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Title Page (with authors and addresses)

Volatilised Pyrene: A Phase 1 Study Demonstrating a New Method of Visualising Fingermarks with Comparisons to Iodine Fuming

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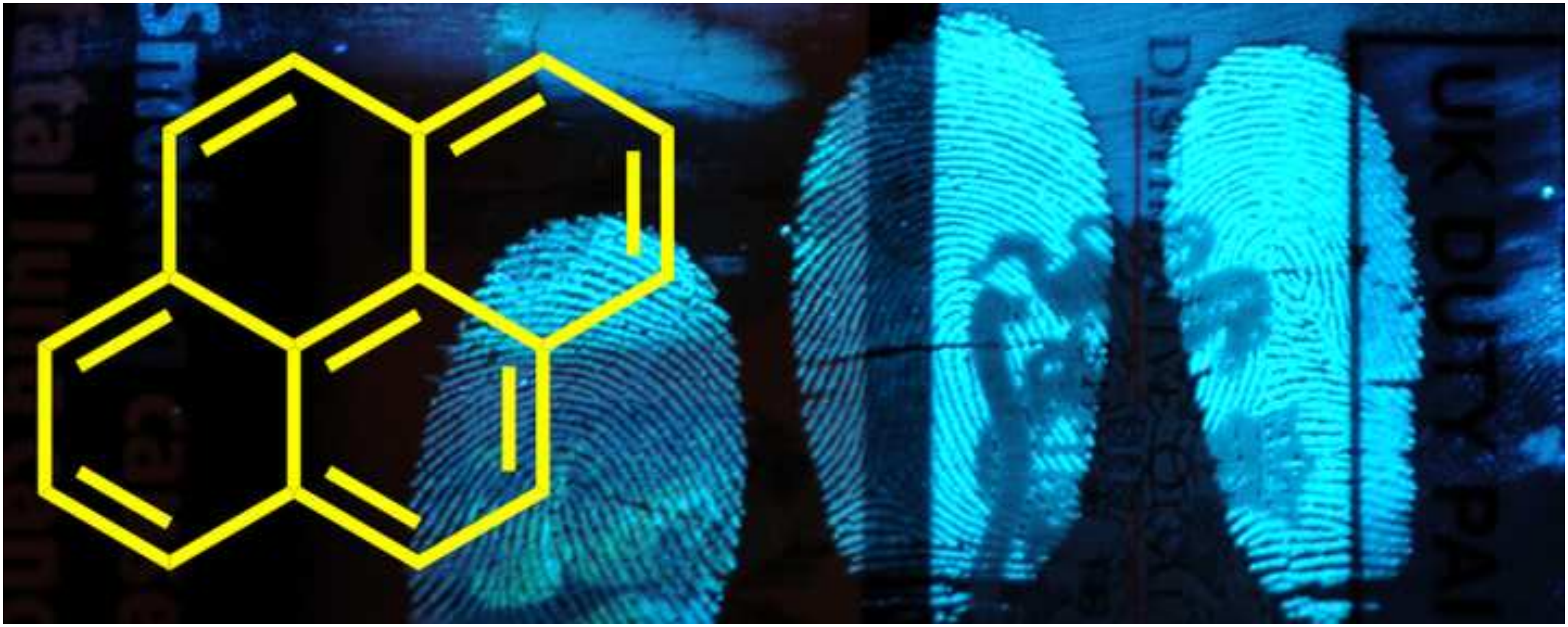
Graphical Abstract (for review)



*Highlights (for review)

- Pyrene fuming is a simple method of fingermark visualisation on nonporous surfaces
- Phase 1 trials demonstrate the method's effectiveness on aluminium foil and glass
- Pyrene-fumed fingermarks retain fluorescence for many hours
- Fumed pyrene appears to have affinity for the sebaceous components of fingermarks

Volatilised Pyrene: A Phase 1 Study Demonstrating a New Method of Visualising Fingermarks with Comparisons to Iodine Fuming

ABSTRACT: Pyrene is a fluorescent polycyclic aromatic hydrocarbon that can be volatilised under mild conditions. When fumed, pyrene is rapidly absorbed into the sebaceous residues of fingermarks, enabling their fluorescent visualisation upon excitation with ultraviolet radiation. This new means of fluorescent fingermark detection is more sensitive than the non-fluorescent iodine fuming approach for nonporous surfaces. This is demonstrated here in a phase 1 study using split-print comparisons on metal and glass surfaces. Pyrene-treated fingermarks also retain the volatile fluorophore for comparably long time periods relative to iodine fuming (in the order of hours). The phase 1 study comprised four donors, and 80 natural fingermarks that were grouped into two time periods; aged 24 hours and 1 week. Iodine fuming was chosen as a reference to showcase the effectiveness of pyrene given it is the most closely-related chemical fuming method in routine use. This study demonstrates that pyrene fuming increases the quantity and quality of fingermark visualisations relative to iodine fuming, and is free of many of the latter method's drawbacks. Preliminary results shown here also show the effectiveness of pyrene fuming on highly patterned surfaces, and its compatibility with the use of gelatine lifters. Pyrene fuming is thus easy to effect, low-cost, and shows great promise as a new means of visualising fingermarks on non-porous surfaces.

1. Introduction

Developing methods that clearly visualise latent fingerprints (*i.e.* fingermarks) is of fundamental importance to forensic science. This is because fingermarks are capable of establishing links between specific individuals, objects, and locations. Given the invisible nature of most fingerprint evidence, coupled with the widely variable scenarios in which fingermarks are found, there is a need to refine and develop new visualisation techniques that broaden the capabilities of forensic practitioners who perform this task [1]. New fingermark visualisation treatments typically target a single characteristic of the fingermark, be it a physical or chemical attribute. As a methodology, fuming broadly targets the chemical attributes of fingermarks, and is undertaken in multiple established techniques such as iodine fuming, and cyanoacrylate fuming [2]. The latter method could be made fluorescent by subsequent dye staining [3,4], and more recently inherently luminescent cyanoacrylate fuming reagents, such as Polycyano UV, have been developed that enable one-step fluorescent fuming [5,6]. Recently newer, more experimental means of fuming have been investigated, such as ninhydrin sublimation [7], lanthanide complex sublimation [8], and sublimation of purely-organic fluorophores such as 9-fluorenone [9]. This premise was established by Almog and Gabay who explored fuming of fluorophores such as anthracene and perylene to visualise fingermarks using UV radiation, with a particular emphasis on development of paper substrates [10].

Pyrene is a polycyclic aromatic hydrocarbon (PAH) with a long history of use in the fields of host-guest chemistry, photochemistry, and electrochemistry [11–21]. In host-guest chemistry, book chapters have been dedicated solely to the properties of pyrene, making it one of the most well-characterised PAHs known [11]. It emits long-lived fluorescence in either the monomeric, or excimeric form, dependent on its molecular environment [12,13], and the absorption and emission behaviour of each pyrene form is well characterised [14]. The excitation spectrum of the monomeric form has maxima at 241, 273 and 335 nm, and an emission spectrum spanning 375 – 405 nm. An additional maximum is seen at *ca* 460 nm associated with excimer formation should it occur [15]. Solid-state photoemission of pyrene crystals is dominated by a static excimer form, with a broad emission feature from 390 – 650 nm, and a maximum at 485 nm [16]. These emission characteristics result in solid pyrene exhibiting blue fluorescence upon excitation with ultraviolet (UV) radiation in the range of 253 – 375 nm. In solution studies, pyrene delivers a quantum yield of 0.32 [14], however this value is lower in the solid-state owing to reduced fluorescent efficiency of the excimer form [17]. These photo-properties have led to pyrene being incorporated into many luminescent materials and dyes [18–21], and make pyrene an exceptionally promising candidate for implementation in new fingermark development treatments. With the exception of a study exploring powder formulations containing pyrene [22], limited work has been done in this area.

The volatility of pyrene is similarly well-characterised owing to past investigations of common combustion processes, such as the burning of plant material (in particular tobacco), combustion of coal, oil and gas, and the cooking of meat. Many of these, as well as more recent studies looking at biochar [23], were undertaken to assess the environmental impact that results from the release of pyrene. The enthalpy of sublimation for pyrene has been measured at 103.25 ± 2.05 kJ/mol^{-1} [24], and the vapour pressure of pyrene measured between 50 – 150 °C [25,26]. These values provide a useful guide for optimising the temperature range when assessing the volatility of pyrene as part of a fuming study. Vapour phase processes, coupled with the inherent lack of chemical reactivity of pyrene, are attractive for fingermark visualisation because they reduce the likelihood of reaction with constituents in fingermarks residues. Inhibition of reactivity between fingermark and developer may be beneficial for subsequent processes such as further treatments in a detection sequence, or analytical approaches for determining fingermark age [27].

Of the established fingermark treatments, iodine fuming is chemically the closest analogue to pyrene fuming, recommending it as a comparative methodology. Iodine fuming is one of the oldest chemical techniques for visualising latent fingermarks [2,28], and is performed by subliming solid iodine crystals to produce iodine vapour, which is then delivered to the fingermark. Upon encountering fingermark residue, iodine fumes are transiently absorbed, resulting in a yellow / brown staining. Iodine fuming is thought to target the sebaceous components of the fingermark, and given the nonpolar nature of pyrene, this same preference is likely shared by pyrene fuming. Iodine fuming is typically combined with a secondary treatment, such as a solution of 7,8-benzoflavone (α-naphthoflavone) [29,30], which serves to fix the iodine within the fingermark following chemical reaction [31,32]. The fixing agent also generates a coloured product, thereby also improving the contrast of the iodine fuming method. Iodine fuming is classed as a Category B process according to the Fingermark Visualisation Manual [33], with niche uses for fragile and valuable items, or for rapid evaluation of large surfaces at a crime scene. While iodine fuming is typically applied to porous surfaces, there have been reports of the method being successfully applied to nonporous surfaces such as brass [34]. Iodine fuming is most effective for fingermarks aged up to one week, and is notably less efficient for fingermarks aged beyond two weeks, however in combination with added water vapour, its effectiveness can be expanded to include fingermarks that are months-old [35].

This work serves to showcase the effectiveness of pyrene fuming as a means of visualising fingermarks relative to iodine fuming in a phase 1 study. It should be noted that this work is preliminary, and that further work is needed to evaluate the detection sequence, expand the number of substrates and donors, and fully validate this method prior to implementation in an operational setting. However in spite of this, pyrene fuming was found to reliably and rapidly impart highly-visible blue fluorescence in treated fingermarks placed on a range of nonporous surfaces, and this fluorescent response could persist for multiple days post-treatment. The pyrene methodology is simple to effect, the cost of reagents is minimal [36], and the number of detections and average CAST (Centre for Applied Science & Technology) scores [37] of the pyrene treatment were consistently better than that of iodine fuming for natural fingermarks aged one or seven days.

2. Materials and methods

2.1. *Chemicals*

Pyrene (98%) was purchased (£10 / gram) in the United Kingdom from Fluorochem. HFE-7100 (95%) was sourced from Fluorochem. Iodine beads (99.999%), and α-Naphthoflavone (98%) were sourced from Sigma Aldrich. Dichloromethane (AR grade) was purchased from Fisher Scientific. All chemicals were used as received.

2.2. *Safety considerations*

According to the Safety Data Sheet [38], pyrene is toxic to the environment and aquatic life, however it is not listed as a known carcinogen, and there is minimal evidence for toxicity in humans. Pyrene is rapidly absorbed through the skin, hence standard protective equipment should be worn as a minimum, and is it recommended that fuming be undertaken in a sealed fuming chamber. It is important to note that the SDS information provided relates the use of pyrene as a solid, not in the gas phase. Items treated with pyrene should be handled in a well-ventilated area, or on a downdraught bench.

The use of UVA radiation to visualise treated fingermarks can cause damage to eyes and burn skin, particularly from high-power sources. Appropriate eye and face protection that incorporates UVA filters should be worn, and exposed skin should be covered with personal protective clothing. Where possible, engineering and administrative controls should be put in place to protect against accidental UVA exposure.

2.3. *Substrates*

A split fingermark study was performed on two pristine surface types, glass (Thermo Scientific™ microscope slides), and metal (Fisherbrand™ aluminium foil). Other surfaces given a preliminary evaluation during this study included a ceramic tile, polypropylene plastic, a CD label, a glossy cigarette packet, a brass bullet casing, and the United Kingdom £5 polymer note.

2.4. *Collection of latent fingermarks*

Four fingermark donors were used in this study, including one weak, two medium, and one strong donor. The donors comprised three males and one female, three of which were aged 20-24 years, one of which was aged 30-34 years. It was ensured that donors did not have contact with food or chemicals, had not worn gloves, and had not washed their hands in the preceding one hour prior to depositing their fingermarks. The fingermarks were obtained in a natural condition, in that no grooming to increase the about of sebum on the fingerpads was performed. The donors were instructed to sequentially apply their fingerpads to separate areas of the dictated surface type with light pressure.

2.5. *Phase 1 laboratory trials*

Each of the four donors contributed twenty fingermarks in two batches of ten, collected over two days. This pool of 80 fingermarks was divided into two groups according to date of collection, one of which was aged for 1 day prior to treatment, the other was aged for 7 days prior to treatment. The upper aging time of 7 days was chosen to approximately match the upper limit that iodine fuming is typically considered to be effective, with some sources recommending 3-5 days [2,28]. This degree of aging is thus well suited to enable a new method to demonstrate greater efficacy relative to iodine fuming. Each fingermark was divided in two lengthways for aluminium foil in the case of the metal surface, or was laid across two side-by-side microscope slides for the glass surface. The two halves of the fingermark were then separated and subjected to a unique visualisation treatment, pyrene for one half, iodine fuming for the other. This provided 80 fingermarks divided so that half were pyrene fumed and compared against the other half that were iodine fumed, of which 40 had been aged 1 day, and 40 had been aged 7 days. This study has endeavoured to follow recommendations of the International Fingerprint Research Group for phase 1 Projects [39]. The preliminary nature of this research recommends caution when judging these findings, given that further validation studies are required to optimize and benchmark this methodology prior to implementation in an operational capacity.

2.6. *Fuming procedure with pyrene*

Given that pyrene is known to exhibit environmental toxicity, a fuming chamber was used to contain the pyrene fumes during treatment (See ESI, Fig. S1). The fuming chamber contained a hotplate, on which a glass beaker was placed containing solid powdered pyrene (0.2 g). In a typical experiment the pyrene in the beaker was heated to 75 °C, and the surface to be fumed placed across the opening of the glass beaker, orienting the fingermark downward to face the pyrene. Provided the hotplate and glassware were at 75 °C, the fuming process was found give excellent results after only 10 minutes for fingermarks aged 7 days, with shorter fume times possible for more-recently deposited fingermarks. A video showing the entire process is provided as supporting information. The developed 24 hour old fingermarks were typically visible by eye, however the 7 day aged fingermarks often required oblique lighting to observe evidence of the fingermark.

2.7. *Fuming procedure with iodine*

Iodine fuming was also conducted in a fuming chamber, located in a fume hood. Iodine crystals were sublimed at 60 °C and the vapour applied to the fingermark. After a few minutes the fingermark was quickly (<1 minute) transferred to a photography station and documented prior to noticeable loss of absorbed iodine from the residue.

2.8. *Fixing treated iodine prints with 7,8-benzoflavone*

Where fingermarks were treated with 7,8-benzoflavone (α-naphthoflavone) solution post-iodine fuming, that solution comprised: 0.030 g 7,8-benzoflavone dissolved in dichloromethane (1 mL), which was then diluted with HFE-7100 to a volume of 10 mL [2]. The resulting solution was allowed to stand for a few minutes before being filtered, and was finally applied to the fingermarks as a fine spray using a 200 mL Spray Bottle sourced from kiloline PRO.

2.9. *Gellatine Lifts*

A gel lift of a pyrene-treated palm mark was performed using Gellifters Black sourced from BVDA.

2.10. *Documentation of treated fingermarks*

Samples were photographed using a Nikon D300 camera coupled with an AF-S DX Micro NIKKOR 40 mm Lens without the use of filters. Pyrene fingermarks were illuminated using a LUMATEC Superlite S04 portable forensic light source set to the UVA wavelength range of 320–400 nm, which was further filtered to 320–350 nm using a HS0350 Shortpass Filter (see ESI for transmission profile) obtained from Asahi Spectra. Iodine fingermarks were documented while illuminated with white light (400–700 nm).

Rapid screening and imaging of pyrene-treated fingermarks was also conducted using a Foster+Freeman VSC4CX video spectral comparator. An excitation wavelength of 313 nm was found to promote the strongest fluorescent emission from pyrene, thereby maximising contrast.

2.11. *Evaluation of fingermark quality*

Treated fingermarks were evaluated using the CAST grading scheme [37], which is summarised below in Table 1. The data from all the donors was averaged within each study and depicted graphically. Standard deviations have not been included owing to expected variation in quality of fingermarks between donors, which is consistent with other studies of this type [40].

Table 1: CAST grading scheme used in this work [37].

Grade	*Detail Visualised*
0	No evidence of a fingermark
1	Some evidence of a fingermark
2	Less than ⅓ clear ridge detail
3	Between ⅓ and ⅔ clear ridge detail
4	Over ⅔ clear ridge detail

3. Results and discussion

3.1. *Optimisation of pyrene fuming conditions*

The reported vapour pressure data for pyrene in the range of 50 – 150 °C provided a useful guide for assessing pyrene's suitability as a fingermark reagent [25,26]. This study commenced with an evaluation in the range of 25 – 75 °C, as milder conditions may have wider applicability for fragile evidence types. Fresh fingermarks were laid on an aluminium foil surface, which was then suspended over a bed of pyrene crystals heated to the desired temperature. Exposing the fingermark to pyrene heated at 75 °C for 10 minutes resulted in excellent visualisation upon exposing the treated print to 313 nm UV radiation (Fig. 1). The temperature was next lowered to 50 °C which resulted in a slight decrease in fluorescent intensity from the treated fingermark, which could likely be offset by longer fume times. Attempting to fume at 25 °C, even with a greatly extended fuming duration of 18 hours, resulted in both reduced and uneven uptake of pyrene across the fingermark. However when the sublimation temperature of pyrene was supressed by conducting the fuming experiment under vacuum (4.0×10^{-1} mbar), similar results to the 75 °C experiment were obtained in 10 minutes. Given the ease of conducting pyrene fuming without vacuum equipment, the optimal conditions used in this study were to heat pyrene at 75 °C with a 10 minute exposure time, unless otherwise stated.

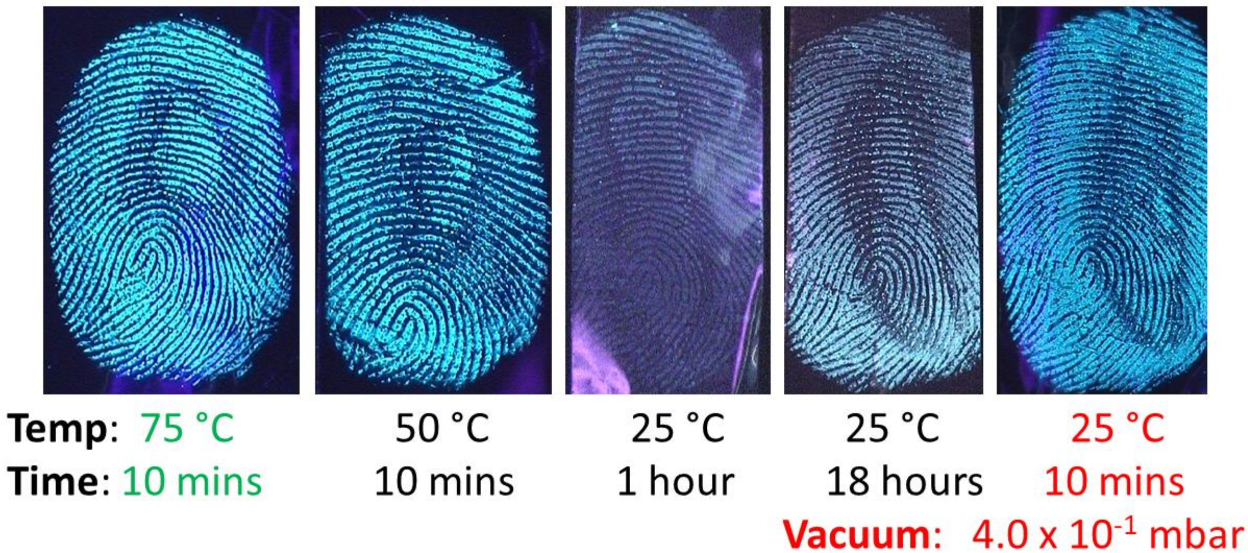


Figure 1. Optimisation of pyrene fuming conditions using fresh fingermarks on aluminium foil, and visualised with 313 nm UV radiation. The optimal fingermark fuming treatment (highlighted in green) was exposure to pyrene fumed at 75 °C for 10 minutes. Heating at 25 °C was insufficient to deliver good visualisation, unless vacuum conditions were also applied (highlighted in red).

To better understand the affinity of pyrene towards specific fingermark components, an aluminium foil surface bearing residues of squalene, glycine, alanine, and sodium chloride were fumed using the optimised conditions. Squalene, which here is acting as a simulant for sebaceous secretions, showed clear evidence of pyrene uptake, with a small amount of uptake also observed for the nonpolar amino acid alanine (See ESI, Fig. S3). This result strongly suggests that the nonpolar fingermark components are responsible for the affinity for volatilised pyrene.

3.2. *Preliminary evaluation of surface types*

With optimised fuming conditions determined, a preliminary evaluation of various surface types using fresh fingermarks was undertaken to narrow the focus of the phase 1 study. This included a range of nonporous and semi-porous surfaces inspired by items potentially found at crime scenes (Fig. 2). Both nonporous and semi-porous surfaces yielded promising preliminary results using fresh fingermarks, however the nonporous surfaces provided exemplary results for prints that had been aged. The treatment of polymer surfaces, particularly the polymer £5 note, declined in effectiveness once the fingermarks were aged beyond 24 hours, which may be due to polymer surfaces being more prone to absorbing the nonpolar pyrene molecules, leading to greater background fluorescence. Consequently, depletion of the fingermark residue with time will have a more noticeable adverse effect on contrast for polymer surfaces. Some porous surfaces were also evaluated (paper, cardboard), which showed indications that pyrene fuming was effective, however the fluorescent emission properties of pyrene closely match those of optical brighteners used in paper manufacture, hence the usefulness of this method is greatly diminished for these material types (see ESI, Fig. S4).

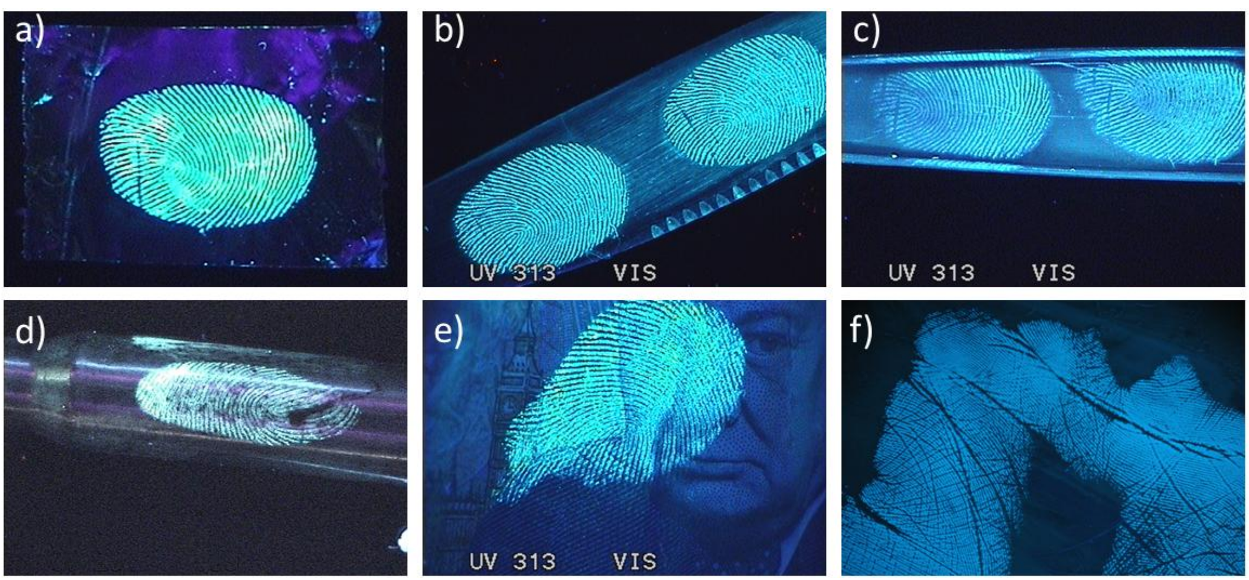


Figure 2. A selection of archetypal surface types bearing a fresh fingermark(s) (a-e) or palm print (f) and treated using the optimal pyrene fuming conditions. a) aluminium foil; b) stainless steel knife blade; c) polypropylene spoon handle; d) brass bullet casing; e) UK £5 polymer note; f) ceramic tile. In each instance the fingermark was fumed with pyrene heated at 75 °C for 10 minutes and visualised with 313 nm UV radiation.

These preliminary results guided the selection of metal (aluminium foil) and glass (microscope slides) as surfaces for investigation during the phase 1 study. Using glass slides simplified creation of split-print comparisons and provided a high degree of clarity when documenting the pyrene and iodine fuming results.

3.3. *Pyrene phase 1 study*

A phase 1 study was conducted to ascertain the effectiveness of pyrene under controlled conditions. The study made use of natural fingermarks, *i.e.* fingermarks not artificially charged with sebaceous or sweat deposits, that had been aged 1 or 7 days prior to investigation. Details of the donors and deposition methods are provided above. Two pristine nonporous surfaces were investigated by the study, glass and metal, in the form of microscope slides and aluminium foil.

As mentioned previously, split fingermarks treated with pyrene were compared against those treated by iodine fuming, it being the most closely-related established method to the new method under investigation. Iodine fuming as a methodology has drawbacks, such as facile loss of absorbed iodine after fuming, and a need for secondary chemical treatment with a fixative such as benzoflavone [29,30], both of which may be overcome by using pyrene as an alternative. Figure 3 compares pyrene and iodine fuming in terms of number and quality of visualisations on metal and glass surfaces across all the donors, inclusive of both aging periods. These results show that pyrene was particularly effective for metal surfaces, with 90% of the 80 fingermarks classed in the range of 3-4 according to the CAST scale, and consistent detection of the fingermark. The percentage classed 3-4 dropped to 66% when the metal surface was substituted for glass, with the decline equating to a drop in fingermark quality that was made up for in the

CAST 1-2 range (28%). Pyrene was thus found to consistently outperform iodine on both glass and metal, with the iodine fuming more frequently resulting in instances of no evidence of visualisation, and approximately double the number of marks assessed in the 1-2 range at the expense of the higher-quality 3-4 range.

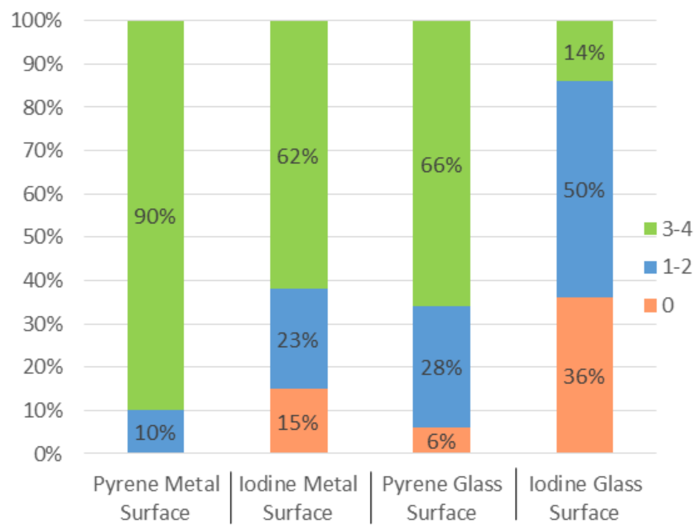


Figure 3. Collated results of all donors comparing the effectiveness of pyrene fuming against iodine fuming on metal and glass surfaces as an overall fraction of CAST score (0 = no evidence of a fingermark; 1-2 = Evidence of a fingermark but less than ⅓ clear ridge detail; 3-4 = Between ⅓ and full visualisation of fingermark ridge characteristics).

Figure 4 expands the analysis of average CAST scores by separating the data for prints aged 1 and 7 days, and also partitioning by surface type. Each category is derived from 40 fingermarks per fuming method, sourced from 4 donors. Again pyrene fuming was found to give consistently higher average CAST scores relative to iodine fuming in each category. Pyrene fuming typically yielded an improvement of *ca* 1 on the CAST scale relative to its iodine counterpart, with a slight decline in effectiveness for fingermarks aged 7 days (↓0.13 on metal, ↓0.20 on glass). A similar decline with aging for iodine fuming was observed on metal surfaces (↓0.30), however an unexpected increase in average CAST score was observed for the glass surfaces (↑0.52), which appeared to stem from an anomalously weaker set of 1 day aged fingermarks sourced from the weak donor that could be visualised with the pyrene treatment, but not with the iodine treatment. This was not seen for the 7 day aged prints, resulting in suppression of the 1 day iodine average CAST grading for glass.

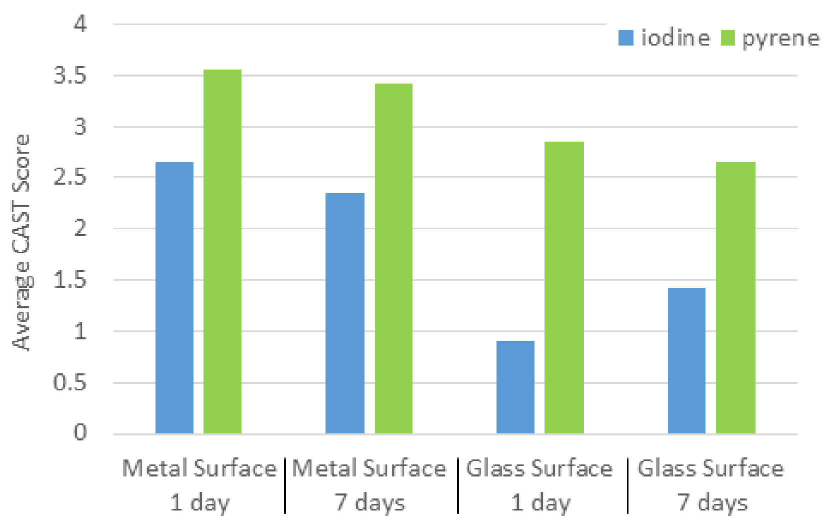


Figure 4. Average CAST scores comparing pyrene fuming (green bars) against iodine fuming (blue bars) showing the influence of surface type (metal, glass), and fingermark aging (1 day and 7 days).

Figure 5 shows representative examples sourced from both metal and glass surfaces to demonstrate typical visualisations produced by strong, moderate and weak donors during this work. Four sets of three fingermarks are provided, showing each surface type and aging period. Each set has a representative strong donor sample (left), a moderate donor sample (centre), and a weak donor sample (right). Close inspection of Figure 5 shows possible evidence of pyrene over-development in certain ridge patterns, such as for the strong donor on glass slide aged 1 day, or the weak donor on aluminium foil aged 7 days, however the possibility that these marks were smudged upon deposition also cannot be excluded. Certain semi-porous surfaces like plastics were found to be susceptible to pyrene over-development, possibly because these materials have more permeability towards pyrene, resulting in enhanced background emission leading to reduced contrast (See ESI, Fig. S5).

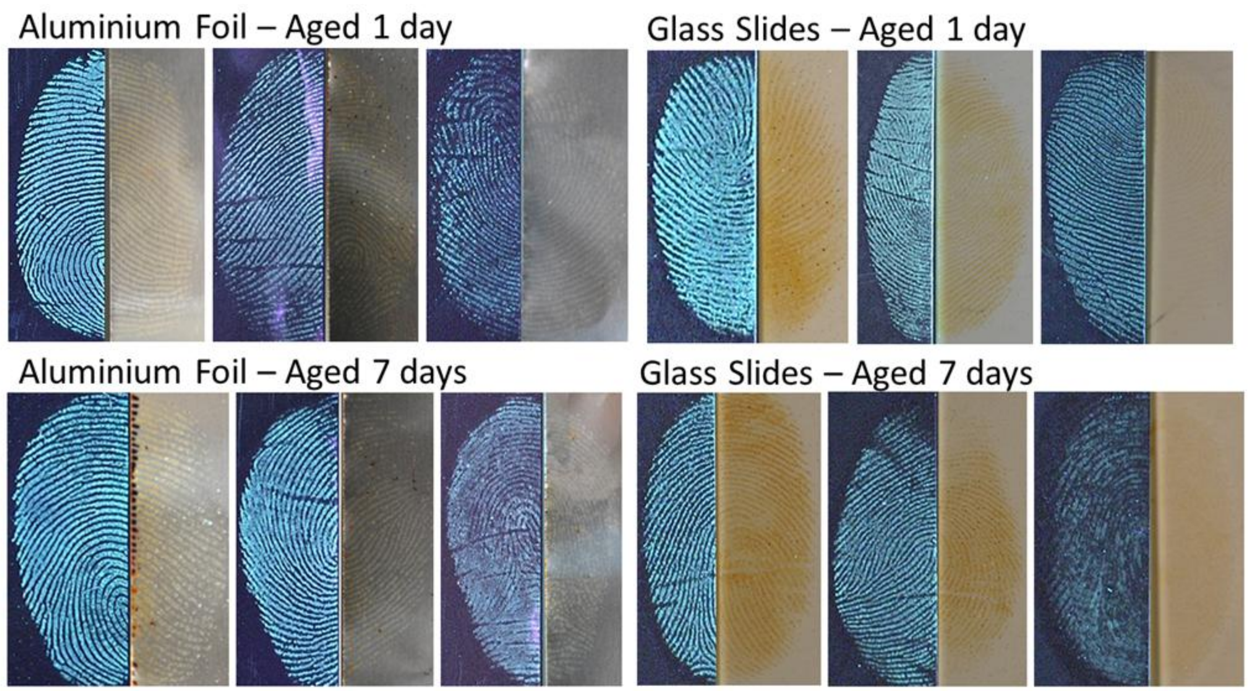


Figure 5. Four representative sets of three split fingermark images (Aluminium foil aged 1 & 7 days; Glass slides aged 1 & 7 days). Each set shows a strong donor (left), a moderate donor (centre), and a weak donor (right).

3.4. *Comparison of pyrene treatment to benzoflavone-fixed iodine treatment*

One major benefit of the pyrene treatment is that it requires no secondary treatments to fix or improve the contrast, unlike iodine fuming. However, to demonstrate the efficacy of the pyrene treatment relative to fixed iodine prints, a 7-day aged natural fingermark was placed on both metal foil, and glass slides, for a split-print comparison. Figure 6 shows a comparison of both treatments. Even after application of benzoflavone enhancement to iodine-fumed prints, pyrene fuming was found to give superior visualisation on the aluminium foil surface, even yielding evidence of pore locations within the fingermark ridges (ESI – Fig. S6). The lower half of that same fingermark appeared to overdevelop using the iodine/benzoflavone treatment, resulting in loss of ridge characteristics. For glass surfaces ridge detail was visualised using both treatments, however pyrene treatment appeared to give a more even distribution throughout the fingermark relative to the iodine/benzoflavone treatment. This uneven distribution appears to match the observed variation in iodine uptake evident in Fig. 5, suggesting that the iodine uptake step is the cause of this effect. This observation justifies the decision to compare the pyrene results in the phase 1 study against the initial iodine fuming step.

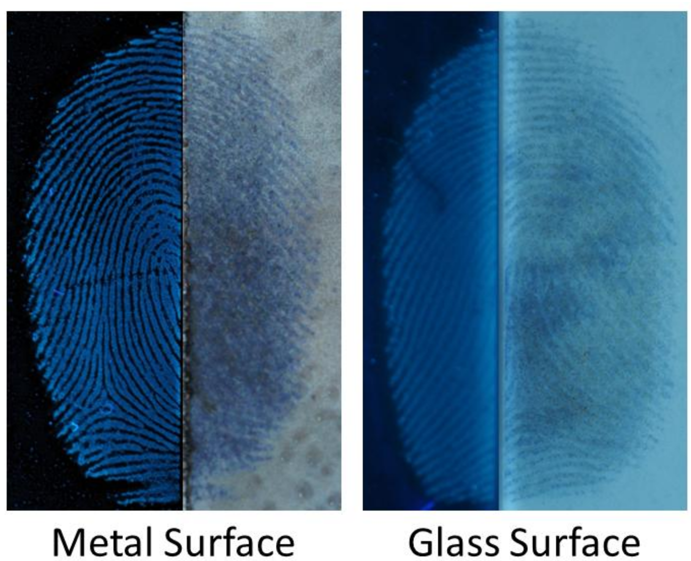


Figure 6. Two 7-day old, split fingermark images for aluminium foil (left), and glass slides (right) comparing the pyrene treatment to the iodine/benzoflavone treatment. The benzoflavone reagent was applied to the surfaces using a fine-mist sprayer sourced from kiloline PRO.

3.5. *Retention of Pyrene within the Fingermark*

A second drawback of the iodine fuming method is that iodine adsorbed by fingermark residues is rapidly lost after the treated fingermark is removed from the iodine fumes. Pyrene is less volatile than iodine vapour, meaning that once uptake of pyrene has occurred, its loss from the fingermark residue should be slower. To demonstrate this, two fingermarks from an average donor, made equivalent by rubbing the fingerpads of the two fingers together to homogenise residue prior to deposition, were placed on aluminium foil. The fingermarks were each aged for 24 hours, after which they were treated identically with pyrene using the optimised conditions. One of the treated fingermarks was sequentially imaged after the passing of 0, 2.5, 10, 24, 48, and 72 hours, and finally again after 16 days, whereupon there was no longer any evidence of fluorescent emission (Fig. 7.). Re-fuming that fingermark 16 days after the initial fuming resulted in restored fluorescence for that fingermark, albeit to a lesser, but still useful, intensity. It should be noted that the amount of residue deposited on a surface will vary according to the individual and situation, which in turn will influence the amount of pyrene uptake, and thus the duration of fluorescence post-treatment. The other pyrene-treated print was concurrently assessed using fluorimetry to accurately gauge the loss of fluorescence (see ESI, Fig. S7). The decay in fluorescent intensity post-fuming resembled an exponential function that yielded a 50% decrease in intensity after approximately 12 hours. This dropped further to a 90% decrease in intensity after approximately 48 hours. The ability of pyrene-treated prints to generate clear fluorescent emissions hours after treatment is a considerable improvement over the iodine fuming method.

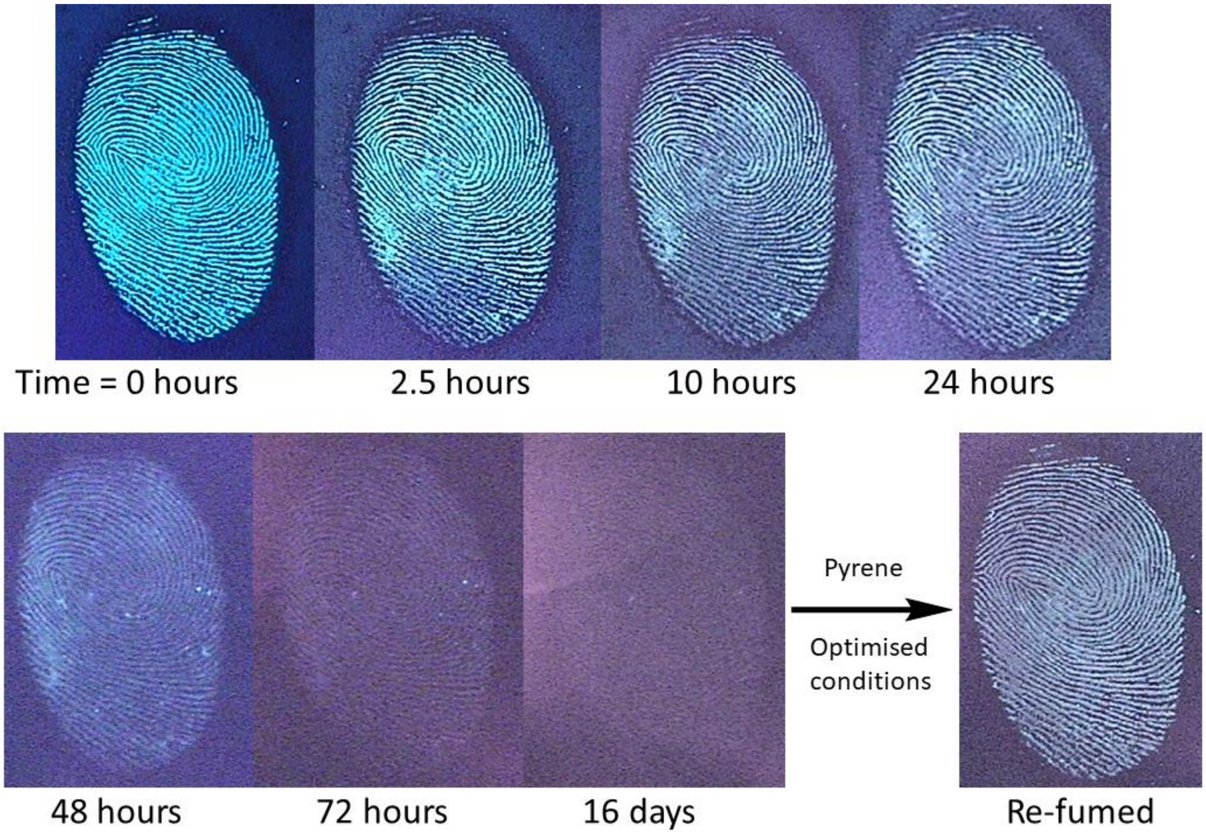


Figure 7. The observed decrease in fluorescent intensity of a fingermark post-pyrene fuming from T = 0. The same fingermark was re-imaged 2.5, 10, 24, 48, 72 hours, and finally after 16 days after treatment. After 16 days the fingermark was re-fumed using the optimised pyrene conditions to partially restore the fluorescent emission.

3.6. *Assessment of highly patterned surfaces*

Given that most surfaces encountered by crime scene investigators are not pristine and featureless, three highly patterned materials were chosen to assess the effectiveness of pyrene fuming on more challenging substrates. The materials chosen were an M&Ms confectionary wrapper, shown in Figure 8, a glossy cigarette packet, and the label of a CD (ESI, Fig. S8&S9). Natural fingermarks from a single average donor, aged for one day were used for this part of the study. The patterned surfaces posed a challenge for pyrene treatment given that many dyes, inks and optical brighteners also fluoresce when exposed to UV irradiation, thereby competing with pyrene emissions. This was particularly problematic for white surfaces, given they have a high likelihood of containing optical brighteners that emit blue visible light upon UV excitation that is difficult to distinguish from pyrene emission. The darker regions of the M&Ms wrapper also negatively impacted the intensity of pyrene emission likely as a result of absorption of the emitted fluorescence, however increasing the power of the light source could partially offset this effect (ESI, Fig. S10). Similar observations were made for the cigarette packet and the CD label.

Figure 8. A highly patterned surface depicting the challenges posed for the pyrene treatment. Left: Treated M&Ms packet viewed under white light; Right: viewed using filtered UV radiation with the HS0350 Shortpass Filter. The M&Ms packet was fumed using the optimised conditions.

3.7. *Transferring pyrene treated prints onto a gelatine lifter*

A common means of lifting treated fingermarks from nonporous surfaces is to apply a gellatine lifter that adheres to both fingermark residues and applied visualisation treatments that, when lifted, result in an impression of the fingermark being transferred. This is commonly done after applying fingerprint powders, but can also be applied after a chemical treatment such as cyanoacrylate fuming, or even in the absence of any visualisation treatment [28]. Here the compatibility of pyrene treatment with a gelatine lifter evidence collection method is evaluated. A natural palm print on a ceramic tile (*i.e.* Fig. 2f) was chosen for this experiment, which was treated using the optimised pyrene fuming conditions. Figure 9 shows a sequence starting with the ceramic tile post-pyrene treatment under ambient lighting, visualisation of the palm mark with UV radiation filtered through a HS0350 Shortpass Filter, a gel lift of the pyrene-treated palm mark under ambient lighting, and finally that same treated palm mark viewed on the Gellifter under HS0350 Shortpass-filtered UV radiation. This demonstrates that gelatine lifters can be used to lift pyrene-treated residues from a nonporous surface with retention of luminescent properties, although it should be noted that gelatine lift will produce a mirror image of the original. One identified challenge for this method was that the cover sheet of the gel lifter prevented much of the UV radiation from penetrated to the pyrene, hindering visualisation after the cover was reapplied to the gel lift. Low level of background fluorescence was also observed from the Gellifter, despite choosing the black-backing. These factors show that this type of gel lifter is best imaged either prior to applying the cover sheet, by later removing the cover sheet, or by sourcing a cover sheet that is transparent to UV radiation. Work is ongoing to ascertain whether this drawback is common amongst gelatine lifters, or specific to only certain brands.

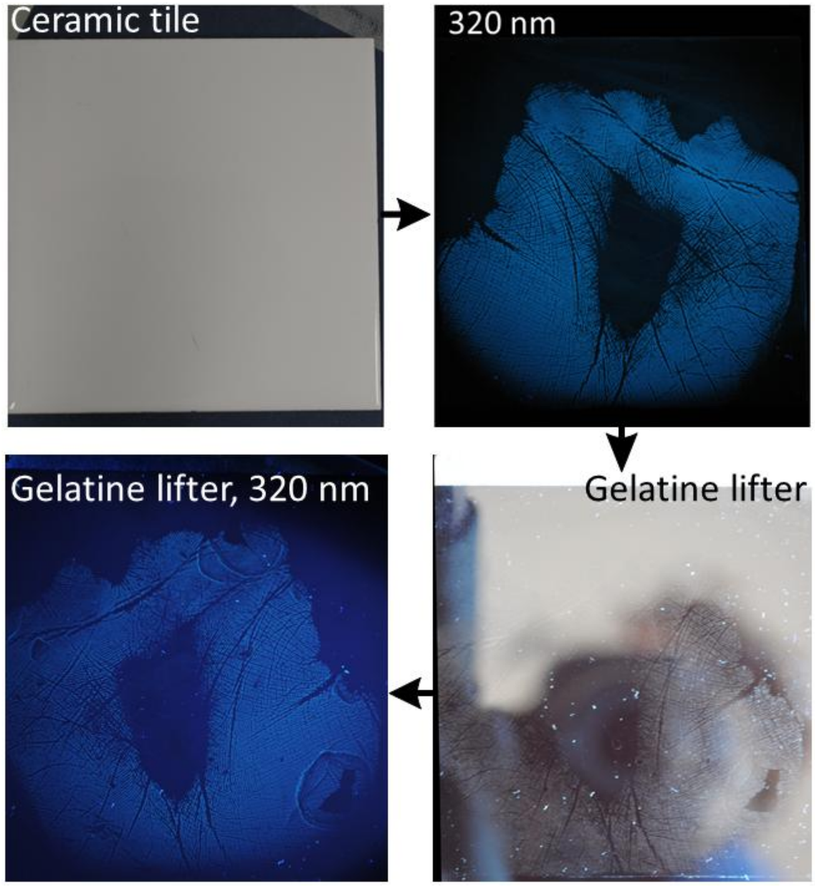


Figure 9. The application of Gellifters Black to a palm mark placed on a white ceramic tile. Clockwise from top left: A pyrene-treated ceramic tile viewed under white light; the same tile under HS0350 Shortpass-filtered UV illumination; a gel lift of the tile under white light; the same gel lift under HS0350 Shortpass-filtered UV illumination. Note that the gelatine lift images are mirror images of the original treated palm mark.

This study highlights the promise of fumed pyrene as a new means of visualising latent fingermarks on nonporous surfaces, particularly metal surfaces, however additional work exploring the versatility of this method is needed. What is clear is that fumed pyrene is rapid to effect, inexpensive, can be undertaken with minimal training, and be performed using equipment that is widely available in most laboratories. Under controlled conditions, pyrene fuming was more effective than iodine fuming in terms of more visualisations with better quality after 1 and

7 days, and pyrene fuming is free of the drawbacks of rapid reagent dissipation from fingermark residue post-treatment, does not require secondary chemical treatments to fix and improve contrast, and completely eliminates the need to use flammable or toxic solvents. The main drawback for pyrene fuming is that it produces fluorescent emission closely matching the background emission for certain surface types, particularly if those surfaces contain optical brighteners.

It is our belief that these results merit continued evaluation in phase 2 studies, however it should be stressed that this work equates to a pilot study, and that further studies involving larger sample sizes of fingermarks and donors are needed. In addition, further work evaluating a greater number of surface types, environmental conditions, and investigating the placement of pyrene fuming within a fingermark detection sequence should all be undertaken to finalise validation of this method. In addition, pseudo-operational studies (phase 3) are recommended prior to implementing this research in any real-world capacity. Recommendations outlining these further activities can be found in the guidelines provided by the International Fingerprint Research Group [39].

4. Conclusion

This research has identified fumed pyrene as a promising new method for visualising both latent fingermarks and palm marks on nonporous surfaces, as demonstrated in this work using a metal substrate (aluminium foil), a glass substrate (glass microscope slides), and a ceramic substrate (porcelain tile). The optimised method described here requires only ten minutes to effect, is inexpensive, and outperforms its closest analogous chemical treatment, iodine fuming, in terms of both number of visualisations, and quality of those visualisation. This was quantified by average CAST scores for prints aged 1 and 7 days. Under idealised conditions a fingermark may retain pyrene for up to 7 days, however during routine assessment of natural marks no appreciable loss in fluorescent intensity was observed 1 hour post-fuming, circumventing a major drawback associated with iodine fuming. This work also showed the effectiveness of pyrene fuming on challenging surfaces, and the ability to perform gelatine lifts on pyrene-treated fingermarks.

ASSOCIATED CONTENT

Supporting Information

A video showing the pyrene fuming process is supplied as supporting information, as well as more detailed setup of the fuming chamber, the spectral characteristics of the shortpass filter used, and further images documenting the effectiveness of pyrene on porous and patterned surfaces.

ABBREVIATIONS

CAST, Centre for Applied Science & Technology; CD, Compact Disk; IFRG, International Fingerprint Research Group; PAH, Polycyclic Aromatic Hydrocarbon; UK, United Kingdom; UV, ultraviolet.

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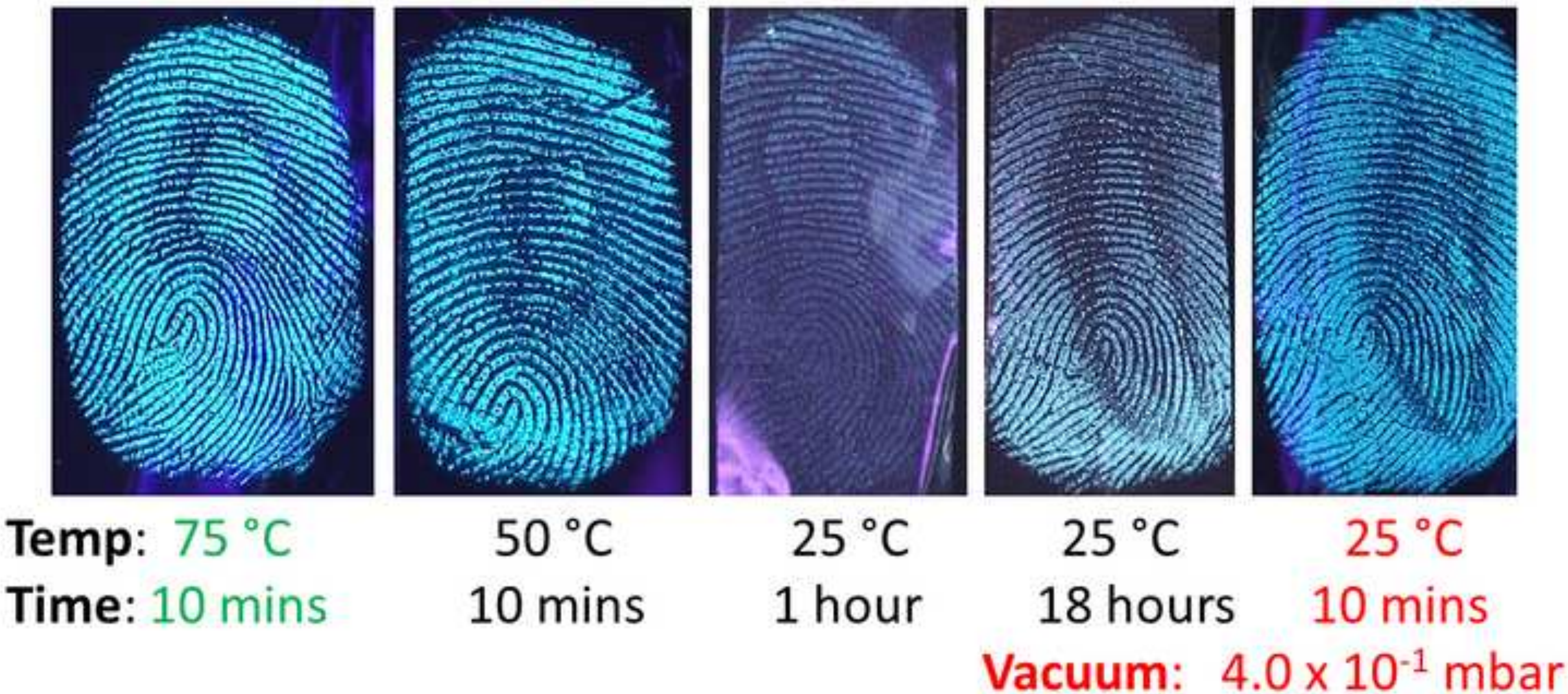

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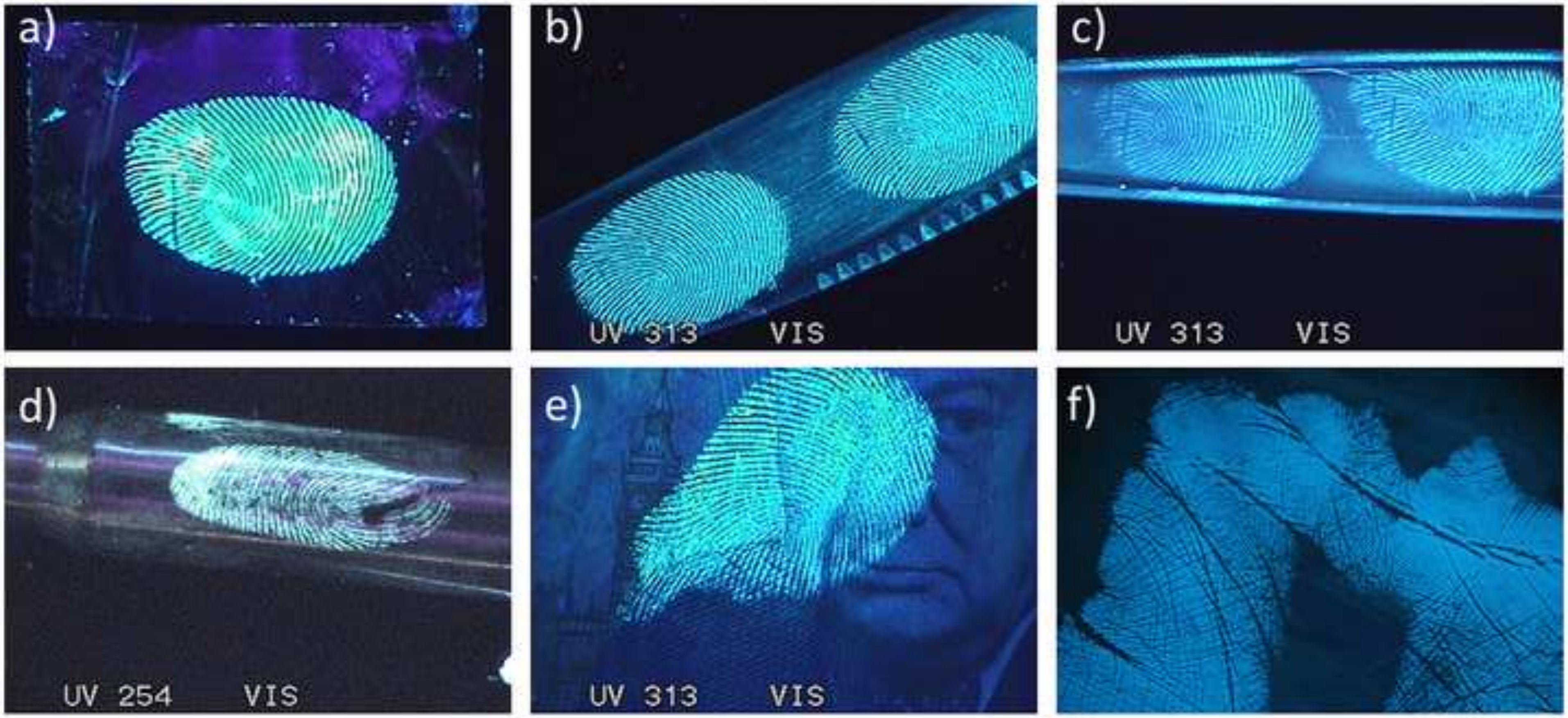

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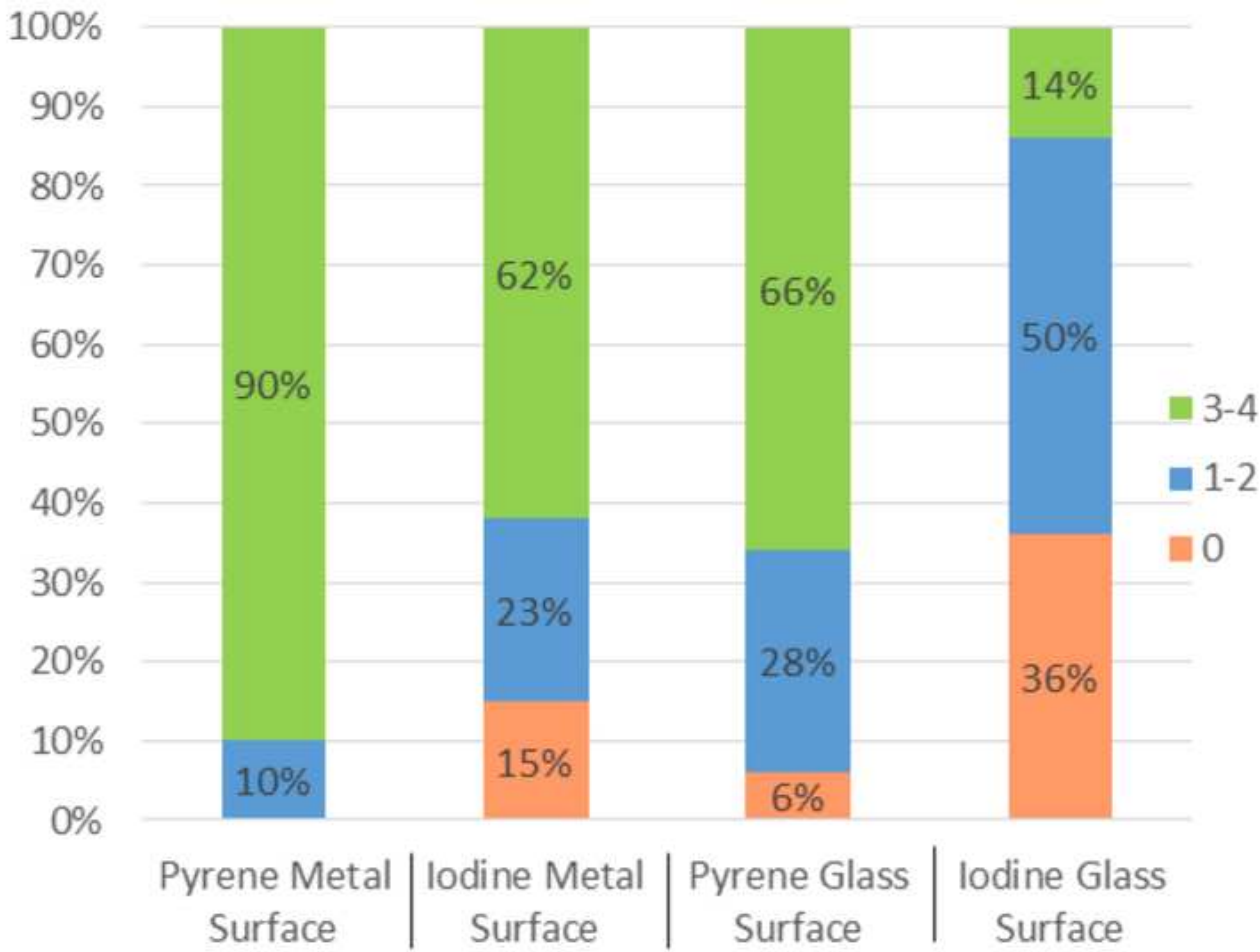

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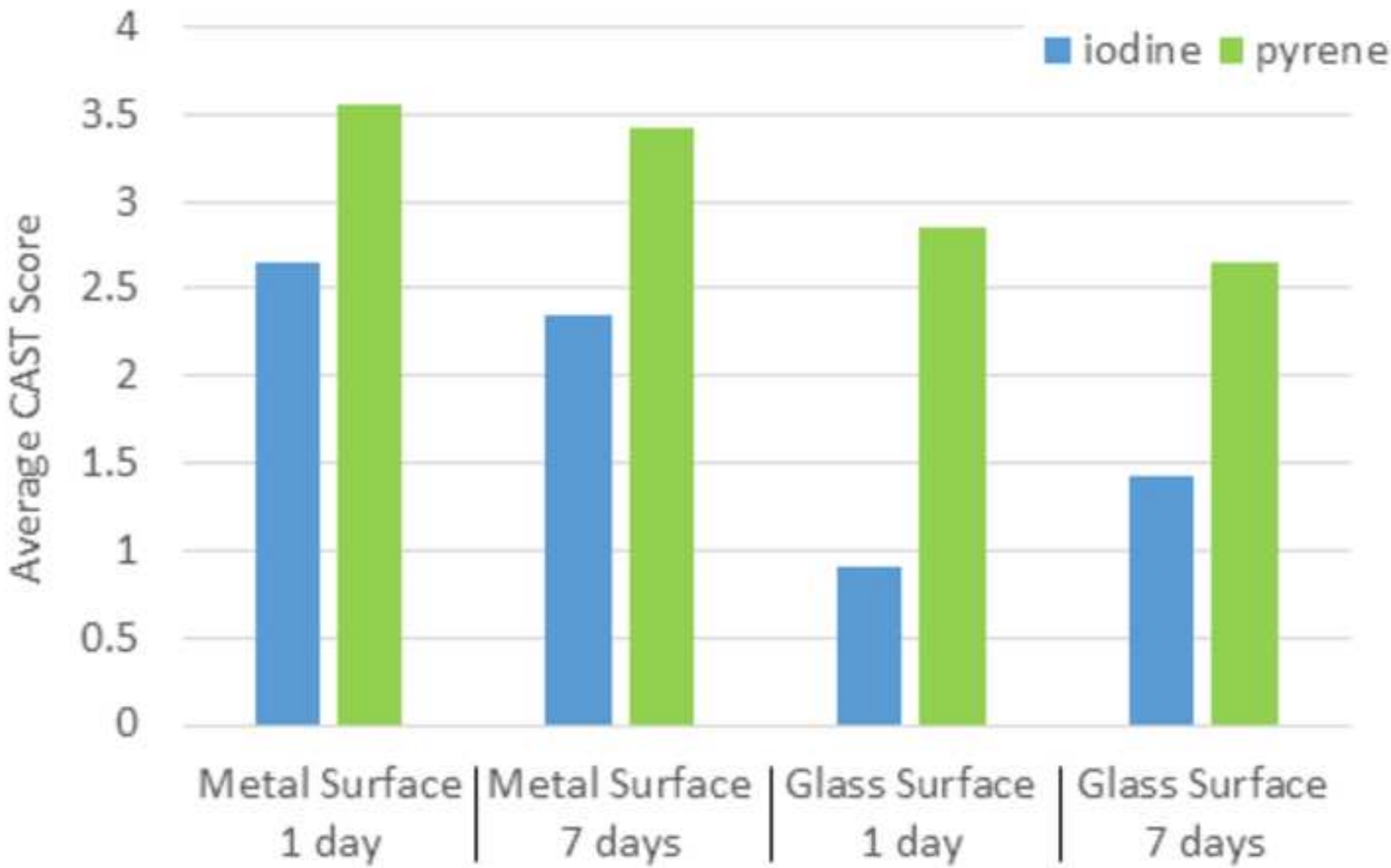

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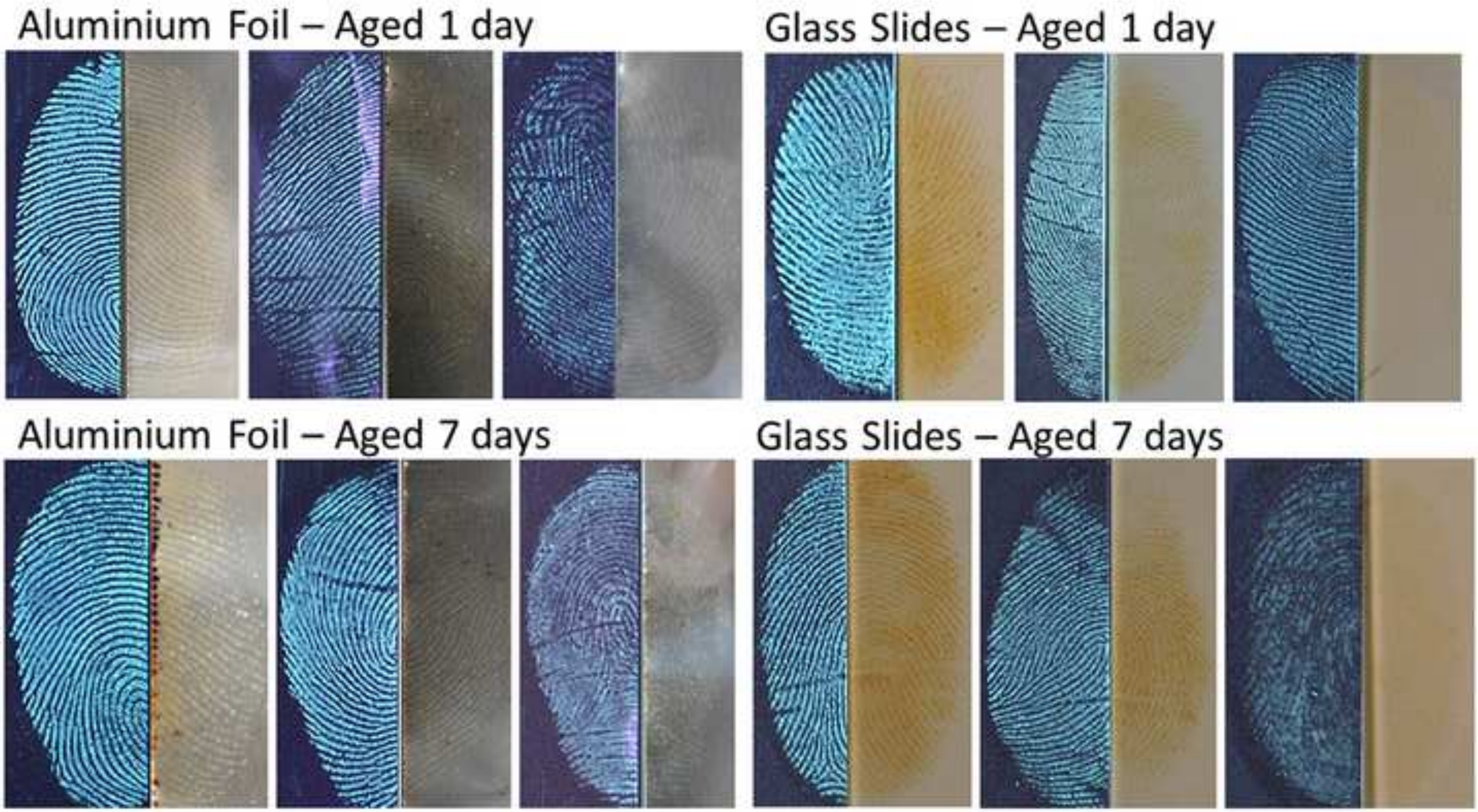

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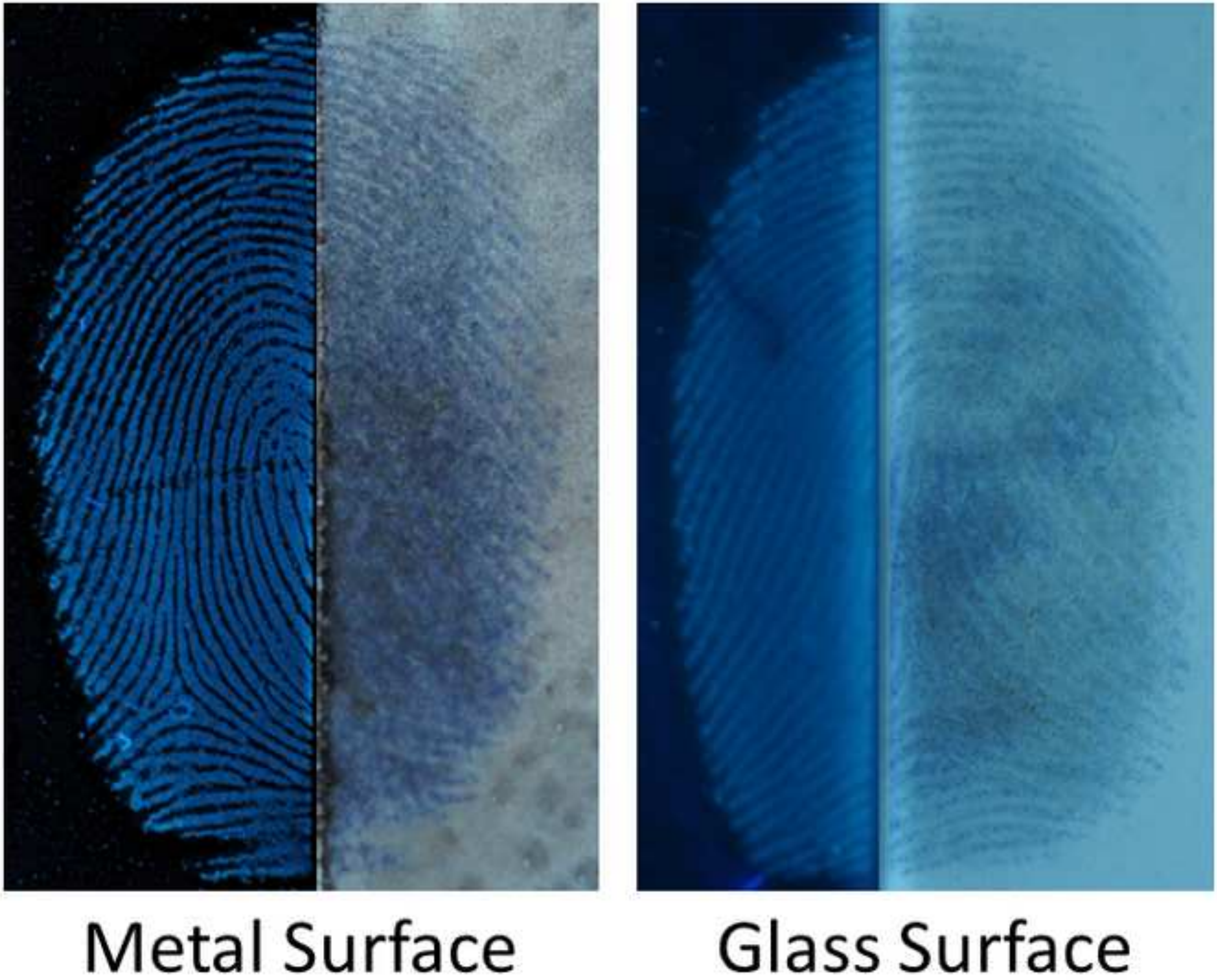

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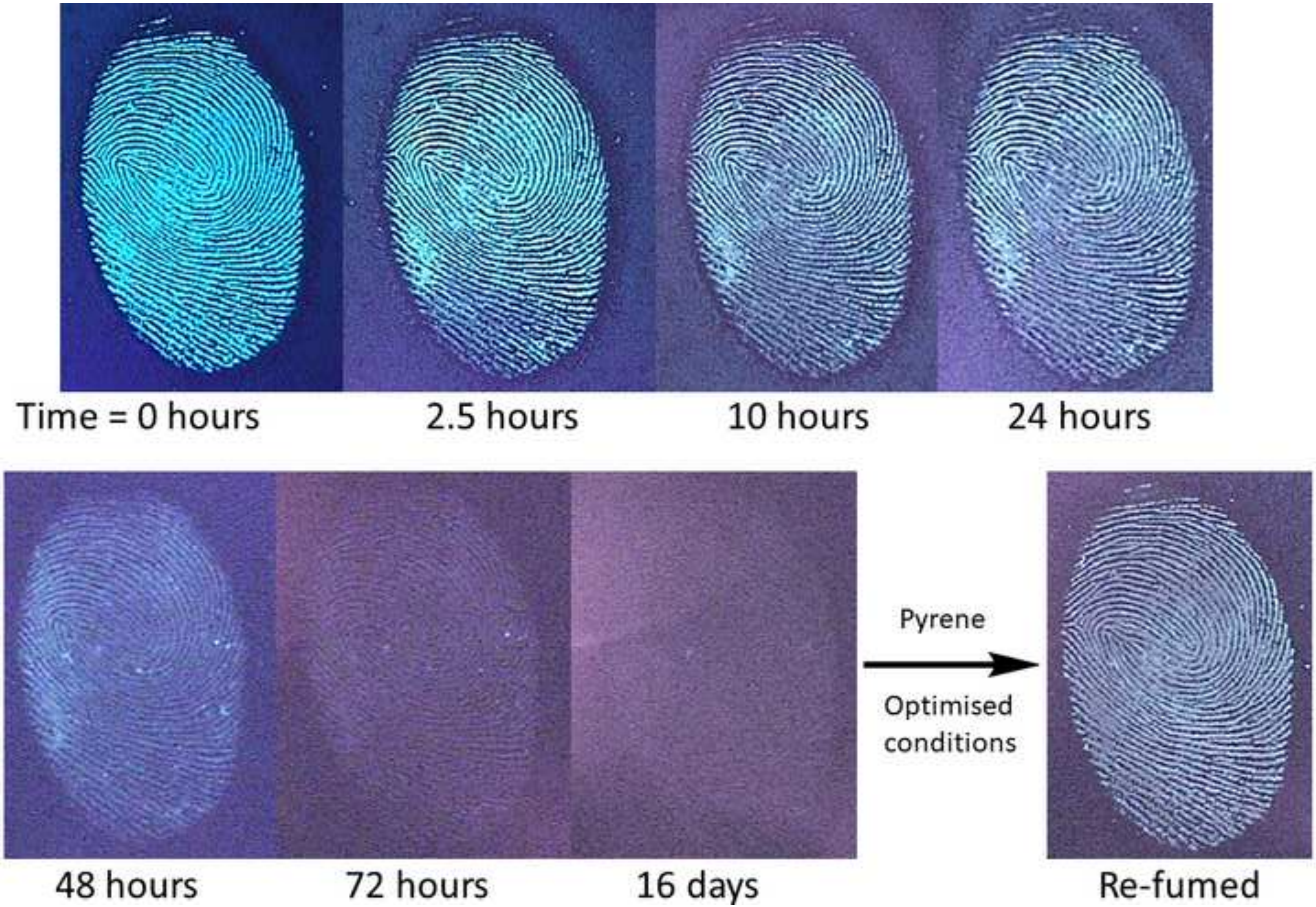

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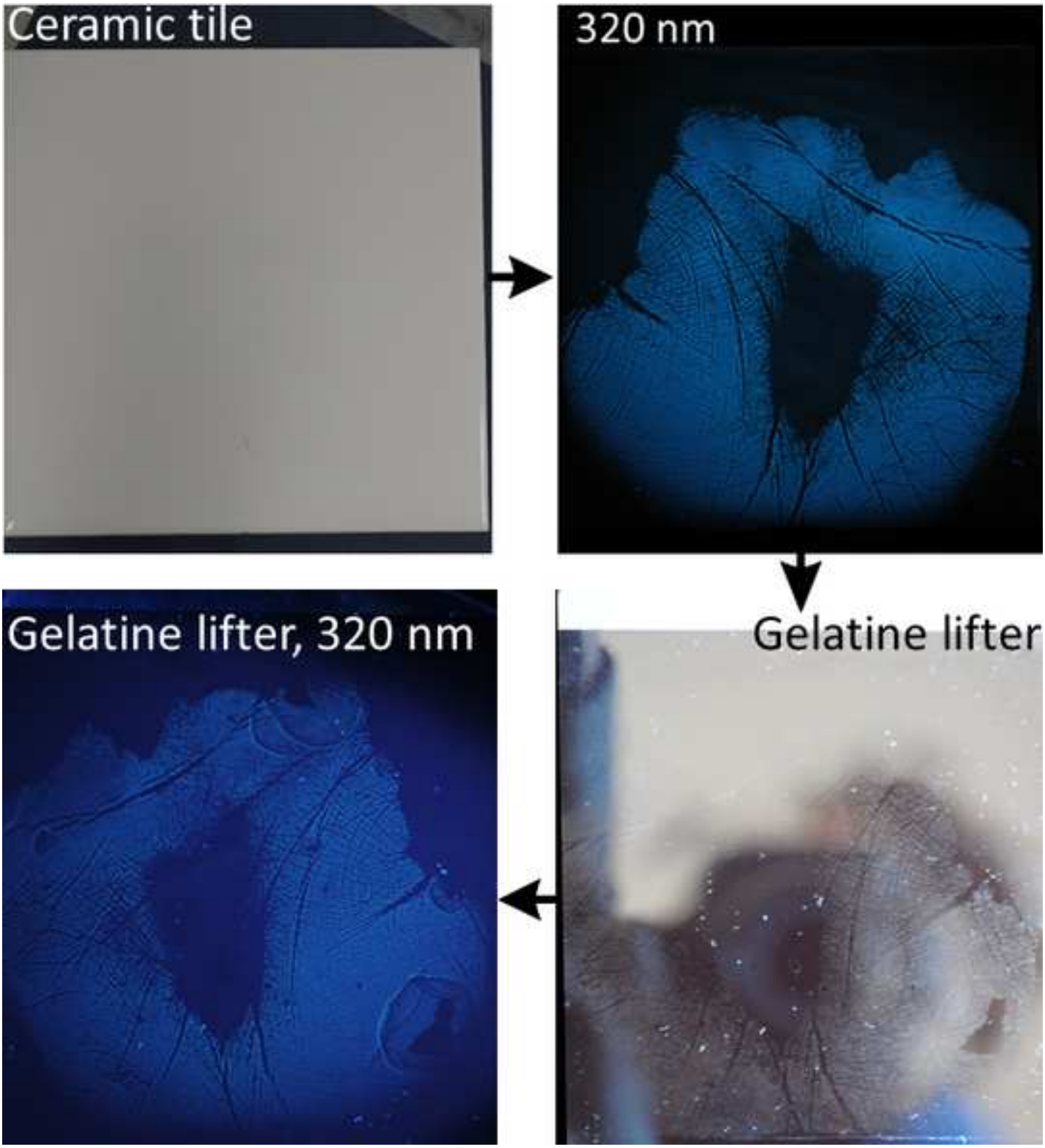

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