyrządów wybuchowych | dr Anna Trynda, podinsp. Wiktor Dmitruk, mgr inż. Łukasz Matyjasek | 23 czerwca 2021 r. |
| 6. | Akwizycja pamięci ulotnej w wybranych systemach rodziny Windows | st. asp. Marzena Holak, sierż. Rafał Czech | 8 września 2021 r. |
| 7. | Podciśnieniowa komora do ujawniania śladów linii papilarnych związkami organicznymi w fazie gazowej | podkom. dr Krzysztof Klemczak, dr Marta Olejniczak | 22 września 2021 r. |
| 8. | Charakterystyka chemiczna materiału roślinnego pochodzącego z konopi | dr Robert Bachliński | 13 października 2021 r. |
| 9. | Genetyczna identyfikacja narkotycznej odmiany konopi | podinsp. Żanetta Makowska | 17 listopada 2021 r. |
| 10. | Zastosowanie technik sekwencjonowania następnej generacji | podinsp. dr Ewa Kartasińska, dr inż. Kamil Januszkiewicz, mgr Michał Boroń, mgr Anna Woźniak | 8 grudnia 2021 r. |

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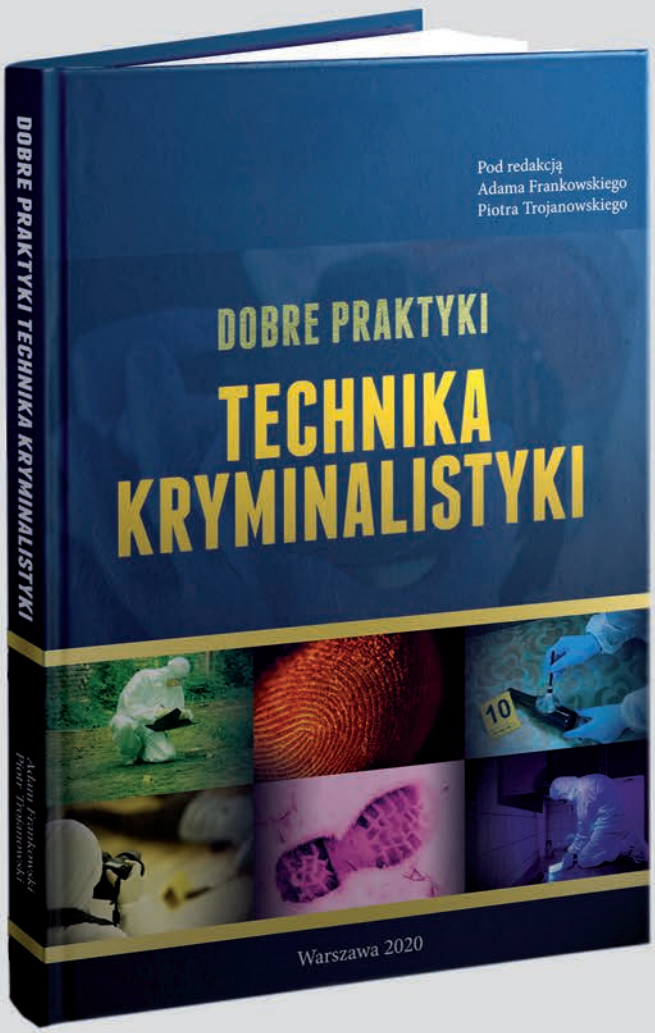


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