THE PENNSYLVANIA STATE UNIVERSITY
SCHREYER HONORS COLLEGE

DIVISION OF SCIENCE

# GUN RESIDUE ANALYSIS USING PAPER MICROFLUIDICS

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## ABSTRACT

At borders, security checkpoints, and crime scenes, officials need an efficient test for explosive, gunpowder, and gunshot residues. This work describes the development and evaluation of a microfluidic paper-based analytical device (μPAD) that is intended to be used for the rapid detection of aromatic compounds present in gunpowder and gunshot residue. The μPAD offers benefits of rapid prototyping, reduced cost, disposability, and ease of transport, which is important for an application that is designed for use in the field. Similar work has been reported in the literature for a μPAD-based colorimetric assay that detects aromatics from explosives. The μPAD presented here was designed using AutoCAD software and patterned onto chromatography paper of various grades with a dedicated printer using wax-based ink to define fluidic flow paths. A panel of common residues was evaluated on paper in order to better understand experimental limitations, prior to the acquisition of real samples from local authorities (e.g., campus police) that were able to provide substrate containing residue. A colorimetric response on paper was thoroughly explored for each residue. The latter analysis could prove beneficial for follow-up analysis after an initial colorimetric screening "in the field."

# TABLE OF CONTENTS

# LIST OF FIGURES

# LIST OF TABLES

## ACKNOWLEDGEMENTS

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# Chapter 1

## Introduction

### Forensic Analysis

Forensic analysis has come a long way since its origins around 44 B.C. The first signs of forensics were seen with the Romans, where a physician performed an autopsy on Julius Caesar and determined that of the 23 wounds on his body, only one was fatal.[1] At around 221 B.C – 220 A.D. fingerprints were used as a form of identification and were analyzed to establish ownership for documents and sculptures in China.[2] In 1000 A.D., a Roman attorney showed that bloody prints left at the scene of the crime were meant to frame an innocent man in a murder.[1] A Chinese book published around 1248 A.D. described how to distinguish between drowning and strangulation. This book was the first known record of medical knowledge used to solve criminal cases.[1]

Forensic analysis started to define itself as a field in the 1800s, where the need to solve crimes in a timely matter became a prominent goal. During the 1840s, arsenic was the primary poison used, but there was no reliable way to test this poison for its use and presence. Mathieu Orfila, the father of forensic toxicology, developed a technique that successfully detected arsenic in the body and other suspected surfaces. Orfila published the first scientific treatise on the detection of poisons and the effect it has on animals shortly after his success in developing the technique. Due to his work, Orfila was able to convict a man for murdering his wife using arsenic.[3]

In 1879, Alphonse Bertillon, father of criminal identification, developed the science of anthropometry, which is a systematic procedure by taking a series of body measurements as a means of distinguishing one individual from another.[3] For two decades, Bertillon's method was

considered the most accurate method of identification but was replaced in the late 1900s by fingerprinting.

Richard Saferstein[3] notes in *Criminalist: An Introduction to Forensic Science* that Sir Arthur Conan Doyle had considerable influence in forensic and crime-detection methods through his fictional character Sherlock Holmes. It was in his 1887 book that Doyle describes scientific methods of detection years before they are actually implemented. Sherlock Holmes recognizes potential usefulness of forensic serology and anthropology throughout the book as he continues to solve difficult and uncanny crimes.

Francis Galton had determined from his studies that no two fingerprints are alike and that a person's fingerprints remain constant throughout the person's lifetime.[4] He published a book in 1892 called *Finger Prints*, which contained the first proof supporting the uniqueness of his methodology in identifying and classifying fingerprints. In his book, it listed the most common types of fingerprints, whorl, arch, and loop, and described the basic principles of fingerprint identification and classification that are still used today.[3]

In 1901, Dr. Karl Landsteiner discovered that human blood could be grouped into different categories as well as discovered the Rh factor in human blood. This discovery intrigued Dr. Leone Lattes, who developed a procedure for determining the blood group of a dried bloodstain.[3] Some forensic scientists continue to use this technique, known as serology, as a means to identify blood types between suspect and victims. As forensic analysis was modernized and used more frequently, gunshot residue analysis became equally useful in the advancement of forensic analysis.

Preceding modern methods of gunshot residue analysis, there was more emphasis on the physical aspects of ballistic mechanics, and consequently, identification with respective firearms.

In the nineteenth century, an early detective by the name of Henry Goddard, led an investigation regarding firearms.[5] Goddard deduced the identity of the culprit through ballistic analysis of the projectile, and the mold marks imprinted on the gun. Because of the mold mark being a unique intrinsic property of the firearm, Goddard was able to successfully identify the culprit through means of physical ballistic analysis.

Calvin Goddard[5], not related to Henry Goddard, refined the technique to compare a fired bullet with a test-fired bullet from the suspect weapon in 1925. His technique used a comparison microscope, which showed the obtained bullet and the test-fired bullet side by side. This technique is considered by many to be the hallmark event in the science of identifying firearms.[5] Not soon after, the Bureau of Forensic Ballistics was established, a service that provides firearms identification throughout the United States.

As time progressed, methods of physical ballistic analysis became more complicated, especially with increasing amounts of standardized weapons technologies. At this point, forensic scientists were more preoccupied with the chemical composition of the residue, as opposed to the aforementioned “tangible” methods of observation/analysis. Wolten et al.[6] were the first to provide, in full detail, the chemical composition of gun residue, using scanning electron microscopy. The four recurring elements in gunshot residue are lead, antimony, barium, and copper. Varying amounts of these elements, along with the presence of trace elements like sulfur, aid forensic scientists in determining whether gunshot residue is present at the scene of the crime, and possibly, the type of gun used. Modern advancements in gunshot residue analysis have proven useful; however, explosive residue analysis has proven just as effective in ascertaining the weapon used during the criminal event.

Preceding the discovery of nitrate explosive compounds, the only explosive available before the 1900s was black powder, which is a mixture of charcoal, sulfur and potassium nitrate.[7] The use of explosives were manifested in the military as shells that burst upon contact. By the 1900s, the Russians began using picric acid (2,4,6-trinitrophenol) (Figure 1) as their shell fillings, and other countries soon followed in the use of this compound. By World War I, the commonly known explosive, TNT (Figure 2), became the primary explosive used in the military.

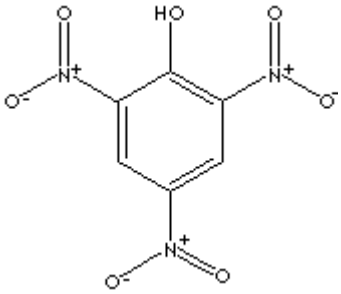

**Figure 1: Picric Acid**

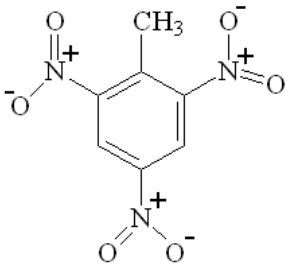

**Figure 2: Trinitrotoluene (TNT)**

As time progressed, new explosive compounds were produced. Competition between nations engaged in war, either domestic or international, ultimately led to innovation in weapons technologies with respect to explosive ammunition. Prior to 9/11, devices used to detect explosive residue were scarce. Yet after the terrible attack, the need to detect hidden explosives became imperative because of increasing security. The task to find hidden explosives has

become more complex due to expanding scenarios and threats, but the challenge to find these hidden explosives is offset by the increase in technological advancement.[7]

With regard to the modern practices associated with forensic science, and by extension, forensic analysis, it is evident that both disciplines have undergone respective changes through the progression of technology. As technology advances, new forms of analysis will continue to be developed. This thesis consists of gunshot residue, explosive residue and paper-based diagnostics that contribute to the history mentioned above. To contribute to the advancement of forensic analysis, a paper-based device is developed to detect gunpowder and gunshot residue.

**Explosive Residue**

With respect to forensic analysis of gunpowder and gunshot residue, yet another method of substance detection exists: explosive residue. Explosives are classified according to the speed of their reaction. High explosives such as trinitrotoluene (TNT) react quickly and therefore cause loud detonations, whereas low explosives such as fireworks react slower and cause deflagration.[8]

Ion mobility spectrometry or IMS is the common instrument used to detect explosives. This type of instrumentation is not only used in the laboratory, but it is also used at airports and border crossings. The IMS works by introducing a sample into the instrument, which will cause a soft ionization to occur via interactions with beta particles, and then separating ion-molecule clusters by their size-to-charge ratio (Figure 3).[9] As the particles move through the instrument, the ions are recorded based on the time it takes to reach the detector. The detector then sends out spectra to the computer for the scientist to analyze. Different ions will display different spectra.

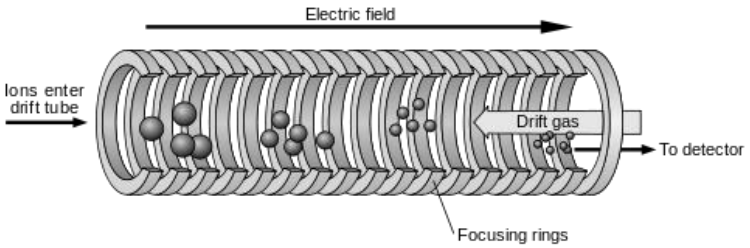


**Figure 3: Ion Mobility Spectrometry (IMS) Separating Particles by Charge and Size**

The IMS provides accurate determination of ions and compounds but the results take time to surface. Because of this, there is a growing need for a portable, quick, and efficient method for determining the presence of explosive residues.[10] Recently, Ma et al.[11] developed a fluorescent paper-based sensor that detects trinitrophenol (TNP) (Figure 4), a nitroaromatic explosive. They found that polymer-coated nanocomposites with aqueous solution are highly

luminescent when mixed. This led to the production of a rapid, cheap and convenient paper sensor for TNP detection.

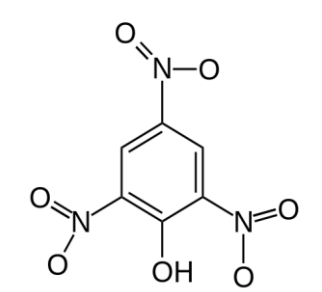


**Figure 4: Trinitrophenol (TNP)**

**Gunpowder and Gunshot Residue**

Gunshot residue or GSR refers to residues traceable to the primer used to ignite the propellant.[9] The traceable primers that identify GSR contain the inorganic residues of lead (Figure 5), antimony (Figure 6), barium (Figure 7), copper (Figure 8) and gunpowder residue.


**Figure 5: Lead**


**Figure 6: Antimony**


**Figure 7: Barium**


**Figure 8: Copper**

Gunpowder residues contain organic residues that are derived from the propellant powder. These include cresol, resorcinol, carbazole, carbanilide, nitrotoluene and many others.[12] Some of the common tested ones include diphenylamine (Figure 9), nitrodiphenylamine (Figure

10), and N-nitrosodiphenylamine (Figure 11), because diphenylamine is listed on the FBI's list of organic compounds.[12]

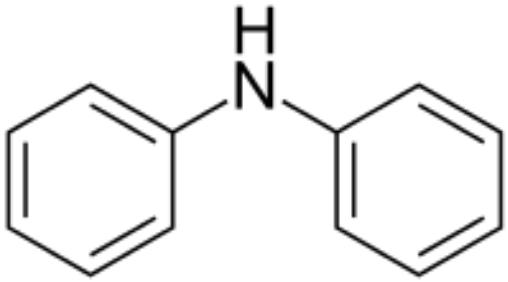


**Figure 9: Diphenylamine**

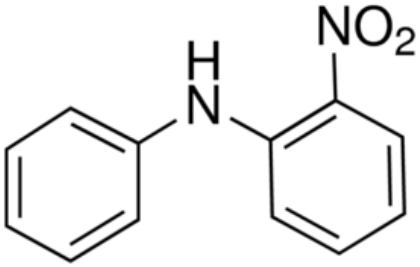


**Figure 10: Nitrodiphenylamine**

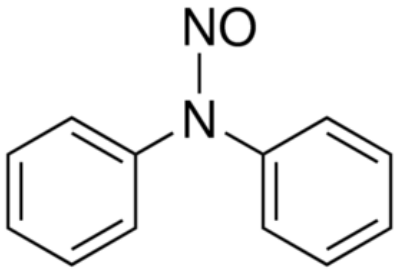


**Figure 11: N-Nitrosodiphenylamine**

The most common technique to analyze GSR involves the SEM/EDX (scanning electron microscopy-energy-dispersive X-ray). The GSR is collected using moistened swabs containing nitric acid and then it is introduced to the SEM/EDX. The instrument then uses its two functions to facilitate the examination of the suspected GSR. The SEM facilitates examination of the particulates under high magnification, while EDX allows the elemental analysis of the particles.[9]

Although the SED/EDX instrument is well established and frequently used, it does have its drawbacks. The technique the instrument uses is expensive and is very slow when analyzing the suspected GSR. The slow analyzation causes drawbacks in progression of evidence as well as delays in the advancement of cases. In addition, since not all primer residues from GSR are unique, it poses a problem when identifying the specific discharge of the firearms.[13]

**Paper-Based Diagnostics**

Paper-based analysis began in the field of medicine as a means to provide disposable, affordable, quick, and efficient diagnostics to places where medical practice is scarce. Although paper-based devices show potential within forensics, many paper-based diagnostics and devices have been fixated on point-of-care diagnostics. An example is Rohrman and Richards-Kortum[14], who developed a device that can conduct early HIV diagnosis for hospitals and facilities with limited resources. Their device, made of multilayer paper and substrate chips, analyzes dried blood drops and provides results more quickly than traditional methods.

Additionally, Pollock et al.[15] developed a device that can evaluate liver function. Their paper-based device uses a finger-stick specimen to detect measurements of aspartate aminotransferase (AST) and serum glutamic-pyruvic transaminase (SGPT or ALT) in the blood. The results in the device present themselves in 15 minutes, faster than regular laboratory testing. Khan et al.[16] use antibodies embedded in the paper to determine blood type. When the red blood cells encounter the paper, the antibodies begin to recognize the proteins in the blood and causes the blood to clump. This process will help determine what blood type was being tested. This device will help in quickly assessing a person's blood type.

Similarly, as to how medicine can use paper-based diagnostics to detect liver function and blood typing, forensics also has potential in using such devices to accelerate the analysis of evidence. Although no record of such advancement has been shown, this thesis will show that paper-based devices can also be used in the field of forensics. The paper-based device developed in this thesis will function by the use of microfluidics.

## Microfluidics

### Brief History of Microfluidics

Microfluidics is the science of manipulating small amounts of fluids in channels or zones for further analyzation. Due to its cheap and efficient use, microfluidics have become an attractive piece of technology for many researchers. Many forms of microfluidics have surfaced over the years since its first origin. In 1938, Izmailov & Shraiber[17] developed a device that separated plant extracts into different zones that could be analyzed from thin layers of absorbents. Izmailov called this a spot chromatographic method of analyzing data. Development of this device allowed the fabrication of zones or barriers on paper-based devices possible. The concept of having barriers and zones to retain liquids will be used in the development of the paper-based device used in this thesis.

T.I Williams[18] in 1947 developed an improved version of Izmailov's device, in which the absorbent layer was covered with a glass plate that contained a hole through which samples could be applied. This experiment provided a new improvement for efficient separation and quantification of organic and inorganic substances. The absorbent layer provided a means for scientists to be able to place compounds upon a layer that would not spill any liquid evaluated. Instead, the absorbent layer would retain the compound within the layer for later scientific use. A similar concept, in which an absorbent layer is used, will be used in this thesis as means to develop a functioning paper-based device.

By the beginning of the 1960s, microfluidic experiments plummeted. It was not until the late 1960s that N. Anderson used centrifugal microfluidics to create a clinical chemical analyzer. This device would measure the absorbance of light in order to further study the progress of

mechanical reactions. By using this device as a means of analyzing absorbance of light, Anderson proved microfluidic analysis can be performed in the device itself.[19]

In 1975, the first publication concerning the construction of a microfluidic separation device was fabricated in which the device, in a matter of seconds, was able to separate compound mixtures with a silicon wafer. The device consisted of an open-tubular capillary column, two sample injection valves, and a thermal conductivity detector, all fabricated on silicon wafer.[20] This experiment allowed further fabrication of microfluidic separation devices as well as proved that compounds can be manipulated without the need of using excessive amounts of liquid compounds.

In 1979, another microfluidic device fabricated with silicon wafer was developed. The device was built based on gas chromatography and consisted of a sample injection valve, a capillary column fabricated on substrate silicon wafer, and a mounted output thermal conductivity detector.[21] The system not only provided a complete analysis system in smaller quantities, but it proved to be useful in analyzing air quality monitors, such as portable contaminant analyzers, and planetary probes.

A manifold of capillary channels in a planar glass substrate was fabricated in 1992. With the fabricated device, the separation of fluorescein and calcein within the channels was achieved by using electrophoresis.[22] The flow of the solvent could be directed using the appropriate voltage so that switching of fluid between capillaries could be done. The results of this device provided a foundation for the design of more complex sample treated and separation systems that are integrated on glass and silicon substrates.[22]

By 1998, Duffy et al., created a procedure that made it possible for one to design microfluidic devices in PDMS, an elastomeric material-poly. The system proved to be useful in

prototyping microfluidics, which are widely used in drug screening, clinical diagnostics, and environmental monitoring.

In 2001, Dolomite, the world's first microfluidic application Centre, was founded. By this foundation, and the opening of The Dolomite Centre in 2005, the company has expanded the study of microfluidics by selling microfluidic devices to over 50 countries (Dolomite, 2015). Dolomite increased the availability of microfluidic device for others to use as the company continued to expand.

In 2007, Martinez et al.[23] fabricated the first paper-based microfluidic device, in which they described how to pattern paper with hydrophobic polymer to create a disposable assay system. The device, which made multiple diagnostic assay testing in one strip of paper possible, was able to simultaneously detect glucose and protein in artificial urine. With this fully developed paper-based technology, many scientists have found this device has provided a cheap, transportable, and compatible method to conduct many lab experiments on a smaller scale and for the ability to be used in less-industrialized countries.

**Paper-Based Microfluidics**

The earliest forms of μPADs were fabricated using photoresist, which formed a patterned coating on the surface and defined the barriers. Unfortunately, due to the cost of photoresist, scientist found this method to be undesirable and tried developing other methods to control the flow of fluids.[24] Today, μPADs can be created using wax patterning, inkjet printing, ink stamping and much more. By creating barriers using wax patterning in particular, scientists find this method provides a low cost, easily accessible fabrication technology that can contain fluid flow.[24] Wax patterning can be applied to paper with a multitude of techniques and can easily melt through the paper with heat to provide the desired barrier.

In order to detect and analyze compounds absorbed by the μPADs, colorimetry is used due to its simplicity. In addition, colorimetry technology is compatible with many smartphone-based reporting systems and therefore phone-based camera technology can be used within laboratories as an alternative to other expensive equipment.

Recently, a group of scientists have begun using paper-based microfluidics as a means of forensic analysis.[25] In this experiment, Pesenti et al.[25] use μPADs to detect explosive residues on surfaces. They found that the μPAD, on contact with aromatic explosive particles, will fluoresce, signaling the presence of an explosive. The creation of this device opens new opportunities to provide safety within our world, including airports, trains, grand events, and more. The simple process of having security officials rub the μPADs against luggage, purses, and book bags will reduce the need to search inside them. This reduces long wait lines as the μPAD will either quickly fluoresce, detecting the aromatic compounds of a dangerous device, or remain blank detecting no explosive.

**Purpose of Research**

Research concerning both microfluidics and forensic analysis are scarce in journal publications. In light of this, microfluidic devices that aid in forensic analysis remain an underdeveloped opportunity for new research and development. SciFinder, a chemical abstracts service website, was used to create the keywords shown below (Figure 12). While the number of publications featuring "microfluidics" have increased drastically over a decade, the number that feature "microfluidics and forensics" have remained consistently low.

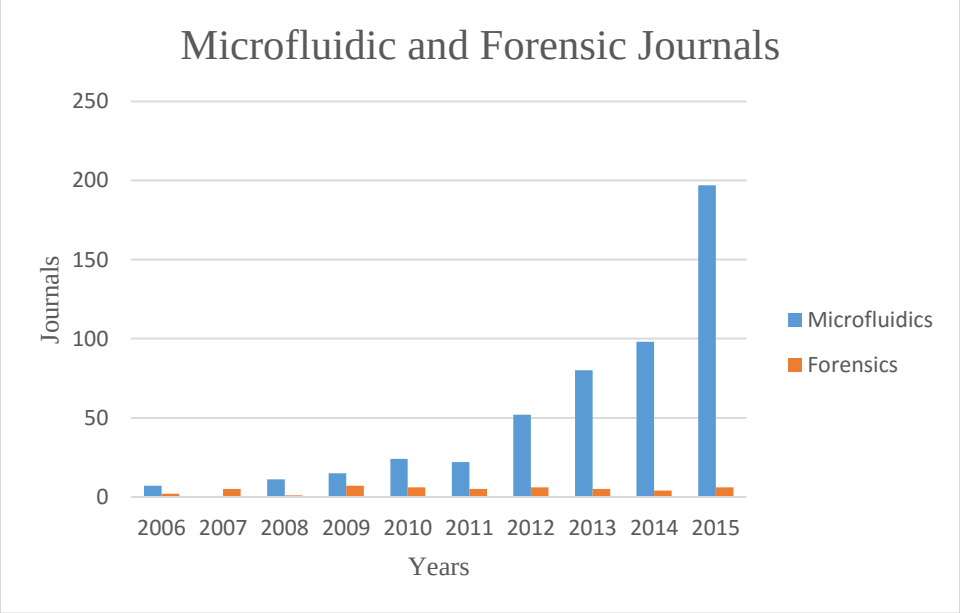


**Figure 12: Microfluidic vs. Forensic Journals**

Although there is research concerning a microfluidic device that can detect explosives, [25] there have been no reports to date of a microfluidic device's application for gunpowder and gunshot residue. Therefore, the goal of this thesis is to develop and analyze a paper-based microfluidic device that will be able to detect gunpowder and gunshot residue. The finalized

μPAD should change color upon detection of selected compounds. Success in developing this device will provide a disposable, cheap, and effective way to promote safety.

# Chapter 2

## Paper-Based Microfluidic Methodology

### Creation of Paper-Based Microfluidic Device

The purpose of creating a paper-based microfluidic device is to be able to isolate and analyze small quantities of fluids. AutoCAD was used to design a pattern of circles within a grid. The circles were designed to have diameters ranging from 6 mm to 8 mm, and hydrophobic borders with a 0.3 mm to 0.7 mm line width (Figure 13). The testing design was printed, using wax printer Xerox ColorQube, on multiple types of Whatman filter paper in order to compare absorption and reaction times in developing the paper based microfluidic device (Table 1). Full pictures of the microfluidic device were taken with a Samsung Galaxy S6 camera. Close up pictures of the microfluidic device were taken using a Bodelin ProScope Mobile Digital Microscope camera mounted onto the back camera of an iPAD.

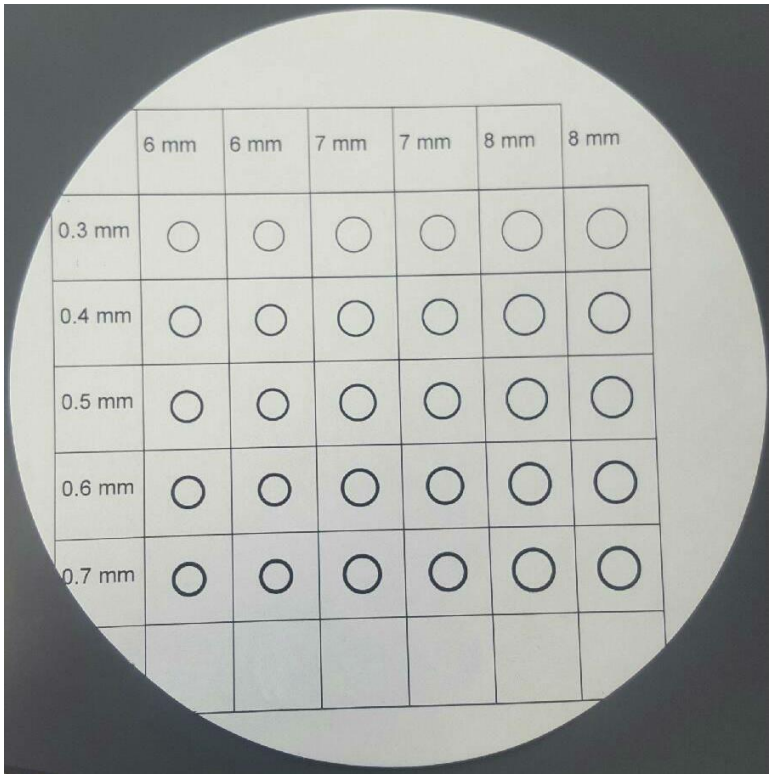


**Figure 13: Paper-Based Device Design**

| Grade | Paper Type | Diameter (mm) | Thickness (µm) | Pore Size (µm) |
|---|---|---|---|---|
| 1 | Qualitative | 150 | 180 | 11 (particle retention) |
| 4 | Qualitative | 150 | 205 | 20-25 (particle retention) |
| 5 | Qualitative | 150 | 200 | 2.5 (particle retention) |
| 41 | Ashless | 150 | 220 | 20-25 (particle retention) |
| 541 | Hardened Ashless | 150 | 155 | 22 (particle retention) |

**Table 1: Whatman Paper Types**

After the testing pattern was printed onto each type of filter paper with wax ink, they were transferred to an oven and heated for 15 minutes at 110°C to allow the wax to melt and flow through the fibers of the paper. This created a barrier that would prevent any liquids from passing through (Figure 14). After 15 minutes, the papers were removed from the oven and allowed to cool for approximately one minute before use. Closer examination of the wax paper showed the consistent expansion of the wax. The wax expanded outwards, leaving the size of the inside of the circle the same, adding an additional 0.20 cm to the overall border size.

**Figure 14: Whatman Paper Before Heating (left) and After Heating (right)**

After the paper cooled, the columns and rows of the microfluidic device were labeled to allow for easier explanation of the results. The 6mm to 8mm sets of columns were labeled 1-6, and the letters A-E were used on each row to designate the 0.3mm to 0.7mm borders (Figure 15).

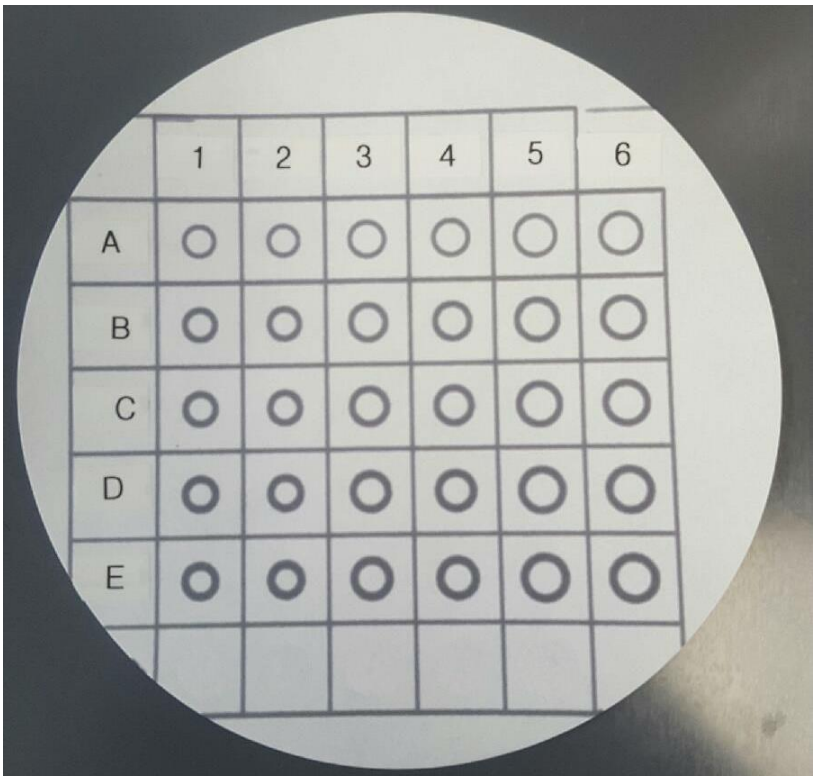


**Figure 15: Microfluidic Device Baked and Labeled**

**Chemicals**

All reagents and chemicals were of different grades. Gunpowder residues Diphenylamine, of reagentplus grade, and N-nitrosodiphenylamine, of technical grade, were purchased from Sigma-Aldrich. All certified reference material explosive solutions (1000 µg/mL) in acetonitrile comprising 2,4,6-trinitrotoluene (TNT), 1,3,5-trinitrobenzene (TNB) and 2,4,6-trinitrophenylmethylnitramine (tetryl) were also purchased from Sigma-Aldrich. Sodium

hydroxide (NaOH), of reagent grade, and concentrated sulfuric acid ($H_2SO_4$), of reagent grade, were obtained from Penn State Berks lab provided by the lab technician, Greglynn Gibbs.

**Preparation**

Explosive residue can be detected using basic reagents.[25] With this knowledge, 20.000 g of NaOH was mixed with 250 ml deionized water in a volumetric flask to prepare a 2 M solution of NaOH. The flask was capped and sealed to ensure the solution did not evaporate.

Gunpowder residues such as diphenylamine and N-nitrosodiphenylamine, can be detected using acidic reagents.[12] Dilutions of sulfuric acid were prepared for testing as listed in Table 2.

| Reagents |
|---|
| 18 M Sulfuric Acid ($H_2SO_4$) |
| 9 M Sulfuric acid ($H_2SO_4$) |
| 4.5 M Sulfuric Acid ($H_2SO_4$) |
| 2.25 M Sulfuric Acid ($H_2SO_4$) |
| 1.125 M Sulfuric Acid ($H_2SO_4$) |
| 0.5625 M Sulfuric Acid ($H_2SO_4$) |

**Table 2: Acidic Reagents**

**Testing**

For the explosive residues, 1,3,5-trinitrobenze, 2,4,6-trinitrotoluene, and 2,4,6-trinitrophenylmethylnitramine (tetryl), 3 µl of 2 M NaOH was applied inside each circle. In column 1 the NaOH was applied and allowed to dry for 1 minute. Then, 2,4,6-trinitrotolune was applied to column 1. NaOH was then applied to column 2 and immediately, while still wet, 2,4,6 trinitrotoluene was applied. In column 3 the NaOH was applied and allowed to dry for 1 minute. Then, 1,3,5-trinitrobenze was applied to column 3. NaOH was then applied to column 4 and immediately, while still wet, 1,3,5-trinitrobenze was applied. In column 5 the NaOH was applied and allowed to dry for 1 minute. Then, tetryl was applied to column 5. NaOH was then

applied to column 6 and immediately, while still wet, tetryl was applied. Table 3 provides a reference for the application of each reagent on Whatman paper.

| **Column** | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| **Application** | Dry | Wet | Dry | Wet | Dry | Wet |
| **Reagent** | TNT | TNT | TNB | TNB | Tetryl | Tetryl |

**Table 3: Column Reference for Explosive Residue Reagents**

The effect of sulfuric acid on the microfluidic device differed as the molarity of the $H_2SO_4$ increased. As shown in Figure 16 through Figure 21, as the molarity of sulfuric acid is increased, the more reactive the acid is with respect to the microfluidic device. Since 18 M, 9 M and 4.5 M of $H_2SO_4$ tremendously deteriorated the microfluidic device, they were not used for the remainder of the experiment. 2.25 M of $H_2SO_4$, a dilution of the acid that did not burn through the paper, was chosen to be used for the remainder of the experiment.

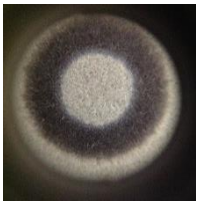

**Figure 16: 0.5625 M of $H_2SO_4$**

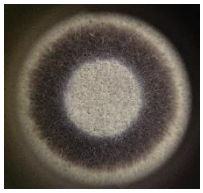

**Figure 17: 1.125 M of $H_2SO_4$**

**Figure 18: 2.25 M of $H_2SO_4$**

**Figure 19: 4.5 M of $H_2SO_4$**

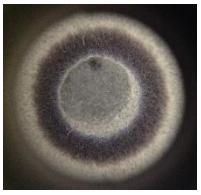

**Figure 20: 9 M of $H_2SO_4$**

**Figure 21: 18 M of $H_2SO_4$**

Gunpowder reagent, diphenylamine and N-nitrosodiphenylamine, were analyzed on different types of Whatman filter papers by first applying 3 µl of 2.25 M $H_2SO_4$ inside each circle. $H_2SO_4$ was applied to columns 1 and 2 and was allowed to dry for 1 minute. Then, gunshot reagents were applied to each column. Column 1 contained diphenylamine and column 2 contained N-nitrosodiphenylamine. Additionally, $H_2SO_4$ was applied to columns 3 and 4 and gunshot residue reagents were then applied immediately while still wet. Lastly the gunshot residue reagents were applied to columns 5 and 6 followed by $H_2SO_4$. Table 4 provides a reference for the application of each reagent on the Whatman paper. Abbreviations on the table are as followed: DPA – Diphenylamine and NDPA – N-nitrosodiphenylamine.

| **Column** | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| **Application** | Dry | Dry | Wet | Wet | Applied | Applied |
| **Reagent** | DPA | NDPA | DPA | NDPA | DPA | NDPA |

**Table 4: Column Reference for Gunshot Residue Reagents**

# Chapter 3

## Results and Discussion

Upon initial development, Whatman papers were first heated using a hot plate. Different temperature settings were used to determine the best temperature for the wax ink to melt into the Whatman paper. Unfortunately, when applying the reagents to these Whatman papers, it was discovered that some of liquids were seeping through the wax circle and spreading through the entire paper. It was also discovered that, if left too long or if the hot plate temperature was too high, the Whatman paper would burn. Additionally, since the hot plate has its main source of heat focused in the middle, any wax on the corners of the Whatman paper would be left improperly melted. To solve this issue, the Whatman papers were heated in an oven, where the overall heat would hit all parts of the paper. The oven was used for the remainder of the experiment to heat up all Whatman papers used in this experiment.

### Explosives

The motivation for the proposed work was the report of Pesenti's experiment for explosive residue analysis, in which a microfluidic paper device was created to detect and analyze explosive residues, specifically TNT, TNB, and tetryl.[25] 1,3,5-trinitrobenze, 2,4,6-trinitrotoluene, and tetryl, 3 µl of 2 M NaOH was applied inside each circle. In column 1 the NaOH was applied and allowed to dry for 1 minute. Then, 2,4,6-trinitrotolune was applied to column 1. NaOH was then applied to column 2 and immediately, while still wet, 2,4,6 trinitrotoluene was applied. In column 3 the NaOH was applied and allowed to dry for 1 minute. Then, 1,3,5-trinitrobenze was applied to column 3. NaOH was then applied to column 4 and immediately, while still wet, 1,3,5-trinitrobenze was applied. In column 5 the NaOH was applied and allowed to dry for 1 minute. Then, tetryl was applied to column 5. NaOH was then

applied to column 6 and immediately, while still wet, tetryl was applied. Table 5 provides a reference for the application of each reagent on Whatman paper.

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Whatman Paper | Dry TNT | Wet TNT | Dry TNB | Wet TNB | Dry Tetryl | Wet Tetryl |
| 1 | 1 minute | 1 minute | 1 minute | 1 minute | Immediate | Immediate |
| 4 | 1 minute | 1 minute | 1 minute | 1 minute | Immediate | Immediate |
| 5 | 1 minute | 1 minute | 1 minute | 1 minute | Immediate | Immediate |
| 41 | 1 minute | 1 minute | 1 minute | 1 minute | Immediate | Immediate |
| 541 | 1 minute | 1 minute | 1 minute | 1 minute | Immediate | Immediate |

**Table 5: Explosive Residue Reagent Reaction Times**

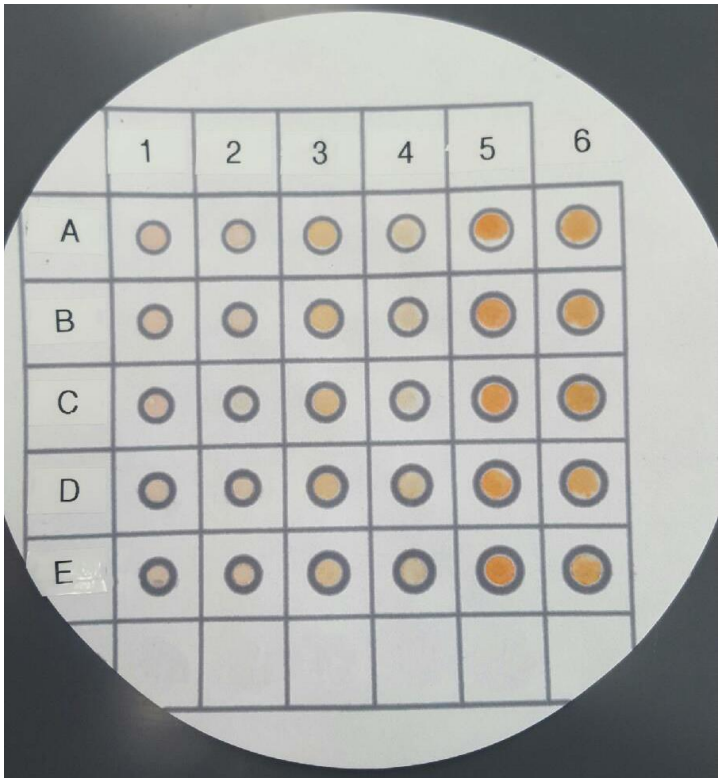


**Figure 22: Explosive Residue Reagent Results**

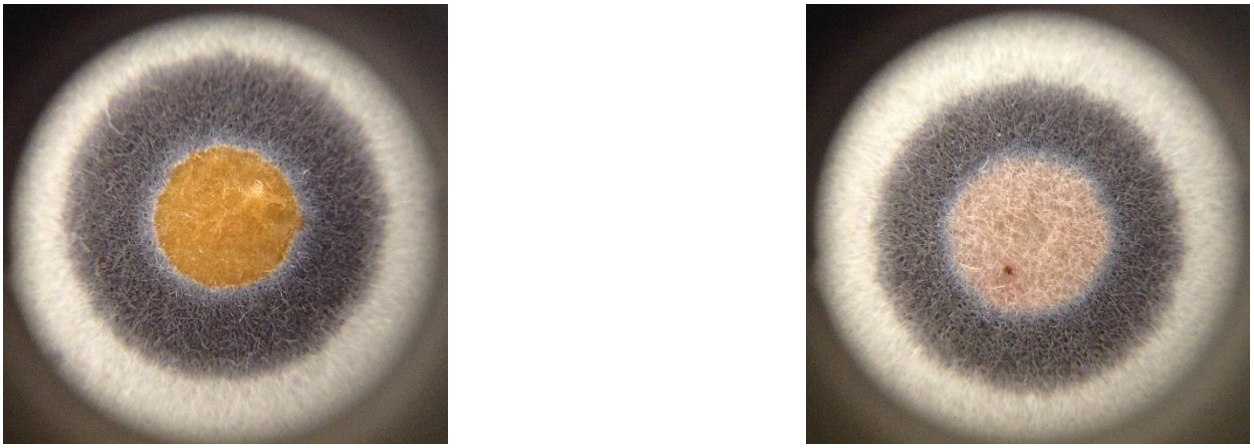

**Figure 23: Circle 1E: TNT Reaction after 1 Minute (left) and after 10 Minutes (right)**

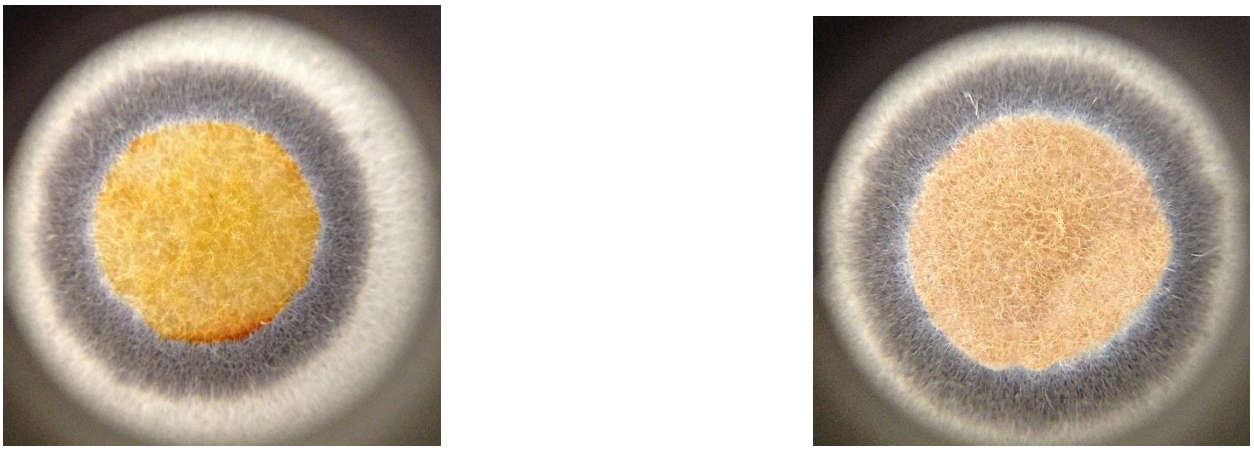

**Figure 24: Circle 3A: TNB Reaction after 1 Minute (left) and after 10 Minutes (right)**

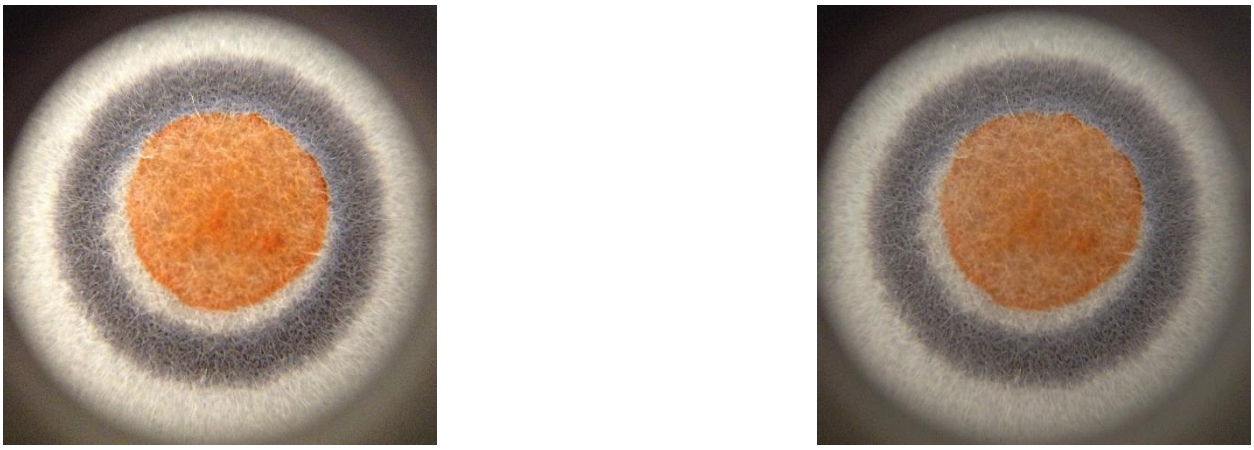

**Figure 25: Circle 6A Tetryl Reaction after 1 Minute (left) and after 10 Minutes (right)**

**Gunpowder and Gunshot**

Gunshot residue analysis is no longer routinely performed by the FBI due to the inability for gunshot residue to determine firing angles, the handedness of the shooter, the hand used to discharge the weapon, and the type of weapon used.[26] On the other hand, several laboratories across the U.S. still continue to examine gunshot residue due to its continued improvement through research, advancements, and more integrated communication among analysts.[27] Gunpowder residue, on the other hand, continues to be routinely performed in laboratories that conduct these type of examinations. The work performed here was modeled after the previously shown explosives analysis by looking at diphenylamine and N-nitrosodiphenylamine, applied to the paper device using three different methods and monitoring reaction time (Table 6). Abbreviations on the table are as followed: DPA – Diphenylamine, NDPA – N-nitrosodiphenylamine and NR – no reaction.

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| Whatman Paper | Dry DPA | Dry NDPA | Wet DPA | Wet NDPA | Applied DPA | Applied NDPA |
| 1 | NR | Immediate | NR | 3 minutes | NR | 3 minutes |
| 4 | NR | Immediate | NR | 3 minutes | NR | 3 minutes |
| 5 | NR | Immediate | NR | 3 minutes | NR | 3 minutes |
| 41 | NR | Immediate | NR | 3 minutes | NR | 3 minutes |
| 541 | NR | Immediate | NR | 3 minutes | NR | 3 minutes |

**Table 6: Gunshot Residue Reagent Reaction Times**

Upon testing the microfluidic device, only N-nitrosodiphenylamine reacted with 2.25 M $H_2SO_4$ by indicating a blue color as seen by Figure 26. Column 2, which was the dry column,

showed a reaction immediately after the application of the reagent. Figure 27 shows a close up of circle 2A for better visibility of the reaction. Column 4, which was the wet column, showed a reaction 3 minutes after the application of the reagent. Figure 28 shows a close up of circle 4D, which presents a darker blue color than Figure 27. Column 6, which was the applied column, showed a reaction 3 minutes after the application of the acid to the reagent. Figure 29 shows a close up of circle 6D for better visibility of the reaction. The reaction between the acid and N-nitrosodiphenylamine only lasts 17 minutes. As shown in Figure 30, columns 2, 4, and 6 stop showing the faint blue color that was previously seen in Figure 26.

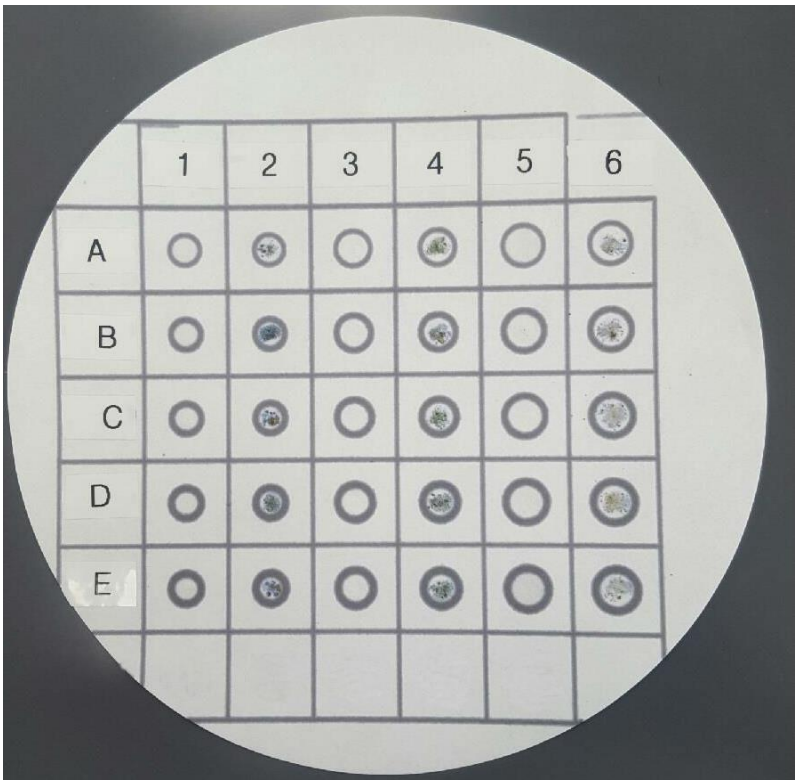


**Figure 26: Gunshot Residue Reagent Results**

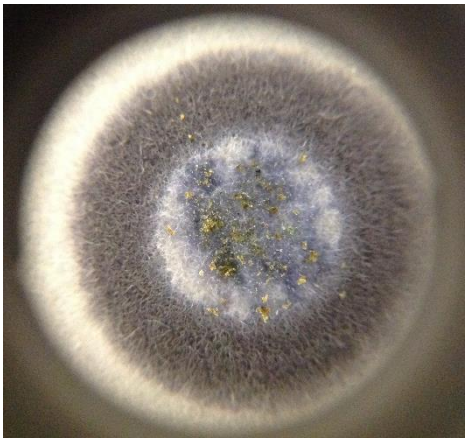

**Figure 27: Circle 2A: N-Nitrosodiphenylamine Close Up (Dry)**

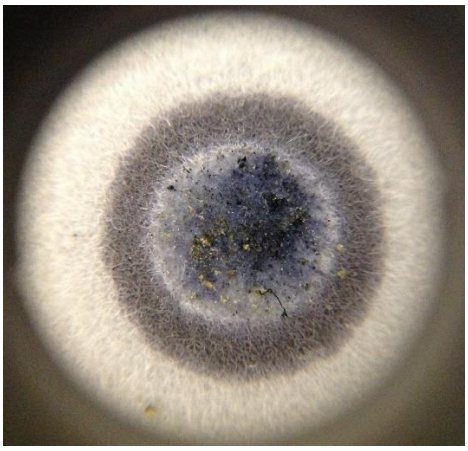

**Figure 28: Circle 4D: N-Nitrosodiphenylamine Close Up (Wet)**

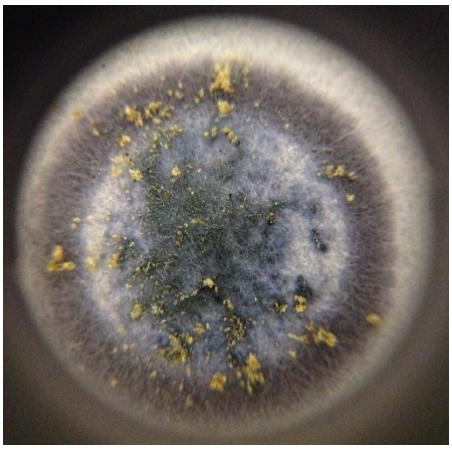

**Figure 29: Circle 6D: N-Nitrosodiphenylamine Close Up (Applied)**

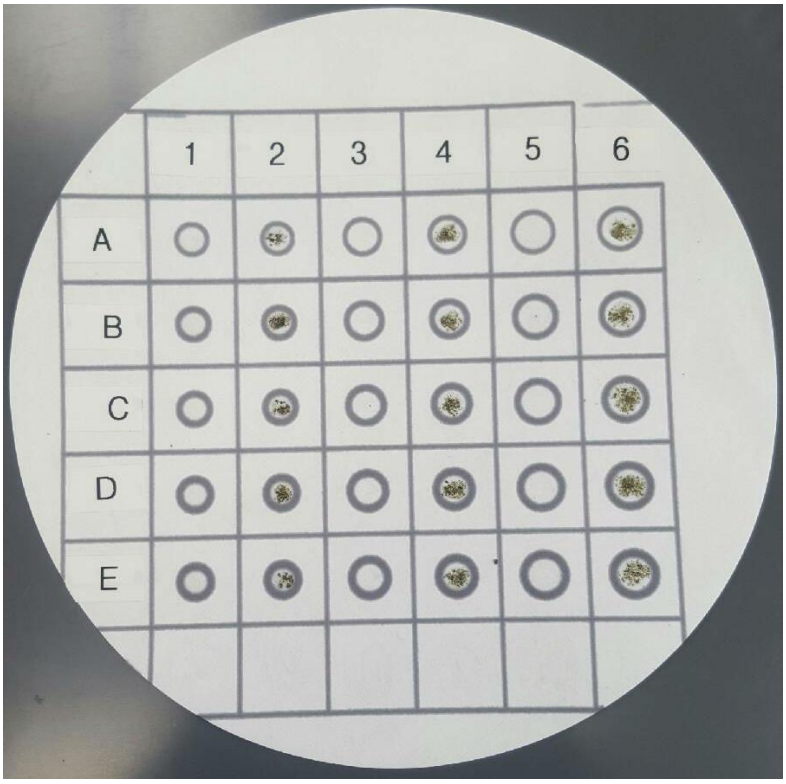


**Figure 30: Gunshot Residue Results after 17 Minutes**

Diphenylamine was the gunshot residue reagent that did not react to the 2.25 M of $H_2SO_4$. The reason for this lies within the overall structure of diphenylamine, Diphenylamine has no unpaired electrons in which the hydrogen ions in the acid could react to, therefore making it harder to bind the acid to the reagent. Instead, $H_2SO_4$ will have to break a double bond in order to bond with diphenylamine. The result is a colorless solution of diphenylamine and sulfuric acid. However, if we add nitric acid to the mixture, then a blue coloration will be given. The reason for this coloration is that in the presence of nitrates, diphenylamine is oxidized. The oxidation of diphenylamine is what gives the blue coloration that was previously seen in N-nitrosodiphenylamine.

N-Nitrosodiphenylamine on the other hand did show a reaction to 2.25 M of $H_2SO_4$. By looking at this reagent's structure the double bond O is a perfect place for the hydrogens in

$H_2SO_4$ to bond. By pushing the electrons in the double bond O, the reagent becomes negative. This negative charge causes the hydrogen in $H_2SO_4$ to become highly attracted to N-nitrosodiphenylamine. The breaking of the bonds causes the reaction to give a blue coloration. Once there are no more hydrogens for N-nitrosodiphenylamine to react with, the perceived blue coloration disappears.

Although the reaction for the explosive residues was a success, it must be noted that the microfluidic device might only detect explosives from the trinitro- family. Unless further testing is done with different types of explosive residues to say otherwise, the microfluidic paper may fail to detect homemade explosives. This, however, does not mean that explosives made specifically for massive destruction cannot be detected by the microfluidic device.

By examining the effect of each concentration of $H_2SO_4$ on each Whatman paper, it was determined that the color changing reaction occurred on each microfluidic device at the same rate. This indicated that the type of Whatman paper used did not affect reaction time. The only difference seen between the Whatman papers was the time it took for each reagent to absorb and dry into the paper. Whether or not the reagent was allowed to dry had no effect on the overall quality of the assay; it did, however, increase the time it took for the reactivity to be observed.

# Chapter 4

## Conclusion

This work represents a relatively simple and low-cost approach to making a detection device for gunpowder residue. The protocol for device preparation and testing was established as the experiment progressed using Pesenti's experiment of explosive residue as motivation for this work. The vision of this experiment was to see if this inexpensive, quick, and easy to use microfluidic device could be applied to the routines for crime scene investigation in law enforcement. The format of the microfluidic device was based on situations, which law enforcement officers might face during crime scene process.

Focusing on just gunshot residue, for the dry columns, an officer encounters a suspect who could be at fault for shooting a gun. The officer could pull the microfluidic device from the gunshot kit, rub the paper against the suspect's clothing, and wait for a possible reaction. For the wet columns, the officer, in the same situation, could apply a drop of $H_2SO_4$ on the paper and then rub the suspect's clothing and wait for a reaction to occur. For the applied columns, the officer could apply $H_2SO_4$ on the suspected object and wait for a reaction to occur.

The color reactions given by these microfluidic devices could be used as primary testing before the objects are packaged and sent to a lab for further processing. Although the microfluidic paper does not detect the exact gun that was used, it can provide the knowledge that a gun was used in the area or by the suspect. Future work into this area could possibly lead to a correct identification of a firearm used in a crime scene.

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## Education

The Pennsylvania State University — Spring 2016
B.S. in Science with Life Sciences Option — Chemistry Minor
Schreyer Honors College — 2012-present
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## Awards

Penn State Berks Science Program Award — April 2016
Undergraduate Research Fund Award — 2016-present
Penn State Berks Chancellor Scholarship — 2012-present
Academic Excellence Scholarship — 2012-present
Bunton-Waller Scholarship — 2012-present
Dean's List — 2012-present
Edwin S. Youse Scholarship — 2012-present

## Forensic Experience

Berks County Forensic Science Unit — January 2016-present
Internship run by the Berks County District Attorney's Office in which interns work closely with detectives of the Forensic Science Unit assigned to various disciplines including forensic photography, digital forensics, crime scene reconstruction, blood spatter analysis, fingerprint comparison and identification, crime scene investigation and processing, AFIS/IAFIS services and shooting reconstruction.

## Presentations

"Gun Residue Analysis Using Paper Microfluidics" — March 2016
Pittcon Conference & Expo at Georgia World Congress Center
Atlanta, GA

"Colorimetric Assay Using Indicator Dye Immobilized on Chromatographic Paper" — April 2015
Higher Education Council of Berks County's 16th annual Undergraduate Research and Creativity Conference at Albright College
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## Publication

"Stories Behind and Beyond the CPAAM Exhibits" — Fall 2012
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## Professional Affiliations

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## Club Activities

Penn State Berks Honors Club (Secretary) 2015-present
Penn State Berks Chemical Society (Secretary/Treasurer) 2014-present
Berks Orientation Leader 2014-present

## Community Service

Citizenship Day August 2014
Community Justice Projects June 2014-August 2014
Relay for Life 2012

## Language Proficiency

English and Spanish