Article

The Use of Oil Red O in Sequence with Other Methods of Fingerprint Development

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Abstract: Oil Red O (ORO) has been proven to be equal to or better than physical developer for the recovery of latent fingerprints on white, kraft, and thermal papers that have been wet. To extend these findings, we investigated whether ORO interferes with or improves results when used in sequence with other methods. Our results indicate that on its own, ORO gives excellent quality fingerprints, and further development is often unnecessary. However, if physical developer is needed, it can still be used as a final procedure in the sequence without being influenced by the insertion of ORO.

Introduction

In the past, physical developer (PD) has been the method of choice for developing fingerprints on paper that had been wet and as a final processing technique on papers that had not been wet. However, the PD technique is potentially destructive to the evidence and it often leaves a permanent stain on the surface that obliterates any printing on the evidence [1]. Oil Red O (ORO) is less damaging to the evidence and yet provides good results in a comparison to PD. This research explores whether it would be beneficial to use ORO as an additional processing sequence on both dry and wet surfaces prior to the use of PD.

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Source of Fingerprint Residue

The eccrine glands are found everywhere on the human body, but they are mostly active in hairless regions such as the palms of the hands and the soles of the feet. Friction ridges have tiny pores that release eccrine sweat, which, according to Greenshields and Scheurman [2], is composed of 99.0% to 99.5% water. Solids that make up the remaining 0.5% to 1.0% are half inorganic substances (sodium chloride, potassium chloride) and half organic materials (amino acids, urea, albumin). When left on paper surfaces, amino acids can be targeted by such processes as 1,8-diazafluoren-9-one (DFO) or ninhydrin. In fact, DFO and ninhydrin are currently the two most commonly used methods for the development of fingerprints on porous surfaces [3].

Sebaceous secretions from areas of the body containing hair follicles are known as sebum. The sebaceous glands are especially active on the face and scalp [4]. Sebum is usually found on hands because of contact with those other parts of the body. Because lipids and other water-insoluble fingerprint residues from sebum sweat remain on paper that has been wet, the PD and ORO methods are most widely used for these.

Oil Red O

As its name suggests, Oil Red O stains the oil in fingerprints red; essentially, it is a fat-soluble dye (or lysochrome). Lysochrome is a generic term used to describe compounds that have the ability to dye fatty acids [5]. Lysochromes contain a portion that dissolves in contact with fat (lyso) and another that is responsible for color (chrome). Essentially, lysochromes stain lipids by dissolving in them.

Dyes are organic chemicals that may be classified according to their chemical structure or by their application method, but the latter is the principal system adopted by the Color Index [6]. According to either classification method, ORO is an azo dye; it contains at least one azo group (– N=N –) attached to one or often two aromatic rings [7]. Azo dyes have structural confirmations that prevent them from ionizing, thus facilitating their dissolving in lipids. They include ORO, Sudan III, Sudan IV, and Sudan black B, all of which are lysochromes often used to stain tryglycerides or lipid-bound proteins (lipoproteins) for microscopy visualization or for isolation purposes in electrophoretic separation [8]. Recently, their use has been extended to fingerprint detection as well [9].

The dye color depends on how strongly it absorbs radiation in the visible region of the light spectrum (400 to 700 nm). Table 1 summarizes absorption ranges and staining colors for some common azo dyes.

Azo dye	Empirical Formula	Absorption (nm)	**Stain color**
Sudan III	$C_{22}H_{16}N_4O$	507-510	Orange-red
Sudan IV	$C_{24}H_{20}N_4O$	520-529	Orange-red
Oil Red O	$C_{26}H_{24}N_4O$	518	Red
Sudan black B	$C_{29}H_{24}N_6$	596-605	Blue-black

Table 1

Some commonly used azo dyes and their empirical formulas, absorption ranges, and staining colors. [8, 10].

Chromophores are the part of a molecule responsible for these absorption values and, hence, the dye color. They are unsaturated groups within the molecule capable of selective light absorption and are often in the form of conjugated pi systems sometimes consisting of alternating single and double bonds [7]. Figure 1 shows some examples of common chromophores.

Dye color can also be influenced by auxochromes. These are generally electron-donating chemical groups that, in combination with chromophores, may cause an increase in absorption to darken the color already created by the chromophores [7]. Figure 2 shows some common auxochromes.

Figure 3 compares the chemical structure of Sudan III, Sudan IV, ORO, and Sudan black B. All structures are made up of – N=N – and aromatic ring chromophores. Sudan III, Sudan IV, and ORO all have the – OH auxophore and stain red, whereas Sudan black B has two – NHR auxophores and stains blue-black. The – NHR auxophores are therefore responsible for Sudan black B’s high spectral absorption range and, hence, dark blue-black staining property.

Another difference among the structures lies in the number of methyl groups present on the aromatic rings. These essentially help stabilize the aromatic ring by adding more substituents to it. Chemicals with more methyl groups are increasingly sterically hindered, which also influences the absorption of the molecules.

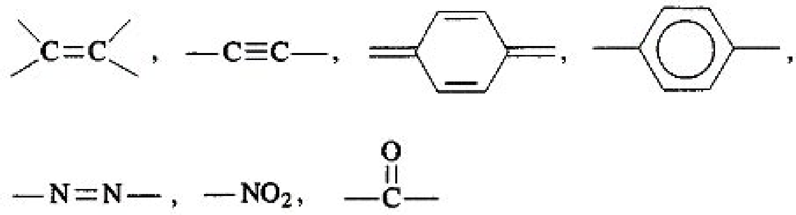


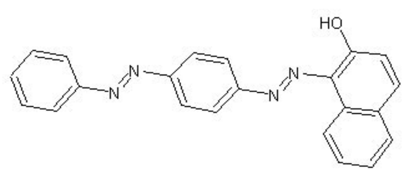
Figure 1

Some common chromophores found in chemical dyes [7].

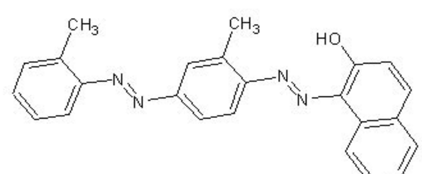
$-OH$, $-OR$, $-NH_2$, $-NHR$, $-NR_2$, $-X$

Figure 2

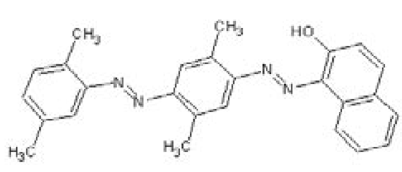
Some common auxophores found in chemical dyes [7].



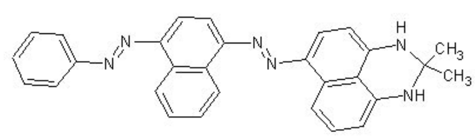
a) Sudan III
1-[4-(phenylazo)phenylazo]-2-naphthol



b) Sudan IV
1-[[2-methyl-4-[(2-methylphenyl)azo] phenyl]azo]-2-naphthalenol



c) Oil Red O
1-[(4-[xylyazo]xylyl)azo]- 2-naphtol



d) Sudan black B
2,3-dihydro-2,2-dimethyl-6-[[4-(phenylazo)-1 -naphthyl]azo]-1H-perimidine

Figure 3

A comparison of chemical structures for common lysochromes.

Interestingly, the first forensic study of ORO was on lip prints, and it compared the effectiveness of Sudan III, ORO, and Sudan black B dyes [11]. The study specifically studied lip prints left by long-lasting lipsticks, because these do not leave visible prints and are very common in the cosmetic industry. Results indicated that all three lysochromes applied as a powder and in solution produced high-quality development of prints up to 20 days old. However, the effectiveness of the development did diminish with the age of the print. ORO produced better quality lip prints than did Sudan III, but Sudan black B gave the best results, with a clear black lip print.

Since this study, a recent one compared PD and ORO on wet paper [12]. Thermal and white paper showed a higher frequency of quality fingerprints with ORO, but no significant differences were found on kraft paper. ORO has now been adopted by many as an alternative method for developing fingerprints on surfaces that have been wet or exposed to high humidity [9], because the result is a clear red print on a pink background.

Oil Red O presents a number of advantages. Apart from being inexpensive, the ORO solution can last up to a year. Furthermore, it only requires two basins: one with the ORO stain solution and the other with a buffering solution. All papers can be treated simultaneously, significantly reducing development time; the technique can essentially be performed in under an hour.

Physical Developer

The physical developer technique was first created in the seventies by the Atomic Weapons Research Establishment as an adaptation of a 1969 publication for photography development [13].

The most important solution mixed into the working PD solution is the redox one. At the Sûreté du Québec, it is made by dissolving ferric nitrate, ferrous ammonium sulfate, and citric acid in distilled water [1]. Ramotowski [13] gives some useful information as to how PD reacts in an oxidative-reductive manner with fingerprint residue. He sums up the net reaction as such:

$$Fe^{2+}{}_{(aq)} + Ag^{+}{}_{(aq)} \longleftrightarrow Ag_{(s)} + Fe^{3+}{}_{(aq)}$$

Ramotowski [13] explains that silver ions (Ag^+) from silver nitrate are reduced to solid silver (Ag), while ferrous ions (Fe^{2+}) from ferrous ammonium sulfate are oxidized to ferric ions (Fe^{3+}). The solid silver precipitates onto water-insoluble lipids, fats, oils, and waxes present in fingerprint residue, and the result is a gray image [14]. Like ORO, it is an excellent method for wet papers where most amino acids are dissolved, and fats become a main target for development.

Unfortunately, this method is quite expensive and cumbersome. Because silver nitrate can be reduced by contaminants, the method requires a large quantity of rigorously clean glassware and chemical products reserved exclusively for PD use. Each paper must be treated individually in five different basins, and extreme care must be taken to avoid any potential contamination. After just the first two basins (acetic acid and maleic acid washes) that serve to neutralize alkaline binders and fillers found in most papers [15], the paper becomes weak and susceptible to tearing. Finally, the process is long and tedious, and any item that comes into contact with the working solution becomes permanently stained [1].

Despite its drawbacks, the method is very useful in situations where the paper is colored, because the fingerprint appears black. Another strength is the stability of the fingerprint after its development [1]. Furthermore, many studies [14, 16, 17] have found innovative ways to shorten some steps or to render some more effective or less expensive. One such example is the additional hypochlorite treatment suggested by Cantu et al. [17] that would intensify the print by converting silver to silver oxide. However, even with such efforts, PD remains a potentially destructive technique. If any step prevents complete neutralization of the paper before the oxidative-reductive step, PD can react with the entire paper, turning it black, and destroying fingerprints that could have otherwise been recovered [15].

1,8-Diazafluoren-9-One (DFO)

DFO is a very effective reagent for amino acids and possibly other components in fingerprints found on porous surfaces such as paper. It is not very useful for wet papers where most amino acids are dissolved and washed away, but if used on its own on dry surfaces, DFO can develop more fingerprints than other methods [18]. The effectiveness of DFO is ultimately dependant on the power and wavelength of the laser or other light source used [19] because the additional fluorescence examination enhances the contrast of the fingerprint.

DFO's forensic application for fingerprint development dates back to the early 1990s [20]. Though DFO reacts with amino acids with no specificity, some studies have followed its reaction with particular amino acids. One example is the reaction mechanism of DFO with L-alanine [21]. Ultimately, if no fingerprint appears with the use of DFO, one can resort to ninhydrin [19].

Ninhydrin

Ninhydrin is an older fingerprint development reagent than DFO. As the latter, it also reacts with amino acids. The developed prints are usually purple (Ruhemann's purple). The color is achieved somewhat similarly to ORO, in the sense that ninhydrin's chemical structure is composed of chromophores (aromatic ring and three ketone functional groups). The reaction mechanism of ninhydrin with amino acids has been extensively studied and is now known in great detail. The multistep reaction can be accelerated by applying heat and moisture [22].

If performed in sequence, DFO and ninhydrin can be considered complementary methods; that is, ninhydrin as a second step can pick up amino acid residues with which DFO may not have reacted [19, 23].

Thermal Paper

Thermal papers are commonly used for facsimiles, printed digitally scanned images, lottery tickets, ATM receipts, and point-of-sale receipts. In forensic terms, they are definitely fair game for fingerprint evidence. The complexity of these papers renders them more difficult to treat than other standard papers.

Thermal paper is made up of five layers, but the topmost layer is of interest in this research because it is the surface for which development techniques must be devised. This coat is known as the active coat, or color-forming layer. It contains sensitizers, stabilizers, dye, and coreactants [24]. The backside of thermal paper is often porous in nature and similar to white paper and will respond to whatever treatment is applied to the top layer.

Thermal papers use heat to produce their images. They are usually thin and have two chemically different sides. The shiny surface on the image side, also known as the emulsion side, is known to stay chemically active after the image has been apposed [25]. For this reason, the images that are present deteriorate quite quickly, and thermal papers are highly unstable. Temperature and humidity extremes, as well as prolonged exposure to light, are a few of the many factors that contribute to this paper's accelerated deterioration [25].

The problem therefore arises as to finding a fingerprint development method that could work with such harsh chemical conditions. Most methods that work on white or kraft paper fail on thermal paper. Ninhydrin, for instance, sometimes causes the active layer to turn dark, rendering fingerprints unable to be detected.

Other methods have proven effective in the past. Exposing the emulsion side of thermal paper to muriatic acid yields impressive ridge detail results [26]. Also, a dipping solution of ninhydrin has been proven more effective than the spraying method in decreasing the amount of darkening [24]. More recently, a new method consisting of fuming the thermal paper with acetic acid has also been proposed, and it seems to give very good results [27]. Unfortunately, the results of this study were presented after ours was conducted.

In our experiment, the dipping ninhydrin technique [24] was chosen to recover fingerprints on dry thermal paper. Rawji and Beaudoin's [12] study confirmed previous results that PD was a poor method for thermal paper. The study also found, however, that ORO yielded better results than did PD. For these reasons, ninhydrin (dipping method) and ORO were the methods of choice for testing thermal paper.

Oil Red O with Other Methods

The present study examines whether performing ORO in sequence with other methods will improve or interfere with fingerprint quality. In modern crime scene laboratories, fingerprint development involves the use of an orderly approach, which combines methods in a specific sequence [22]. It has been found that the use of more than one technique, when performed in the correct sequence, can increase the total number of fingerprints developed [19].

Sequential studies are therefore crucial to the determination of the correct order of fingerprint development methods, especially considering the risk of destroying evidence.

Kent [19] and Wilkinson et al. [23] discuss the fact that ninhydrin use after DFO improves fingerprint development, whereas the use of DFO after ninhydrin is not recommended. The use of PD should be subsequent to that of ninhydrin [28] and it is a destructive technique and should be used last [1]. Finally, the sequential use of DFO, ninhydrin, and PD is suggested for use in important cases to maximize fingerprint detection [18].

The first consideration when choosing a method for fingerprint development is the surface on which it is deposited [22]. Whether the paper is wet or dry will determine which fingerprint components to target and, hence, the appropriate technique to use. Based on the fact that DFO and ninhydrin both react with amino acids, and that ORO stains lipids and is nondestructive, we have decided to insert ORO just prior to the PD (except on thermal paper based on results by Rawji and Beaudoin [12]).

In each case, in order to see whether inserting ORO in the sequence could harm the results, we compared the sequence containing ORO with one excluding it. The sequences compared for each type of paper are listed in Table 2.

Paper Type	Sequences Compared		
White - dry	DFO → ninhydrin → ORO → PD	v	DFO → ninhydrin → PD
White - wet	ORO → PD	v	PD only
Kraft - dry	DFO → ninhydrin → ORO → PD	v	DFO → ninhydrin → PD
Kraft - wet	ORO → PD	v	PD only
Thermal - dry only	Ninhydrin → ORO	v	Ninhydrin only

Table 2

Different processing sequences performed for each paper type.

For all paper types, we hypothesized that inserting ORO in the sequence will either (1) have no effect or (2) improve the quality of the fingerprints. Furthermore, ORO should work best on wet paper because water in the paper repels the dye from the water, causing the selective coloration of the lipid component of the prints.

Materials and Methods

The ORO stain and buffer solutions were the same as the ones described by Beaudoin [9]. The PD, ninhydrin, and DFO solutions (see Appendix) were all taken from the *Manuel du technicien* [1]. In the specific cases of ninhydrin and DFO, the 3M HFE-7100 was used as a carrier instead of CFC113 [29, 30, 31]. This subtlety is important, because it seems that the petrolium ether carrier interferes with the development of fingerprints with ORO when performed after either DFO or ninhydrin. It is well known that petroleum ether has been used in the past as an extraction solvent for fatty acids [32]. This could explain why the lipid stains (like ORO) are not as efficient for fingerprint development after petroleum ether solution treatments. Furthermore, the formulation of DFO used is an effective and improved one and incorporates methanol and acetic acid, as prescribed by Hardwick et al. [33].

For all three paper types, fingers of test subjects were first wiped on the subject's forehead (to allow sebum deposition) and then rolled on appropriate spots on the papers. At least two test subjects were used on each paper, usually one male and one female. A total of six individuals volunteered their fingerprints for the study. Each paper was cut down the center, such that half of each fingerprint was subjected to one sequence excluding ORO, and the other half was subjected to the sequence including it. Table 2 outlines the varying sequences to which each paper was subjected.

Dry Paper: White and Kraft

Two papers were used for each day tested. The white and kraft papers were divided into five square sections down the middle of the paper where five fingerprints were deposited. The fingerprints were either 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, or 30 days old at the time of testing. The two sequences tested for dry white and kraft paper were DFO, ninhydrin, and PD on one half; and DFO, ninhydrin, ORO, and PD on the other.

Wet Paper: White and Kraft

One paper was used for each day tested. The same preparation as for dry paper was involved. However, on the day of the testing, the papers were submerged in a water basin for one full hour before the testing began. Because amino acids are generally no longer present on porous surfaces that have been wet, DFO and ninhydrin were not used on wet paper. The two sequences tested for wet white and kraft paper were PD only on one half and ORO and PD on the other.

Thermal Paper

Nine point-of-sale receipts were collected from each of seven consenting companies (Canadian Tire, Esso, Future Shop, Loto-Québec, National Bank of Canada, Staples/Business Depot, and Zellers). Each paper was divided into three sections, allowing for three fingerprints to be deposited. The fingerprints were either 1, 5, or 10 days old at the time of testing. For thermal paper, all testing was performed on dry paper.

Thermal paper fingerprints were developed with ninhydrin and ORO. Preliminary testing confirmed Rawji and Beaudoin's [12] results which indicated that PD yielded poor results on thermal paper. It was also confirmed that DFO yielded poor results on thermal paper because of the heating step involved. As such, the two sequences tested on thermal paper were ninhydrin only on one half and ninhydrin and ORO on the other half.

Analysis

At the end of the 30-day testing period, all fingerprints were subject to evaluation. Each half was paired with its original counterpart to allow for direct comparisons of single fingerprints (Figure 4). Two qualified senior crime scene technicians as well as the forensic scientist at the Sûreté du Québec evaluated all fingerprints. If the fingerprint was of higher quality when ORO was included in the sequence, it got a score of +1; if it was of higher quality when ORO was excluded, it got a score of –1; if it was of equal quality with or without ORO, a score of 0 was given. It should be noted that in this study, the evaluation of quality was determined by examination of the contrast difference between the fingerprint and background and the variation in contrast created by the compared techniques. (The clarity or lack of clarity created by excessive fingerprint deposition pressure or movement did not affect the quality evaluations.)

Figure 4

Evaluation method for comparison purposes.

Results

White and Kraft Paper

Figure 5a demonstrates that in 91% and 89% of the cases, the inclusion of ORO resulted in equal or better quality fingerprints for dry and wet white paper, respectively. Furthermore, more fingerprints were of better quality on wet paper than on dry paper (about 40% and 25%, respectively) when ORO was included in the sequence. Therefore, on white paper, ORO improved the quality of the prints tremendously and more so when the paper had been wet.

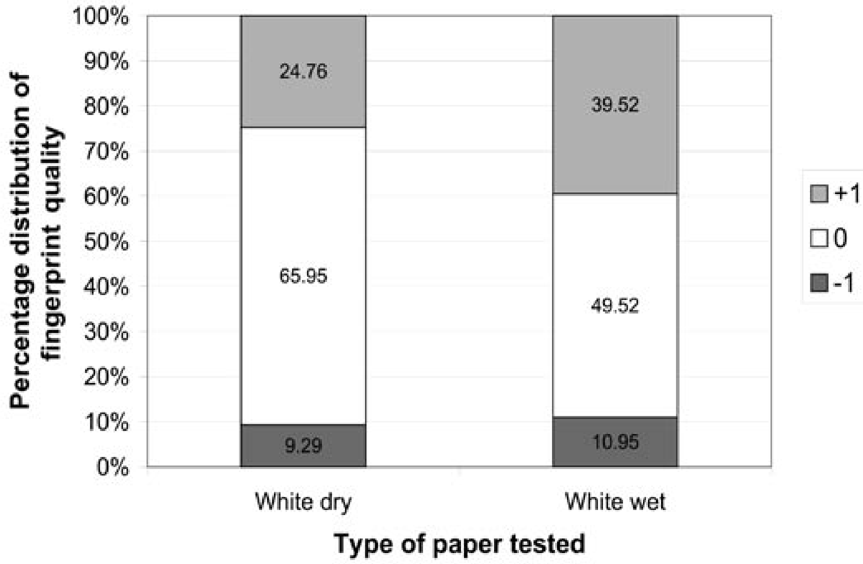


Figure 5a

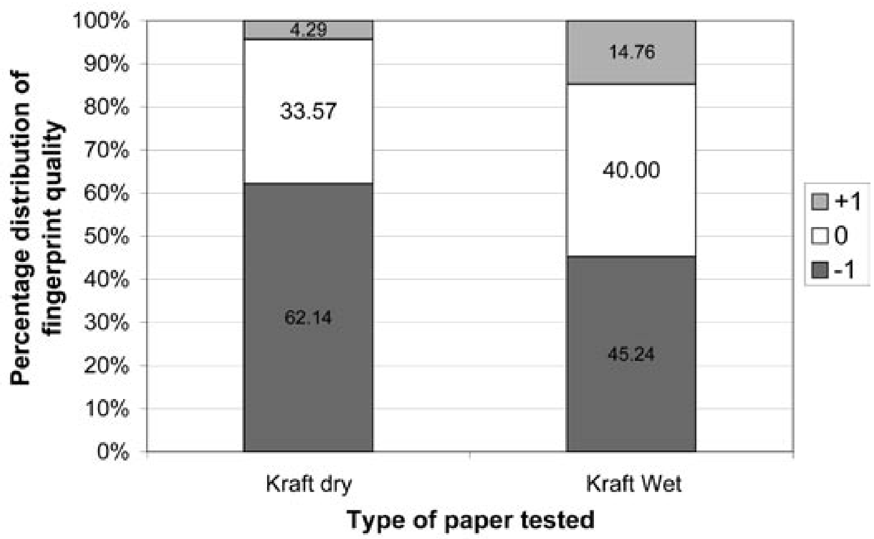


Figure 5b

Comparison of distribution of fingerprint quality for white paper and kraft paper. A score of +1 indicates that the fingerprint was of better quality when ORO was included in the sequence. A score of 0 indicates that both sequences (with and without ORO) yielded equal quality fingerprints. A score of –1 indicates that the fingerprint was of better quality when ORO was excluded from the sequence.

Kraft paper yielded different results. When ORO was present in the sequence, the fingerprints were less visible than on white paper. Figure 5b clearly indicates that in about 62% and 45% of cases, excluding ORO yielded better results for dry and wet kraft paper, respectively. However, in cases where ORO did not yield an identifiable print, it was still useful in locating the fingerprint on the paper. This facilitated subsequent PD use, because the exact location of the fingerprint was predetermined and closely examined for development. Though the results were poor overall, results were still better on wet paper, where 55% of fingerprints were of equal or better quality with ORO use.

Thermal Paper

For fingerprints developed on thermal paper, results varied by company (Figure 6). Staples/Business Depot and Loto-Québec paper had 100% of their prints be of equal or better quality when ORO was included in the sequence, whereas National Bank of Canada had a higher proportion (17%) of better fingerprints when ORO was not used.

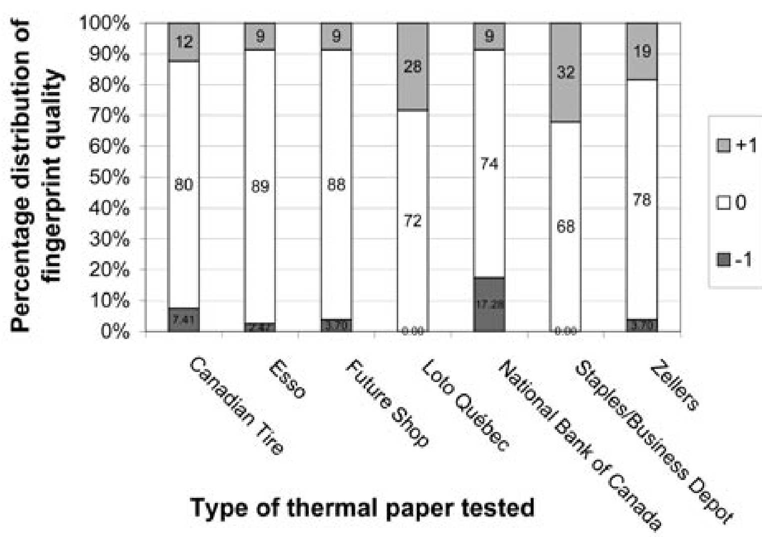


Figure 6

Percentage distribution of fingerprint quality by thermal paper company. A score of +1 indicates that the fingerprint is of better quality when ORO is included in sequence. A score of 0 indicates that both sequences yielded equal quality fingerprints. A score of –1 indicates that the fingerprint was of better quality when ORO was excluded from the sequence.

It should be noted that thermal papers all had a high rate of failure with respect to fingerprint development. That is, regardless of whether ORO was used, 40 to 75% of fingerprints were unsuccessfully developped on all company papers. Figure 7 shows these failure rates, indicating variability among thermal papers. Though Loto-Québec paper had the highest success rate with ORO (Figure 6), it also had one of the highest failure rates to develop fingerprints.

Age and Fingerprint Quality

On white and kraft paper, fingerprints were developed when they were 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, and 30 days old. Figures 8a and 8b both indicate that on white paper, fingerprint quality worsened over time, but more so for wet paper.

On dry kraft paper, fingerprint quality fluctuated dramatically. It improved gradually, peaked at day 9, and then gradually got worse near day 25 (Figure 8c), indicating no observable trend. On wet kraft paper, fingerprint quality improved over time (Figure 8d). Overall, fingerprints degraded less on wet papers, both white and kraft.

Thermal paper was evaluated for fingerprint quality on days 1, 5, and 10. As Figure 8e indicates, quality improved over time.

Table 3 shows how fingerprint quality improved over time for all seven tested thermal paper companies. In most cases, fingerprint quality improved from day 1 to day 5 and stayed the same on day 10. Loto-Québec and Staples/Business Depot were consistently better when ORO was used over the 10-day period; these are the companies that also yielded the highest quality prints when ORO was included. Only Future Shop experienced a slight decrease in quality on day 5 and regained its quality on day 10.

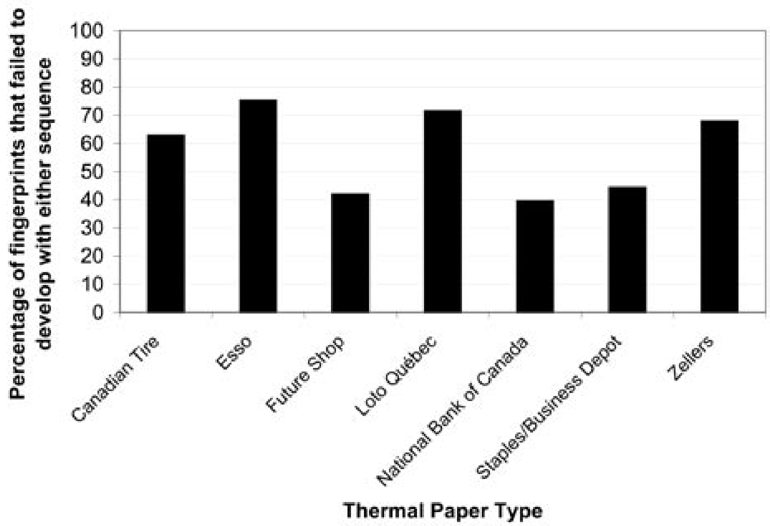


Figure 7

Percentage of fingerprints that failed to develop with both sequences (with and without ORO) on thermal paper across 7 companies.

	Fingerprint age (days)		
	1	5	**10**
Canadian Tire	77.78	100.00	100.00
Esso	92.59	100.00	100.00
Future Shop	100.00	92.59	100.00
Loto-Québec	100.00	100.00	100.00
National Bank of Canada	59.26	96.30	96.30
Staples/Business Depot	100.00	100.00	100.00
Zellers	88.89	100.00	100.00
Average	**88.36**	**98.41**	**99.47**

Table 3

Variation of percentage of high-quality prints using Oil Red O over time.

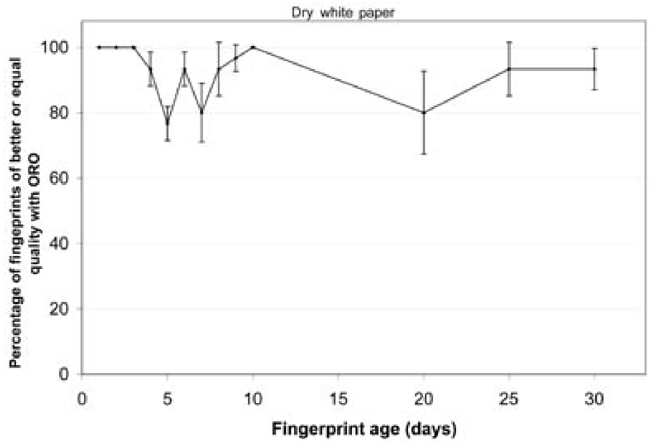


Figure 8a

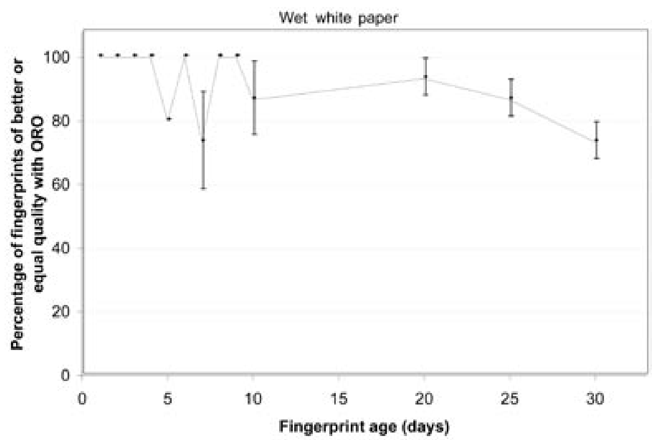


Figure 8b

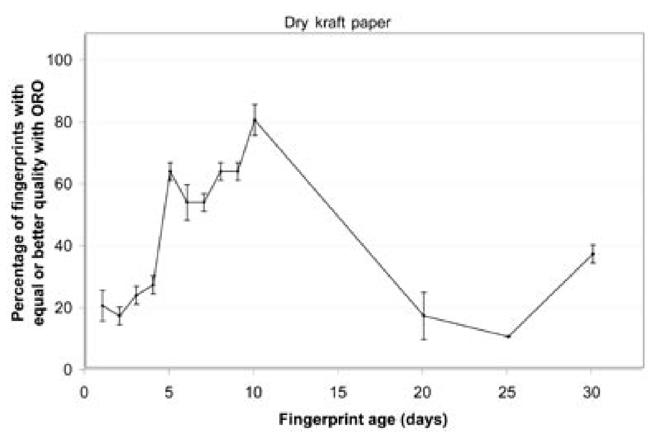


Figure 8c

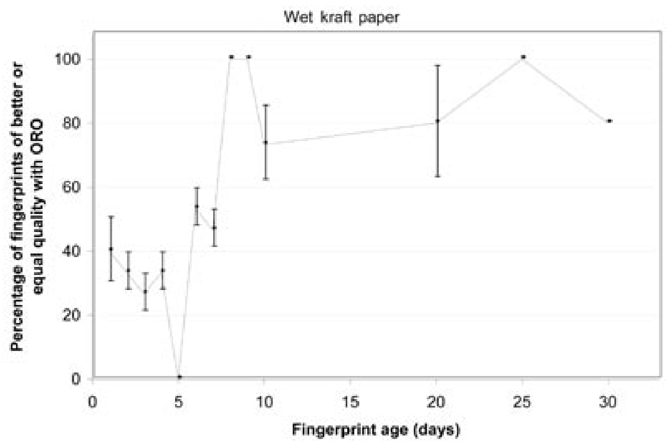


Figure 8d

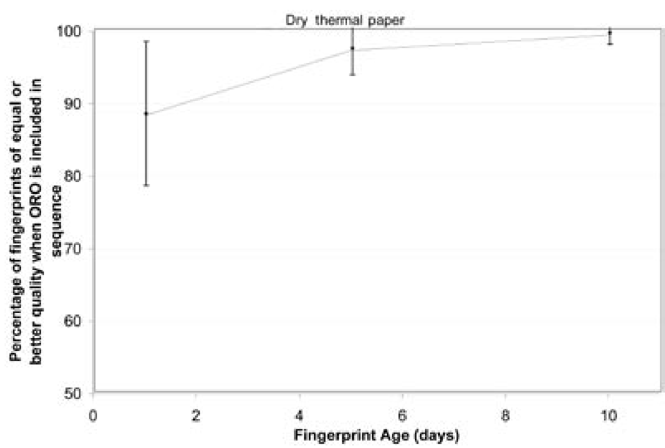


Figure 8e

Variation of fingerprint quality over time with ORO present in sequence (a) on dry white paper, (b) on wet white paper, (c) on dry kraft paper, (d) on wet kraft paper, and (e) on dry thermal paper.

Discussion

White Paper

Of all papers tested, white paper yielded the best quality fingerprints. White paper was standard 8 1/2" x 11" used for photocopying and printing. Because this type of paper is extensively used for office documents that often need to be preserved, it undergoes many chemical processes, including a bleaching step, to ensure high quality. The lighter background results in less paper darkening, better contrast, and, hence, better quality prints.

The main distinguishing feature of white paper is the inclusion of fillers by the manufacturers. In essence, these fillers serve to reduce paper porosity to make the paper surface as smooth as possible. They can be a variety of chemicals, such as china clay or calcium carbonate [34]. A smoother surface leads to a clearer fingerprint, because it is applied to the paper more evenly.

Recently, our results on white dry paper were supported by a forensic scientist from the Belgian Federal Police [35]. Based on all these factors, ORO yields excellent results and is strongly recommended for use on white paper, wet or dry.

Kraft Paper

Kraft paper has not been a favorable surface on which to develop fingerprints in the past [9, 12]. In this study, the results were confirmed and further demonstrated that, in most cases, including ORO with other methods could potentially worsen the contrast of the fingerprints.

According to the senior crime scene technicians who evaluated the fingerprints, the most striking factor contributing to kraft paper's weakness is its dark color. They explained that although the fingerprints were more or less the same quality when ORO was used before PD, they favored fingerprints with PD alone because a dark grey print (PD) on a reddened (ORO) brown paper background has lower contrast. They found that PD alone, which results in a higher contrast dark grey print, was more suitable for developing fingerprints on brown paper. Interestingly, the contrast factor could easily be improved to a great extent with the simple use of image enhancing software. Essentially, these would serve to reduce background darkness and to enhance contrast (Figure 9).

It should be noted that in cases where ORO did not yield an identifiable print, it was still useful in locating the fingerprint on the paper. During PD use, the exact site of the fingerprint was known and closely examined for development.

(a) *(b)*

Figure 9

The use of an image enhancing technique to improve contrast on kraft paper. (a) Original picture and (b) picture enhanced with Adobe Photoshop CS2. Contrast is enhanced on the right half where PD was used after ORO, but left the same on the left half where ORO was not used.

Insight into this paper's use and composition can help explain its drawbacks. Kraft paper is mostly used for grocery bags or strong wrapping paper. Its use requires it to be sturdier than white paper. It is therefore unbleached and undergoes fewer chemical treatments than other papers. Kraft paper contains many more impurities. The result is an unlevel surface, with pores of varying sizes unevenly spread on the paper's surface [34]. When a fingerprint is applied to kraft paper's surface, the residue is spread unevenly. The resulting print is of varying contrast and can appear smudged. Based on these findings, ORO can still be useful for fingerprint development on kraft paper. To enhance contrast, image enhancing techniques can be applied, as shown in Figure 9.

Dry Versus Wet Conditions

For both white and kraft paper, including ORO improved results to a greater extent when the paper was wet, regardless of whether the paper was white or kraft. The simplest explanation for these results lies in the hydrophobic nature of ORO. When a paper has been wet, it repels ORO at its surface. The ratio of the concentration of ORO drawn to the hydrophobic fingerprint residues (fats) to that drawn to the paper increases, causing a greater contrast.

Furthermore, when the paper has been wet, amino acids and salt constituents are mostly dissolved and washed away. The proportion of fat component with respect to overall fingerprint residue increases, and a seemingly darker print is observed.

Thermal Paper

As in previous studies performed on thermal paper [12, 36], results varied by company. Although thermal papers have been studied for fingerprint development purposes [12, 24, 26, 36], not much is known about thermal paper composition in the different manufacturing processes. It is known that the paper's composition does fluctuate, mainly in the coating layer where the fingerprint is deposited [36]. There seems to be no set standard method that consistently works well across thermal paper ranges. In the future, it would be interesting to compare the ORO, ninhydrin, muriatic acid [26], and the new acetic acid fumigation techniques [27] in a standard manner and to investigate the failure rates in the development of aged fingerprints across these methods.

Unlike kraft and white paper for which fingerprint quality was consistent on a given day, thermal paper quality was very diverse across all seven companies. For instance, Staples/Business Depot and Loto-Québec paper both had 100% of their prints be of equal or better quality when ORO was included in the sequence, perhaps due to a peculiarity in their composition, whereas 17% of fingerprints were of better quality when ORO was excluded for National Bank of Canada paper.

Furthermore, high variability was observed in the rate of failure with respect to fingerprint development. Some companies exceeded 70% (Esso and Loto-Québec), while others remained below the 40% mark (National Bank of Canada). It should be noted that with ninhydrin, a small percentage of fingerprints can continue to develop for several weeks after the development day [19].

Another factor demonstrating the differences across manufacturers was in quality of the papers after all methods were performed. ORO, for instance, had the effect of removing some background writing on thermal papers. On thermal paper receipts from Future Shop, Loto-Québec, National Bank of Canada, and Staples/Business Depot, the ink remained, and the company logo was clearly identifiable after treatment. However, on thermal paper receipts from Canadian Tire, Esso, and Zellers, all printed ink from the cash register disappeared after ORO's use. It should be noted that due to this fact, all thermal paper receipts were marked in pen prior to treatment with ORO. If the ink had disappeared from the marking, the indentation left on the paper with the pen could easily be visualized under laser light to retrieve the paper details. In the future, different hole punch shapes could be used instead.

All of these factors contribute to thermal paper individuality across manufacturers, and a deeper investigation into the manufacturing processes, if at all possible, could prove very helpful in the future.

Donor Variables

Controlling for donor variables was accomplished by treating two halves of the same print (from the same donor) with the two sequences (one including ORO and the other excluding it). Therefore, each half print was compared with its original other half.

Jones et al. [37] describe a number of factors that influence fingerprint quality, and their recommendation of gathering donor information is promising for future experiments involving comparison studies. They describe the influence of sex, age, racial origin, smoker or non-smoker, medication use, illness, and diet, amongst other factors. For instance, lipid composition is found to be dependant on age and sex. This is especially influencial for ORO use, because its target in fingerprint residue is fat.

Age and Fingerprint Quality

Overall, fingerprints degraded less on wet papers when ORO was used, both on white and kraft papers. These results are due to the fact that on wet paper, amino acid residues had mostly been washed away by being dissolved in water and, as a result, fat proportion increased. With ORO use, higher fat proportion results in better contrast fingerprints.

Studies have shown that changes do occur to fingerprints over time. Specifically, it has been shown that lipid composition varies over time, because of chemical changes influenced by the presence of light and storage conditions of the prints. Archer et al. [38] demonstrated that the fatty acids squalene and oleic acid decreased in concentration over time. It was also found that most saturated fatty acids increased at first and then decreased. These variations can help to explain some of the fluctuation in perceived fingerprint contrast (and quality) when developed with ORO. However, it is important to realize that aside from fingerprint age, composition, and surface of deposition, many other factors contribute to fingerprint quality, and these should be further investigated to enable optimal experimental conditions.

Conclusion

Oil Red O has been proven to yield better quality fingerprints than PD on white and thermal paper that has been wet. In this study, we extended previous findings to dry conditions.

In conclusion, ORO can be inserted in the fingerprint development sequence without harming the results. On white paper, ORO use before PD yields excellent results. On brown paper, although PD results have a lower contrast when performed after ORO, the strength of the fingerprint technique and the contrast quality can both be improved with image enhancement techniques. On thermal paper, even if paper composition highly impedes fingerprint development, ORO does not interefere with the sequence tested.

Finally, results indicate that on dry paper (white or kraft), the sequence DFO, ninhydrin, ORO, and PD is recommended, whereas the sequential use of ORO and PD is appropriate for wet papers. In general, ORO yields excellent results on its own or just after DFO or ninhydrin use. However, if one considers the PD step necessary, it can still be performed last, without interference from ORO.

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Appendix

DFO

Solution:

Add 0.5 g DFO to 40 mL of pure methanol.

Add 20 mL of glacial acetic acid.

Add HFE7100 (NOVEC) solution to make 1 L.

Procedure:

Spray each paper with DFO solution and allow to hang dry in fume hood.

Repeat spraying and drying step.

Heat all papers at 100 °C, under 0% humidity, for 10 minutes.

Visualize and photograph fingerprints under laser light source.

Ninhydrin

Solution:

Add 130 mL Stock solution ninhydrin to 3870 mL HFE7100 (NOVEC) solution, and stir.

Filter solution through filter paper containing sodium sulfate to absorb any excess water.

Store solution in dark bottle away from light.

Procedure:

For white and kraft paper, spray papers with ninhydrin solution and hang to dry.

For thermal paper, dip papers into ninhydrin solution and hang to dry.

Once dry, all papers are placed in oven at 50 °C, under 60% humidity, for 30 minutes.

Oil Red O

Stain Solution:

Dissolve 1.54 g of Oil Red O in 770 mL of methanol.

Dissolve 9.2 g of NaOH (sodium hydroxide) in 230 mL of distilled water and add it to the above solution.

Stir and filter solution.

Store solution in a dark bottle away from light.

Buffer Solution (pH 7):

Dissolve 26.5 g of Na_2CO_3 (sodium carbonate) in 2 L of distilled water.

Slowly add 18.3 mL of 70% concentrated HNO_3 (nitric acid), while stirring.

Add distilled water to make 2.5 L.

Procedure:

Staining – The documents are immersed in the ORO stain solution and shaken for 60 to 90 minutes.

Buffer Solution – The documents are immersed in the buffer solution for a few seconds to adjust the pH.

Drying – The documents are rinsed in distilled water and hung to dry.

Physical Developer

Acetic Acid (25%):

Add 250 mL glacial acetic acid to 750 mL distilled water.

Maleic Acid:

Add 25 mL of maleic acid to 500 mL of distilled water and stir.

Add water to 1 L and invert solution to release pressure build up.

Physical Developer Working Solution:

Detergent Solution:

Add 2.7 g of N-dodecylamine acetate in 500 mL of distilled water.

Add 4.0 g of Synperonic-N.

Redox Mixture:

Add 30 g of ferric nitrate nonahydrate to 900 mL distilled water.

Add 80 g of ferrous ammonium sulfate and dissolve.

Add 20 g of citric acid, dissolve, and stir solution.

Silver Nitrate Solution (to be prepared the same day as its use):

Add 7.5 g silver nitrate to 37 mL of distilled water and stir.

Cover solution with aluminum foil until its use.

Add 30 mL of detergent solution to 720 mL redox solution.

Stir for 2 to 3 minutes.

Add silver nitrate mixture and stir for 1 minute.

Let solution sit for 5 minutes before use.

Rinsing Solution:

Add 500 mL Kodak fixer to 3500 mL distilled water.

Procedure:

Prepare 5 basins, one containing the solution of acetic acid, one containing the solution of maleic acid, one containing distilled water, one containing the PD working solution, and one containing the rinsing solution.

In sequence, immerse papers in each basin, using a different gripping tool for each basin to prevent contamination.

The paper is dipped in each basin for 1 minute, except the basin of the PD working solution, where it is dipped for 5 to 10 minutes or until a clear gray fingerprint appears.