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## COMMUNICATION

## Aqueous Nile blue: A simple, versatile and safe reagent for the detection of latent fingerprints

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**Nile blue A in aqueous solution undergoes spontaneous hydrolysis to the photoluminescent compound Nile red. This reagent provides a simple and safe approach to the detection of latent fingerprints on porous and non-porous surfaces.**

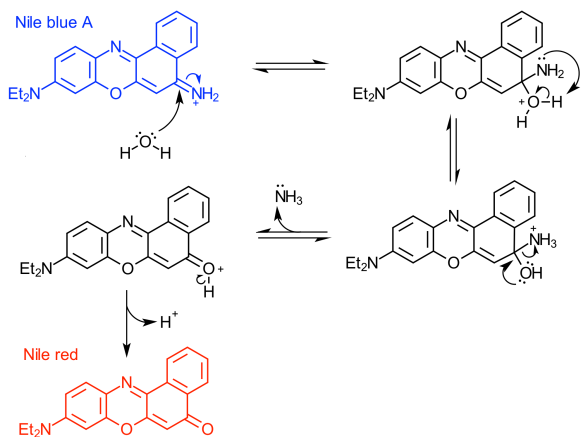
The detection of latent (invisible) fingerprints is highly significant in criminal investigations as a means of establishing connections between individuals, objects and locations. A variety of novel approaches to fingerprint detection have been developed in recent years, in an effort to improve the overall sensitivity of the fingerprint detection process.<sup>1-6</sup>

Detection of latent fingerprints on porous surfaces, such as paper, can be achieved using treatments that target compounds such as amino acids or lipids, that are derived from skin secretions.<sup>1, 7</sup> Commonly used amino acid-sensitive reagents, such as ninhydrin (2,2-dihydroxy-1,3-indanedione), 1,8-diazafluoren-9-one (DFO) and 1,2-indanedione, are not suitable for items which have been wetted or exposed to high humidity, due to the solubility of amino acids in water.<sup>8, 9</sup> Detection of the water-insoluble fraction of latent fingerprints may be carried out with the silver nitrate-based reagent physical developer, or the lipophilic dye Oil Red O (1-([4-(xylylazo)xylyl]azo)-2-naphthol).<sup>6, 10</sup> Oil Red O is ineffective at developing fingerprints on dark or patterned substrates, as the colours conferred upon fingerprints do not provide sufficient contrast. In a similar fashion, physical developer requires additional processing when used on such substrates<sup>11-13</sup>.

Recently the use of Nile red (9-diethylamino-5H-benzo[ $\alpha$ ]phenoxazine-5-one) has been reported as an approach

to the detection of recently deposited fingerprints on porous substrates that have been wetted. This treatment imparts both colour and photoluminescence to lipid-rich fingerprints, allowing their detection on dark and/or patterned surfaces.<sup>14</sup> However, as the operational use of this reagent would be performed by non-scientifically trained personnel, there are concerns regarding the toxicity of the organic solvent required (methanol) due to the poor solubility of Nile red in aqueous solution.<sup>15</sup> There is a general move away from using methanol and other toxic solvents in such situations.

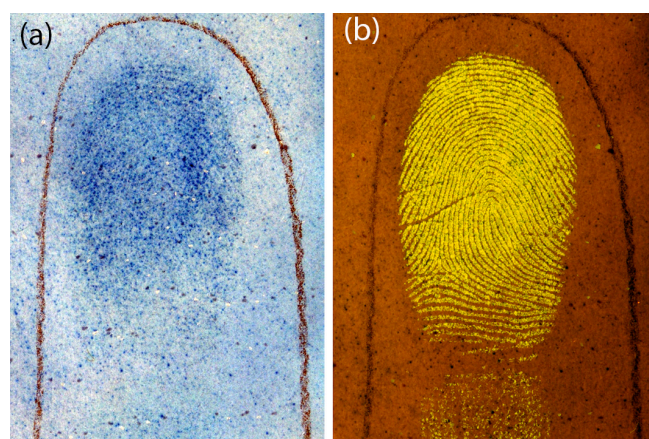
Nile blue A (benzo[ $\alpha$ ]phenoxazin-7-ium,5-amino-9-(diethylamino)-,sulfate (2:1)), commonly referred to as Nile blue<sup>†</sup>, is a basic phenoxazine dye employed in histology.<sup>16-18</sup> When prepared in aqueous solution, Nile blue A stains acidic components, such as phospholipids and nucleic and fatty acids, a dark blue colour, while neutral lipids, namely triglycerides, are stained pink or red.<sup>16, 17, 19</sup> Nile red is the corresponding phenoxazine of Nile blue A, and can be synthesised by refluxing Nile blue A in dilute sulfuric acid.<sup>17, 18, 20</sup> The spontaneous hydrolysis of Nile blue A also occurs in the aqueous Nile blue staining reagent (Scheme 1).<sup>20-22</sup> It is the presence of these two dyes in the one solution that gives Nile blue its dual staining capabilities: Nile blue A forms a salt linkage with acidic moieties, while Nile red dissolves preferentially into neutral lipids.<sup>17, 19</sup> It is accepted that the Nile red component is responsible for the photoluminescence emitted by tissues stained with Nile blue.<sup>20, 22-24</sup>



**Scheme 1** Proposed reaction pathway for the spontaneous hydrolysis of Nile Blue A to Nile Red in aqueous solution.

Nile red is present in the Nile blue histological stain in only trace amounts, but this is sufficient to provide colouration and photoluminescence in stained tissue sections.<sup>24, 25</sup> Nile blue A has previously been investigated in a forensic context as a powder or in alcoholic solution, for the detection of latent fingermarks<sup>26</sup>, including as a post-treatment stain for cyanoacrylate fuming of latent fingermarks<sup>27</sup>, and for the detection of latent lip prints.<sup>28</sup> Here we propose an aqueous Nile blue solution as a treatment for the detection of latent fingermarks that overcomes the issue of using an organic solvent for Nile red for latent fingermark development, while providing a second development reagent in Nile blue A itself.

Latent fingermarks were collected on white copy paper from five donors. For these preliminary experiments, donors were asked to rub their fingers on their face or scalp immediately prior to fingermark deposition in order to 'charge' fingermarks with sebaceous lipids. Preparation of the Nile blue reagent was based on the method described by Cain.<sup>16</sup> Nile blue A (5 mg; Aldrich; Sigma-Aldrich) was dissolved in deionised water (100 mL). The samples were immersed in the Nile blue solution for 20 minutes, before being briefly rinsed in deionised water. Samples were then blotted and left to dry on paper towels at ambient temperature. Both fresh and 24 hour old fingermarks were developed. As shown in Figure 1 and also in electronic supplementary information Figure S1, fingermarks on porous substrates treated with Nile blue appeared as very pale blue-purple impressions on a blue background, although frequently fingermarks were not visible under white light. Strong photoluminescence was exhibited under illumination from a forensic light source (Polilight® PL500, Rofin, Australia), with an excitation wavelength of 505 nm and viewed through an orange camera filter attachment (550 nm barrier filter). The excitation and barrier filter wavelengths are similar to those used to examine fingermarks treated with Nile red.<sup>14</sup> Additional fingermarks on paper were split and the resulting halves treated with Nile Blue and with Nile Red (prepared as described in Braasch et al.<sup>14</sup>)



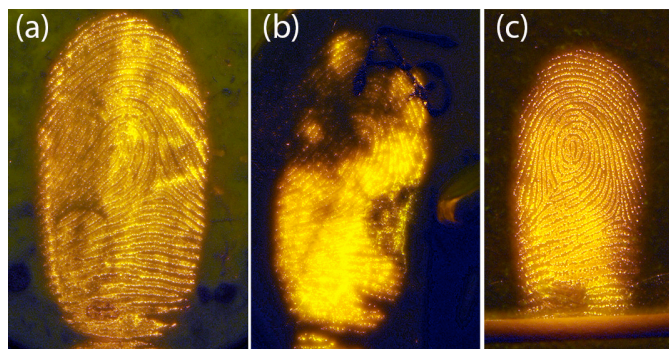
**Figure 1** Latent fingerprint on white copy paper treated with Nile blue, photographed using a Nikon D300 camera a) under white light (aperture f/8, shutter speed 1/20 s) and b) photoluminescence mode (excitation with a Polilight PL 500 at 505 nm excitation and viewed through a 550 nm barrier filter, aperture f/8, shutter speed 2.0 s).

The Nile Blue treated marks consistently gave better contrast, as those treated with Nile Red were effected by a higher level of background luminescence (Figure S1). It should be noted however that further studies are required with a wider range donors and substrates to establish the comparative performance of these two methods.

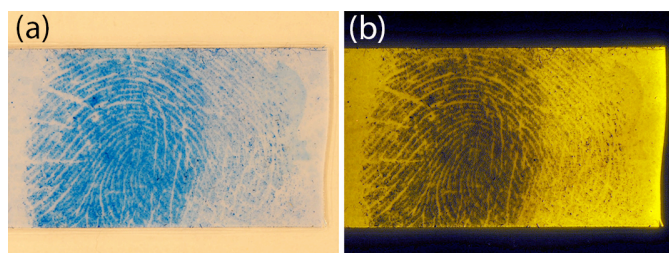
10  $\mu$ L aliquots of 1:1 linseed oil in hexane (Mallinckrodt Chemicals) were pipetted onto white copy paper, dried, and treated with Nile blue. These linseed oil spots all demonstrated strong photoluminescence, indicating that Nile red is present, as Nile blue A does not interact with triglycerides. Fluorescence spectra of fingermarks treated with Nile blue compared very closely with those treated with an organic extract of the aqueous Nile Blue solution, giving an emission maximum at approximately 560 nm with excitation at 490 nm (see electronic supplementary information Figure S2). This is consistent with the viewing conditions required for fingermarks treated with Nile red.<sup>14, 15</sup>

While Nile red is known to dissolve into neutral lipids, it is unclear what exact fingermark compounds are targeted by this reagent, as the linseed oil spots treated with Nile blue produced an emission maximum of 590 nm when excited at 490 nm. The photoluminescence emission and excitation maxima of Nile red are solvent-dependant, so it is possible that the compositional differences between linseed oil (triglycerides) and latent fingermark residue are responsible for this shift.<sup>29, 30</sup> High resolution mass spectra (HRMS) of an organic extract of the Nile blue reagent and a standard of Nile red provides further evidence that Nile red is present in the aqueous solution (see electronic supplementary material, Figure S2 and Table S2). Collision induced dissociation experiments confirmed the identity of Nile red in the sample extract by returning 4 characteristic fragments with the same accurate mass and similar relative abundance respect to those obtained from a standard solution, further confirming the presence of Nile red in the sample extract.





**Figure 2** Latent fingerprints treated with Nile blue on a) plastic lid; b) ceramic crucible lid; and c) glass microscope slide. Photographed using a Nikon D300 camera in photoluminescence mode (excitation with a Polilight PL 500 at 505 nm excitation and viewed through a 550 nm barrier filter, aperture f/8, shutter speed 1/10 s).



**Figure 3** Latent fingerprint on adhesive side of white electrical tape treated with Nile blue. Photographed using a Nikon D300 camera a) under white light (aperture f/8, shutter speed 1/20 s) and b) photoluminescence mode (excitation with a Polilight PL 500 at 505 nm excitation and viewed through a 550 nm barrier filter, aperture f/8, shutter speed 1/20 s).

In addition to the ability to visualise latent fingerprints on wetted paper surfaces, Nile blue was also found to develop latent fingerprints deposited on glossy, non-porous surfaces (Figure 2) and the adhesive side of electrical tapes (Figure 3). The visualisation of latent fingerprints on non-porous surfaces occurred in much the same manner as with porous surfaces. The mode of detection for fingerprints on adhesive surfaces appears to be significantly different, as fingerprints deposited on electrical tape appeared as blue, non-photoluminescent impressions against a highly photoluminescent background. In this case, it would appear that the Nile red component of the reagent partitions into the adhesive of the electrical tape, while Nile Blue A interacts preferentially with the fingerprint residue. When viewed under Polilight illumination, improved contrast between the fingerprints and the background was achieved compared to viewing under white light. Further work is required to determine whether this phenomenon is isolated to the type of adhesive involved here or whether this may be more widely applicable to adhesive tape in general.

The combination of Nile blue A and Nile red in a single aqueous solution enables fingerprint detection on a wider variety of surface types than is possible with Nile red alone. In addition Nile blue A is significantly cheaper than Nile red. The cost of Nile blue A used in this work to make 1 L of working solution is A\$0.30, as opposed to Nile red where the cost is

A\$30. The low toxicity, low cost and simplicity of the reagent creates additional potential for operational use in developing countries or in personnel training. Further investigations into the applicability of Nile blue to a wider range of substrate types, reagent sensitivity and efficacy on natural (uncharged) and older fingermarks are currently underway.

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### Notes and references

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† To avoid confusion, the term 'Nile blue' is used in this paper specifically in reference to the aqueous reagent. The dye compound itself is referred to as 'Nile blue A'.

Electronic Supplementary Information (ESI) available: Fluorescence spectra, high resolution mass spectrometry (HRMS) data. See DOI: 10.1039/c000000x/

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