Investigation of Mass-Manufactured Nanostructured Material for Contamination Detection via Structural Color Analysis

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INVESTIGATION OF MASS – MANUFACTURED NANOSTRUCTURED MATERIAL FOR CONTAMINATION DETECTION VIA STRUCTURAL COLOR ANALYSIS

By

Oisin Biswas

B.S., University of Maine, 2020

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An Abstract of the Thesis Presented in Partial Fulfillment of the Requirements for the Degree of Master of Science (in Biomedical Engineering) December 2021

The spread of the SARS-CoV-2 virus has underlined the importance of monitoring surface contamination of infectious elements on commonly used surfaces. Current monitoring methods of surfaces are both lengthy and expensive. The work in this project took advantage of the optical phenomenon of structural color to develop a rapid, low-cost, and contactless method of surface contamination detection. To accomplish this, a mass-produced material imprinted with a nanostructured pattern capable of exhibiting this structural color phenomenon was used. Structural color, or the bands of color that appear as different wavelengths of light reflect off a textured surface, can vary as a surface becomes contaminated. The diffraction patterns of this material creating these structural color effects were studied, where manual analytical techniques were developed to show quantitative differences in these effects when surface contamination was present. The developed techniques focused on three main features of interest in the diffraction patterns: light intensity, diffraction pattern length, and color presence. Light intensity was found to be the greatest indicator of surface contamination presence. Yet, with all three techniques, it was possible to detect surface contamination down to at least a volume of 1 x 10⁻¹ μL/ 64 cm². Manual detection of contamination was supplemented with machine learning technology as a proof-of-concept. Preliminary results showed the machine learning network’s ability in rapid classification of clean and contaminated diffraction patterns, with a 99% success rate. This work lays the foundation for the development of a rapid,
low-cost, and contactless method of surface contamination detection that could play a role in preventing the spread of infectious pathogens and other infectious materials.
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CHAPTER 1

INTRODUCTION

1.1 Overview

Following the onset of the SARS-CoV-2 pandemic, attention to the rapid assessment of surface cleanliness has intensified. Fomites, or inanimate objects that can become contaminated with infectious materials, can serve as potential avenues of transmission to and from humans.\(^1\)\(^-\)\(^5\) In some cases, these disease-causing pathogens can survive and remain active on fomite surfaces for several days, weeks, or months.\(^6\)-\(^8\) These findings have significant implications for human health, as the physical transfer of pathogens from fomite surfaces to humans can be a key factor in the propagation of infectious disease.\(^9\),\(^10\) Shared surfaces such as countertops, hospital equipment, public transportation interiors, and smart screens are an area of concern as they are in direct contact with a large number of people daily. This is furthermore underlined by the fact that in industrialized countries, around 90% of the average person’s time is spent indoors in close proximity of these kinds of surfaces.\(^11\)

There are currently several methods for assessing surface cleanliness. The first, and perhaps most basic, is visual inspection of a surface.\(^12\) The main advantages of visual inspection are the speed and cost. This form of monitoring can be seamlessly employed for large areas and can be implemented with little to no training. However, the disadvantages are its unreliability and subjectivity. Visual inspection is the most common method for monitoring surface cleanliness but is the least effective. Another method of surface inspection involves fluorescent markers. These markers are applied to a surface, subjected to a cleaning procedure, and then evaluated under ultraviolet light for an assessment of the cleaning method. Fluorescent markers are useful in that they are fast, cheap, and objective evaluators of cleaning techniques.\(^13\) They additionally may improve the quality of surface cleaning practices. On the other hand, the fluorescent markers don’t necessarily inform the user of a surface’s current cleanliness state. They are only used for evaluation of practice, where emphasis is placed on easily visible and accessible surfaces rather than those
that are heavily touched. The final method of evaluating surface cleanliness are microbiological cultures. This method involves using swabs to quantitatively assess the presence of pathogens on a surface.\textsuperscript{13,14} Of the most commonly used practices, microbiological cultures are the most sensitive and specific method of surface assessment. However, there are drawbacks to this solution. The tests to assess the cultures can be both costly and time consuming, requiring a lab capable of running the tests. Personnel involved with the cultures must be adequately trained in lab machinery as well as result interpretation. Additionally, the sample obtained is only an indication of the cleanliness of a small portion of a surface. These disadvantages of all the discussed current methods prevent these current solutions from being ideal continuous monitoring methods of surface cleanliness for a whole host of applications. What is needed is an easily accessible, low-cost, and rapid method of surface contamination detection. A method that is additionally contactless and sensitive enough to detect contaminants that are not easily visible with the naked eye.

1.2 Structural Color

As opposed to color via pigmentation, where color is observed due to a specific material absorbing and reflecting particular portions of the visible light spectrum, structural color arises as a result of the interference of visible light with features of a nano-scale.\textsuperscript{15–21} This phenomenon of rich colors appearing on surfaces is widely present in nature, such as the iridescence observed on select species of beetles,\textsuperscript{22,23} the metallic blue of the \textit{Pollia} fruit,\textsuperscript{24} and the intense blue wings of the \textit{Morpho} butterfly.\textsuperscript{25} As this natural phenomenon has been selected for through billions of years of evolution, great effort has been undertaken to appreciate the complexities surrounding structural color in the hopes of repurposing the concept for new applications.\textsuperscript{26,27} Currently, bio-inspired structural color materials are used in a wide variety of applications but tend to be found in light-emitting sources, fashion materials, cryptography, and sensor technologies.\textsuperscript{28–30} In this project, we aim to utilize the concept of structural color as a rapid, contactless, and low cost monitoring method of surface cleanliness.
As structural color arises from the interference of light with features on a nanoscale, there are multiple types of materials and optical scenarios that can accomplish this. The sources of structural color vary both in material type and specific physical interaction of light. There are four basic sources of structural color: photonic crystals, thin-film interference, multilayer interference, and diffraction gratings. Each source will be discussed in detail in the following sections.

1.2.1 Photonic Crystals

Photonic crystals are a basic source of structural colors. In a general sense, photonic crystals are classified as highly ordered nanostructures of two media, with different refractive index values, arranged in a spatially periodic fashion. Therefore, by the same definition, the multilayer interference systems previously discussed could be classified as 1-dimensional (1D) photonic crystals. Both 2-dimensional (2D) and 3-dimensional (3D) photonic crystals exist as well, where most commonly used photonic crystals are periodic circular holes or rods (Figure 1.1).

These structures contain repeating regions of high and low dielectric constant, where light waves will propagate through the structure or not. The propagation of light through the structure is determined by the cut-off wavelength. Photons that are allowed to travel through the structure are called modes, and...
groups of these allowed modes are referred to as bands. Photons that are not allowed to propagate through the structure are grouped as photonic band gaps. If subjected to light with a frequency within the bandgap, the light will not be allowed to propagate and will instead be reflected. The reflection of this light gives the appearance of a particular color.

1.2.2 Thin-Film Interference

The next, and most simple, source of structural color is thin-film interference. In thin-film interference, light from an incident beam travels through a medium with refractive index $n_1$ and onto a thin film of refractive index $n_2$. As light travels through the medium and boundary of the thin film, each with their own refractive index values, some of the light is reflected and some is refracted at each boundary of the thin film (Figure 1.2). The two reflected waves subsequently interfere with each other resulting in a new unique wave. The optical path difference (OPD) between the two reflected waves is given by

$$\text{OPD} = 2n_2 d \cos \theta_2$$  \hspace{1cm} (1)

Where $\theta_2$ is the angle of the refracted light, $n_2$ is the refractive index of the thin film, and $d$ is the thickness of the film. The condition for constructive interference is satisfied when light travels from a medium of refractive index less than that of the thin film ($n_1 < n_2$) of order and wavelength $m$ and $\lambda$, respectively with the following equation

$$2n_2 d \cos \theta_2 = (m - \frac{1}{2}) \lambda$$  \hspace{1cm} (2)

On the other hand, when light travels from a medium of refractive index greater than that of the thin film ($n_1 > n_2$) of order, $m$, and wavelength, $\lambda$, the condition for constructive interference is described with the following equation

Figure 1.2 Representation of light reflection and refraction in thin-film interference. Reproduced from Fu et al. 2016.
These relationships outline the dependence of the resulting reflected light wavelength to certain dimensions and geometric configurations of a specified material. In this way, it can now be understood how preferential colors are reflected from a material that is subjected to a white light source.

### 1.2.3 Multilayer Interference

As opposed to the basic thin film layer interference, multilayer interference occurs in conditions of periodically stacked thin films with alternating levels of refractive indices.\(^40\) This material, with two distinct layers \(A\) and \(B\) is outlined in Figure 1.3. As light is incident upon this multilayer material, like thin film interference, some light will reflect while other will refract through the first layer. This process of reflection and refraction will now, however, repeat multiple times through the layers present in the material.

![Figure 1.3 Representation of light reflection and refraction in multilayer interference with layers A and B. Reproduced from Fu et al. 2016](image)

In this scenario, the condition for constructive interference is given by\(^39\)

\[
2n_2d\cos\theta_2 = m\lambda \quad (3)
\]

From equation (4) it is apparent that the observed color from the multilayer interference is dependent upon the observation angle. Or, in other words, as the angle of observation changes, a decrease in the wavelength (\(\lambda\)), and a blueshift in resulting color is observed. The resulting coloration of the multilayer interference tends to be more selective, in the colors produced, as the number of layers present increases, yielding sharp reflection peaks with strong angular dependence.\(^38,39\)
1.2.4 Structural Colors in Nature

Structural color has been a trait that has been selected for through evolution, as evidenced by its wide presence in a myriad of organisms.\textsuperscript{41,42} Whether it be attraction for mating or protection from predators, the rich colors produced by this natural phenomenon find themselves ever present in the natural world. One pertinent example of structural color in nature comes from Morpho butterflies which exhibit an intense blue color from their wings (Figure 1.4A).\textsuperscript{43} Examination with transmission electron microscopy (TEM) of the wings from Morpho butterflies reveals the presence of a nanostructure on the surface of the wings.\textsuperscript{25,39,44,45} The nanostructure is specifically observed to be rows of ridges and lamellae (Figure 1.4B). As a result of the multiple rows, the layers of complex wing structures can be treated as a multilayer interference system. The rows of ridges and lamellae are supplemented with air gaps between them, yielding a multilayer interference system that preferentially reflects blue light as described by the interference condition in equation (4). In an evolutionary sense, this trait was selected for several reasons: the bright blue color assists males in attracting a mating partner, the flashing blue color as angle of observation varies can play a role in disorienting predators, and the blue color represents a territorial claim against other male Morpho butterflies.\textsuperscript{38}

\textbf{Figure 1.4} (a) Morpho \textit{rhetenor} butterfly with brilliant blue color in presence of light. (b) Morpho \textit{rhetenor} butterfly scales under low magnification. (c) Morpho \textit{rhetenor} butterfly scales under high magnification. (d) TEM image of the complex nanostructured wing scale with rows of ridges and lamellae. Reproduced from Fu et al. 2016
The *Mycterophallus* cetoniine scarab beetle offers another mechanism of structural color in nature. In the presence of light, the scarab beetle emanates a red iridescence color from the posterior area of the insect. As was the case with the *Morpho* butterflies, this phenomenon is directly related to the complex organization of the beetle’s surface structure. Investigation of this cuticle surface with TEM revealed the presence of many alternating layers of high and low density chitin (Figure 1.5A). The presence of the alternating layers, each with distinct refractive indices, allows us to treat the cuticle surface as a multilayer interference system. Light that interacts with this system is subjected to multiple rounds of reflection and refraction, culminating in constructive interference of preferential colors. This effect can be mathematically described by

\[ n^2 = \frac{l_1n_1^2 + l_2n_2^2}{l_1 + l_2} \]  

(5)

Where \( l_1, l_2, n_1, \) and \( n_2 \) represent the thickness and refractive index, respectively, of alternating layers of chitin. The average refractive index, with the alternating layers taken into account, is represented by \( n \). The wavelength of maximum diffraction, then, is represented by

\[ \lambda = 2(l_1 + l_2)n \]  

(6)

Assuming refractive index values of 1.55 and 1.68 for the alternating layers of chitin, the wavelength of maximum diffraction is predicted to be around 709 nm, agreeing with what is experimentally observed in the wild (Figure 1.5B).
An additional mechanism of structural color in nature involves light’s interactions with periodic surfaces, such as a series of parallel grooves. The seed-shrimp, *Azygocypridinia lowyri*, were one of the first organisms in nature that were found to possess surface structures mimicking diffraction gratings (Figure 1.6A). Its highly ordered, periodic surface structure of antenna hairs had grooves that approximately matched the wavelength of light. As a result of this specific surface structure, the antennas of the seed-shrimp reflected different colors of light as the angle of observation was varied (Figure 1.6B). The appearance of the angle-dependent, reflected light is due to the specific interaction of light with the groove surfaces. As the light hits the grooves, some light will be spread differently. The successive grooves that interact with the light will alter the path length of the incident light. The path difference in the incident light results in the reflection of preferential colors at certain angles. This can be mathematically described as:

\[ 2d(sin\theta_i - sin\theta_m) = m\lambda \]  

(7)

Where \( \theta_i \) and \( \theta_m \) refer to angles of incidence and diffraction, and \( d \) refers to period of the structure. This highlights an important concept, in that the wavelength of light, or color of reflected light, changes depending on the diffraction order and periodicity of the surface. Hypothetically, when dimensions of these quantities change, specifically the periodicity, the observed light wavelength will differ as well. Diffraction gratings will play a large role in the project and will be focused on in greater detail.
1.3 Diffraction Gratings

1.3.1 Basic Principles of Diffraction Gratings

Diffraction gratings are widely used optical components that contain a highly ordered periodic structure on a wavelength scale.\textsuperscript{51} Light that interacts with these kinds of components is reflected or transmitted at specific angles only. The discovery of diffraction gratings began in 1786, when F. Hopkinson observed peculiar light propagation through a silk handkerchief stretched between his hands.\textsuperscript{52} His initial observation encouraged the investigation of light impinging upon periodic structures and further garnered interest for the controlling of geometric features at a nanometer scale. These developments allowed diffraction gratings to be considered the first optical components of modern nanophotonic technology and have played a role in the enhancement of micro and nanotechnologies. The advent of high-powered lasers has allowed diffraction gratings to be developed with the utmost precision, pushing them to the limits in terms of technological influence. Typically, diffraction gratings are used in a wide variety of applications such as spectroscopy, chirped pulse amplification (CPA), sensing technology, telecommunications, and astronomy.\textsuperscript{51} An example of a diffraction grating is outlined in Figure 1.7. The angles of light propagation can be mathematically described by\textsuperscript{51}

\[
\sin \theta_m + \sin \theta_i = \frac{m \lambda}{d} \quad (8)
\]

Where $m$ represents the diffraction order, $d$ represents the grating period, $\lambda$ represents the wavelength of light, $\theta_m$ represents the angle between the diffracted angles, and $\theta_i$ represents the angle between the incident angle and normal to the surface. The diffraction order $m$, is an

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Typical diffraction grating with geometric dimensions represented by $d$ and $h$, representing the period and groove depth, respectively. Light diffraction pattern is also outlined with varying orders of diffraction. Adapted from Bonod and Neauport 2016.}
\end{figure}
integer that when equal to 0, signifies specular order, where no spectral properties occur and the surface acts as a mirror. When \( m \) is not 0, however, a diffraction pattern is observed as shown in Figure 1.7.

Diffraction gratings can be tailored to achieve optimal grating efficiency, for a specific diffraction order \( m \), by achieving an incidence near the Littrow incidence. The Littrow incidence describes the angle of diffracted light, where the diffracted light is opposite to the angle of incident light \( (\theta_m = -\theta_i) \) and is mathematically represented by\(^53\)

\[
2\sin\theta_{m,L} = \frac{m\lambda}{d} \quad (9)
\]

The \( m^{th} \) order reflected beam will reflect in the same direction as the incident beam. As a result of this finding, blazed gratings are specifically designed to achieve incidence approaching the Littrow incidence to achieve the highest diffraction efficiency.

There are several methods used to manufacture diffraction gratings. The first gratings were mechanically ruled until the development of holographic gratings.\(^54\)-\(^56\) In holographic gratings, requiring the use of lasers, a photo-sensitive layer is coated onto a substrate to create an interference pattern by superimposing two coherent beams. In this way, an interference pattern is recorded onto a photographic plate, yielding sinusoidal modulation.\(^51\) The grating period in this manufacturing method is described by\(^57,58\)

\[
d = \frac{\lambda}{2\sin\theta} \quad (10)
\]

Where \( \theta \) corresponds to the angle of the laser beams and \( \lambda \) is the wavelength of the laser beams. Holographic gratings constructed in this fashion are desirable for large surface gratings as the manufacturing process is fast due to the one-time modulation exposure.\(^59,60\) More recently, scanning beam lithography was developed as a way to address one particular issue with holographic manufacturing. In holographic manufacturing, the maximum possible size of grating that is able to be generated is limited by the size of collimators.\(^61\)

Scanning beam lithography takes advantage of benefits from both ruling and holographic grating production.
methods. Similarly, electron beam lithography is another more recent grating manufacturing method and can be used with direct-writing or photo lithographic processes.

1.3.2 Structural Color in Diffraction Gratings

1.3.2.1 Interference of Waves

The behavior of light can generally be classified as wave-like. This finding was initially demonstrated by Young’s slit experiments in 1801. In Young’s slit experiment, a light source illuminates a plane that is pierced by two slits. Light passes through these two slits and is observed on a screen behind the plane (Figure 1.8A). Assuming the light source is emitting a plane wave, once the light passes through the two slits, two spherical waves are generated and interfere with one another. At some points within this space of interaction, the two waves will constructively interfere. At other points, the two waves will destructively interfere. In constructive interference, two waves will exert an additive effect on one another resulting in a bright spot (Figure 1.8B). In destructive interference, two waves will exert a diminishing effect on one another, or in other words, will cancel each other out resulting in a dark spot (Figure 1.8C). It is possible to predict where bright spots, a product of constructive interference, and where dark spots, a product of destructive interference will be observed.

![Figure 1.8](image.png)

**Figure 1.8** A) Light interference from Young’s double slit experiment. The light enters the scene as plane waves and are converted into identical spherical waves from the two slits. The two waves interfere with one another, and an interference pattern is observed from the viewing plane. B) Additive effect of waves constructively interfering. C) Destructive interference of waves, resulting in dark spot.
If $\Delta L$ is defined as the path difference of light travel, then $\Delta L$ must be equal to an integer multiple of $\lambda$, the wavelength of light. Once this case is satisfied, the two waves will constructively interfere, adding with one another, and resulting in a bright spot on the viewing plane. On the other hand, if the path difference $\Delta L$ is not equal to an integer multiple of $\lambda$, then the two waves will not be in phase and will constructively interfere, resulting in a dark spot. This reliance of the path difference of light is represented in Figure 1.9.

1.3.2.2 Separation of White Light into Constituent Wavelengths

With interference of light waves in mind, it can now describe how diffraction gratings can exhibit structural color effects reminiscent of those observed from the natural world. Similar to Young’s slit experiments, light interacting with gratings will constructively and destructively interfere with each other. This interaction is dependent upon the wavelength of light, diffraction order, and period of grating.

The relationship can be represented by

$$\sin \theta = \frac{m\lambda}{d}$$

(11)

where $\theta$ denotes the angle from grating peak to spot of constructive interference, $m$ denotes the diffraction order, and $d$ denotes the grating period. Inspection of equation (11) underlines the reliance of bright spot

\[\text{Figure 1.9 Single and double slit interference experiments. Regions of constructive and destructive interference and represented by presence of red peaks. Constructive interference, or bright spots, will occur when } \Delta L = m\lambda \text{ where } m = 0, 1, 2, \ldots n. \text{ Reproduced from Wu 2020.}\]
occurrence to both the wavelength of light and geometric dimensions of the grating. Furthermore, equation (11) crucially relates $\theta$ to the wavelength of light, $\lambda$. In other words, the regions of constructive interference, regions where bright spots of color are observed, will be different for each distinct color or wavelength of light.\textsuperscript{49,64,65} This means that as white light is sent towards our grating, the white light will be broken up into its constituent colors like red, green, and blue (Figure 1.10).

**Figure 1.10** Large diffraction grating splitting white light at high incidence into its constituent wavelengths. Reproduced from Bonod and Neauport 2016.

### 1.3.3 Rainbow Coated Film 947

The material our team has been using is already mass-produced by Sappi North America, Inc. for use in the textile industry.\textsuperscript{66} This system of interest contains a nanostructured pattern that is coated onto a thin film layer, forming two distinct layers. The highly organized, periodic structure acts as a diffraction grating, where the optical effects associated with diffraction gratings were described in detail in Section 1.3.1.\textsuperscript{67} In other words, when light is incident upon the surface of this textured material, the reflected light is separated into its constituent wavelengths.\textsuperscript{68} The resulting effect enables white light that interacts with the material to reflect towards the viewer’s eye in a whole host of colors such as red, green, and blue (Figure 1.11).\textsuperscript{69}
1.4 Structural Color Analysis

1.4.1 Color Representation

Any analysis with structural color effects will likely include the specific investigation of color. The analysis of color requires the understanding of color representation, or in other words, the formal and mathematical formulas that allow colors to be projected onto computers or other devices. There are multiple color models that use different formulas to represent the color spectrum, with the ultimate goal being to enhance machine representation of the human understanding of colors. Color models essentially explain the way in which colors can be represented, and specify numerical elements of colors to generate a color space that represents the general spectrum of colors. Digital images that are represented through computers have three primary channels: red, green, and blue. These colors are differentiated by the parameters of brightness, hue, and saturation. Furthering this explanation, each color can be represented by two components, luminance and chrominance. Luminance refers to the brightness, while chrominance refers to the hue and saturation. What color models will do, is take the numerical components that represent specific colors, and will use mathematical functions to represent these components as coordinates in a three-dimensional space. Color models will vary in the functions used to attain the three-dimensional representation of the color spectrum.

1.4.2 Color Models

There are three categories of color models: device-oriented, user-oriented, and device-independent models. In device-oriented models, the color representation is affected by the signal of the device. Or in other words, the representation of color varies by hardware used to display digital image. In user-oriented models, the representation of color can be translated between the user and the device used to view the color, usually through specifying chromaticities of the three primary channels. This model is considered to be the middle ground between device-oriented and device-independent models. In device-independent models, the color model is not affected by the device used to obtain images, and will be the same regardless of hardware.
Munsell Color Model.

One of the first color representation models was the Munsell color model, developed by Professor Albert Munsell.\(^1\) A device-independent model, the Munsell model represented the color spectrum as a cylindrical shape with three dimensions. The components constituting these dimensions were value (lightness), hue, and chroma. The value was used to represent lightness, and was divided into eleven sections ranging from 0, black, to 10, white. The hue was a circle shape that was broken into ten sections: red, yellow-red, yellow, green-yellow, green, blue-green, blue, purple-blue, purple, and red-purple. The chroma represents the saturation of the combination of hue and value, with a range of \([0, 12]\).

RGB Color Model.

The RGB model is a device-dependent model derived from the three additive primary colors: red, green, and blue. In the light spectrum, the colors are combined to represent one color. These colors can be mixed in different weights and generate different colors.\(^2\) There are two different forms of the RGB model, linear and non-linear. In both, the color space is represented as a three-dimensional cube. The models differ, however, in the dimensions of the cube. In the linear-RGB model, the values for each component (R,G, and B) have a range of \([0, 1]\) (Figure 1.13). The coordinate \([0,0,0]\) would represent

![Figure 1.12 Munsell’s color system with cylindrical shape in three dimensions. Reproduced from Ibraheem et al. 2012.](image1)

![Figure 1.13 Linear RGB color model. Reproduced from Ibraheem et al. 2012.](image2)
black, while [1,1,1] would represent white. For the non-linear RGB model, the coordinates have a range of [0, 255]. Typically, JPEG file types are stored with RGB data in this fashion.

**HSV Color Model.**

The HSV color model is derived from the HSL family of color representation. The HSL family of color models use a cylindrical representation of the color spectrum, mainly relying on hue and saturation of the chrominance component of color. These models are linear transforms of the RGB models, and are considered user oriented-device dependent models. The HSV model is heavily used in image analysis, especially with segmentation processes. A hexacone is used as the specific, three-dimensional representation of the HSV color space, where the central vertical axis represents intensity. The hue is defined as an angle in the range of [0, 2π]. Saturation is the purity of color and is specifically the radial distance from the central axis to the outer surface. Figure 1.14 demonstrates the color space represented through the HSV model.

**1.5 Goals of the Project**

The goal of this project was to establish a proof-of-concept in using low-cost, easily producible materials as a sensor for surface contamination. Continuous monitoring of surface contamination is of the utmost importance, especially following the SARS-CoV-2 pandemic. However, the discussed solutions to assess surface cleanliness are not convenient for a wide variety of applications. The solutions to this issue can be too time consuming, costly, or ineffective. In developing a low-cost sensor that works at the speed of light, our team will play a major role in delivering a well-needed solution to this problem. To work towards this desired achievement, the structural color effects of Sappi’s Rainbow Film 947 were quantitatively investigated to assess the ability of the material to serve as an indicator of surface contamination.
contamination. A custom-built imaging chamber was designed and manufactured to enhance the investigation of structural color effects. Work also began in conjunction with a machine learning team to lay the groundwork for machine learning integration into the problem solution. Generation of thousands of images of structural color effects from the material were fed into a convolution neural network to train a computer capable of identifying structural color effects indicative of surface contamination.
CHAPTER 2

MATERIALS AND METHODS

2.1 Rainbow Coated 947 Film Material

2.1.1 Manufacturing of Material

The rainbow coated 947 film material was obtained from the Release Paper Group at Sappi North America’s manufacturing plant in Westbrook, Maine. The material is manufactured on an industrial scale through Sappi’s Ultracast process (Figure 2.1). In this process, the highly structured material is generated through wrapping the liquid-coated material against a textured roll, molding the coating against the liquid coating. This detailed coating, is then polymerized through high-energy electron beam. The Ultracast process enables Sappi to produce various materials, such as the rainbow coated 947 film, with the utmost repeatability and replication.

2.1.2 Sample Preparation

The rainbow coated 947 film material was delivered in large rolls. Individual, square samples were generated from these large rolls to run experiments of interest. The bulk rolls of material were rolled out in such a way to ensure that the visible striations of the material were positioned in a North to South fashion. This was done to be sure that all samples were cut with their surface structures oriented in the same direction. From this position, a 3D printed square shape was used to draw guides to cut 8 x 8 cm squares of individual samples from the roll. A black dot was marked in the top left corner of the individual samples to mark the appropriate orientation of the generated samples and their associated surface structure direction.

Figure 2.1 Sappi’s Ultracast process. This manufacturing process enables the production of highly ordered materials on an industrial scale. Reproduced from Reference [70].
On the rolls of bulk material, there were areas where certain bumps or crinkles were present. Individual samples were not obtained from these areas.

2.2 Surface Characterization

2.1.1 Microscope Information

Surface characterization images in this work were obtained from the Keyence VHX-7000 digital microscope. All images were taken at a tilt angle of 0°. The lenses used were: E20:X40, E100:X100, and E500:X2500. The images from the microscope were generated by John Belding from the Advanced Manufacturing and Composites Center (AMC) at the University of Maine.

2.3 Initial Angle Dependence Observations

2.3.1 Homemade Imaging Chamber

Initial investigation of structural color effects of Sappi’s rainbow coated 947 film was obtained through a homemade imaging chamber. A cardboard box that was obtained from Amazon served as infrastructure to house a camera, light source, and sample of the rainbow coated 947 film material (Figure 2.2D). The inside of the box was covered with a matte black acrylic paint from Stuart Semple. A rack originally used as a kitchen cabinet divider was used as a stand and was secured to the top of the box with Elmer’s glue to serve as a platform for the camera (Figure 2.2C). A camera mount was 3D printed and attached to the wire stand to secure the position of image collection (Figure 2.2A). The camera, an iPhone 11 Pro, was placed into the 3D printed phone mount, and was positioned directly above the sample, or in other words, was directly perpendicular to the material of interest. A hole was created into the side of the cardboard box to allow for a light source to introduce light into the box (figure 2.2B). A custom tilt stage was constructed to hold the

Figure 2.2 Homemade imaging chamber. A) 3D printed smartphone holder. B) Hole for light source to introduce light into the scene. C) Wire stand to keep phone holder in the same position. D) Cardboard box used to shield extraneous light and to house necessary components.
material sample at specified angles. All images were collected in a dark room, where no outside light could interfere with image collection.

*Image Collection Device.*

Images were obtained with an iPhone 11 Pro. The 12 MP camera was preferred, with 26 mm lens, f-stop of f/1.8, and optical image stabilization. Although pictures were taken in a fairly dim environment, no night mode feature was used.

*Tilt Stage.*

A tilt stage was constructed out of wood to hold the material of interest at specified angles of inclination. Holes were drilled into the side of a platform wall in 15-degree increments, from an angle of inclination of 15 to 60 degrees. These angles were verified through the Measurement application by Apple. A slab of wood was used as a sample platform, where rubber bands held the material down flat on the surface (Figure 2.3) A bolt was screwed into one of the holes of choice, and the sample platform was rested on top of the bolt. In this way, the sample’s angle of inclination could be held consistent at a desirable angle. For the initial images that will be discussed in this work, the sample was angled at 15 degrees with respect to the position of image collection.

*Light Source.*

The light source used within the imaging chamber was a 5mm white LED from Chanzon. The forward voltage was 3.0 – 3.5V, with a current of 20 mA. The luminous intensity of the
LED was 12000 – 14000 m cd with a wavelength range from 6000 – 9000K. The LED was positioned at approximately 35° with respect to the camera (Figure 2.4).

2.4 Modeling and Development of Automated Image Capturing System

The modeling and development of the automated image capturing system was led by Theo Erikson, an undergraduate mechanical engineering major at the University of Maine.

Computer Aided Design Development.

All 3D modeling in this work was completed with SolidWorks CAD modeling software (SolidWorks 2019-2020). Models were converted to .stl file types within SolidWorks prior to export for printing.

3D Printer Information.

Any objects modeled from SolidWorks were printed with the MakerBot METHOD 3D printer with fused deposition modeling. The printer had a dimensional accuracy of ± 0.2 mm, with a layer resolution of 20 – 400 μm. Polylactic Acid (PLA) was the printing material of choice. The fill density selected for prints was 10% thatch density.

Arduino Software.

Development of a data collection apparatus required code to control operation of the device, and to cater specific rounds of collection to particular experimental questions. An Arduino uno board was used as a microcontroller, where the Arduino IDE was used as the development environment to code controls onto the board. This code was written in Arduino’s own coding language, the Arduino coding language, based on C++. The Arduino IDE was used to control the positioning of all moving parts in the automated image capturing system, such as the sample rotation, lighting angle, and camera angle.
**Light Source.**

The light source used in the automated imaging capture system was the same light source described in detail in Section 2.3.1. The angle of the light source for data collection with the automated image capturing setup was 15°.

**Image Collection Device.**

A smartphone was used once again for image collection purposes. The smartphone of choice was the iPhone Xs Max. Images were obtained with the 12 MP camera, with f-stop of f/1.8. No night mode feature was used for any images. Videos were collected using this smartphone, with 1080p HD video recording at 30fps.

**Sample Preparation.**

When samples of the rainbow coated 947 film material were placed into the setup for imaging purposes, the sample would be placed in such a way that the black dot placed in the top left corner would also be in the top left of the camera’s view. In other words, the surface structure of the material would start at the same position in the North to South orientation. A 3D printed sample holder would then be placed over the sample to prevent any movement or curvature of the material during data collection.

**2.5 Data Analysis**

**Data Extraction.**

Videos were taken through the automated image capturing system and were subsequently uploaded to google drive via the google drive mobile application. The videos would automatically default to YouTube video links, where the quality would be manually set at 1080p. Screenshots, using the print screen keyboard function, of these videos would be taken and used down the line for image analysis. The screenshots would be pasted into a google slides document, then saved to a local file on a computer.
Image Analysis.

All manual analysis of images was completed through an image processing distribution of ImageJ, FIJI. FIJI comes bundled with an abundance of plugins to facilitate quantitative image analysis. Prior to analysis, all images were cropped to 155 x 155 pixels. Quantitative data regarding light intensity of analyzed images was obtained using the draw line function in FIJI. Once a user has drawn a line over a region of interest, the plot profile function was used. This generates a graph of the pixel light intensity over distance. To obtain length related data, the same draw line function was used to manually draw a line over a feature of interest. The measure function was subsequently used to obtain the length, in pixels, of the line that was drawn. Color data was collected through FIJI’s built-in color thresholding function. In color thresholding, the HSV/HSB color space was selected in conjunction with the Moments thresholding algorithm. The sliders controlling the hue would be changed depending on the color that needed to be analyzed. Once the pixels meeting the criteria to be defined as a certain color were highlighted, the select and measure function were used to analyze those specific pixels.

Data Storage and Visualization.

Microsoft Excel (2018 build 14326.20404) was used to store data from image analysis, as well as to visualize data through graphs and charts. The data were copied from FIJI and pasted into an Excel spreadsheet. Excel also offered an additional environment for data-based calculations that were necessary for data presentation.

Computing Environment Software.

For more numerically rigorous analyses, MATLAB (MATLABR2020A) was used as a computing environment. The curve fitting, wavelet signal denoiser, and signal analyzer add-ons were downloaded in addition to the default program. These add-ons were all created by MathWorks, and were installed through MATLAB’s built-in add-ons manager tab.
2.6 Statistical Analysis

All statistical information and tests completed on the image-derived data from this work was completed in RStudio (version 1.3.1056) in the R programming language. Paired t-tests were the statistical test of choice. Prior to t-tests, however, the data were checked for normal distribution via the Shapiro test. If the data was found to be normally distributed, signified by $p > 0.05$, then a t-test would be completed on the data. If the data were found to be significantly different from normal distribution, with $p < 0.05$, then a Wilcoxon test was performed on the data. Once the necessary checks for normal distribution were completed, the paired t-test could be analyzed. For these tests, $p < 0.05$ was considered significant.

2.7 Experimental Methods

2.7.1 Contaminant Addition

2.7.1.1 Sweat and Sebum

The addition of sweat and sebum was used to add a realistic surface contaminant to the samples of the material of interest. The samples were pressed up to one’s face and uniformly spread around the forehead, nose, and cheek areas. Sweat and sebum extraction would take place following exercise of the researcher, in an effort to generate acceptable amounts of the test contaminants.

2.7.1.2 Silicone Oil

Silicone oil was chosen as the standardized contaminant. The silicone oil was purchased from Sigma-Aldrich with a viscosity of 20 cSt. Addition to the surface of the sample was accomplished by use of pipettes. A specified volume of silicone oil would first be pipetted onto the center of the square sample. The pipette was purchased from Eppendorf and was rated for $0.5 – 10 \mu L$ volumes. Once the selected volume of silicone oil was added to the sample surface, the researcher would hold the sample in their non-dominant hand, and with their dominant hand press their thumb down onto the oil, with enough pressure to feel their thumb with the hand holding the sample. The researcher would make an outwards spiral motion to evenly spread the oil around the surface of the sample. The application of the oil was done in such a way to mimic contamination in a real-world public surface.
2.7.2 Experiments

2.7.2.1 Repeatability of Diffraction Pattern Phenomenon

Setup.

For the repeatability experiments, 6 unique samples were tested with two separate trials. The lighting in the automated imaging setup was placed at a 15° offset with respect to the sample. The angle of the light source was handled by the Arduino code software as discussed in Section 2.4. Samples were placed onto the automated imaging setup as described in Section 2.4, ensuring consistent positioning of the surface structure in the rainbow coated 947 film material. A cardboard box and black cloth were placed on top of the imaging setup to prevent extraneous light from interacting with the sample and altering visual results. Furthermore, the data collection occurred in a room with no windows or openings to introduce more light to the scene.

2.7.2.2 Silicone Oil as Contamination

Setup and Contaminant Addition.

In experiments where clean samples were compared to 5 μL of silicone oil, 5 unique samples were used with two trials completed. The 5 samples were selected as the diffraction pattern presence was observed towards the center, diminishing the possibility of edge effects, where they were extracted as described in Section 2.4. The lighting in the automated imaging setup was placed at a 15° offset with respect to the sample. The angle of the light source was handled by the Arduino code software as discussed in Section 2.4. Samples were placed onto the automated imaging setup as described in Section 2.4. Once in the setup, the samples were individually run through a period of data collection. This process would repeat for each of the 5 samples in both their clean and contaminated states.

Silicone oil contamination was added to the rainbow coated 947 film as described in Section 2.7.1.2. For these experiments, 5 μL of the silicone oil was added to the surface of the material. The volume of oil was verified through the volume selector of the pipette.
2.7.2.3 Limit of Detection

Setup and Contaminant Addition.

For the limit of detection experiment, 5 unique samples were used once again. The samples were extracted as described in Section 2.4. The lighting in the automated imaging setup was placed at a 15° offset with respect to the sample. The angle of the light source was handled by the Arduino code software as discussed in Section 2.4. Samples were placed onto the automated imaging setup as described in Section 2.4, ensuring consistent positioning of the surface structure in the rainbow coated 947 film material. The 5 samples were subjected to 8 different levels of treatment, including 2 control treatments and 6 test treatments. Samples with no added solution and samples with pure ethanol constituted the control treatments. For the 6 test treatments, the volume of added contaminant was varied from $1 \times 10^{-5}$ – 1 μL in decreasing increments of magnitudes of 10. The generation of these varied volumes of silicone oil was accomplished through dilutions with ethanol. Anhydrous ethanol was used to progressively dilute the concentration of the silicone oil that was present. Falcon tubes were used to individually group the dilutions. From these tubes, 5 μL of solution were added to the surface of the samples in the way described in Section 2.7.1.2. The samples were first pictured in the control states, and subsequently pictured with the various volumes of silicone oil.
CHAPTER 3

RESULTS AND DISCUSSION

3.1 Surface Characterization

3.1.1 Surface Structure Imaging

To begin to understand the surface properties of the Rainbow coated 947 film, standard characterization experiments were performed. Figure 3.1.A shows a magnified image of the material surface, with periodic striations clearly visible. In Figure 3.1B, the magnification of the image is increased, and the striations previously observed in Figure 3.1A are more pronounced. Some residues from fingerprints are additionally visible on the surface. Finally, Figure 3.1C increases magnification up to 250× where the highly ordered, periodic surface structure of the material is present.

Figure 3.1 A) Microscopic image at 40× magnification of Rainbow coated 947 film with grooves arranged in parallel fashion. B) Microscopic image at 100× magnification of Rainbow coated 947 film with fingerprints. C) Microscopic image at 250× magnification with grating surface structure present.

The highly ordered, nanoscale, periodic structure that was observed on the surface of the material is expected. Although specific studies on Sappi’s Rainbow coated 947 film are not available, as it is a proprietary material, similar materials that exhibit comparable visual effects have been studied and were
observed to have periodic surface structures on a wavelength scale. The presence of this feature promotes the production of the structural color effects observed.

3.1.2 Refractive Index Identification

To further understand the surface properties of the rainbow coated 947 film, specifically light’s interaction with the surface, the refractive index properties of both the substrate and film layers were investigated. These refractive index measurements were completed by Saman Zare, a PhD candidate, under the guidance of Dr. Sheila Edalatpour at the University of Maine. Figure 3.2 displays a plot of the refractive indices of the substrate and film layers that constitute the material of interest. At all wavelengths examined, the two layers have different refractive index values that decrease at similar rates. As the wavelength of light increases, the refractive indices of both the substrate and film steeply decrease from 300 to 500 nm, while decreasing to a lower degree from 600 to 1000 nm.

![Figure 3.2](image)

**Figure 3.2** Graph of refractive index values by wavelength of light. Refractive index of the film (bottom) and substrate (top) vary as the wavelength of light varies.

It is expected that the refractive indices of the two layers will be different across the wavelength spectrum. Similar photonic materials that have been studied were observed to have periodic refractive index variation of alternating layers to satisfy Bragg’s law for diffraction of visible light.39
3.2 Initial Observations of Diffraction Patterns

3.2.1 Development of Home Setup for Material Investigation

To begin preliminary observations of the material of interest, an at-home imaging chamber was developed as described in Section 2.3. The imaging chamber was necessary to vary the angle of inclination of the material reliably and consistently, while holding the position of image collection and light source constant. A smartphone was chosen as the ideal option for image collection, as the end goal of the project planned to incorporate rapid classification of contaminated surfaces with machine learning integration through smartphones. Figure 3.3A shows a schematic of the imaging chamber, with relevant features labeled. A box enclosed the imaging chamber to obstruct extraneous light, where a battery-powered LED served as the only light source through a built-in hole. A wire stand was attached to the top of the box, and a 3D printed, polylactic acid (PLA) phone holder was permanently attached upon it. The phone holder allowed for the stabilization of the position of image collection. To further prevent the possibility of outside light interacting with the sample, the imaging chamber was used within a dark room with no windows or outdoor light present. Figure 3.3B shows an overhead schematic view of the imaging chamber, including the interior that has been painted with a black matte finish to minimize specular reflection and the LED light source. It was also necessary to be able to vary the incline angle of the sample of interest during image collection. Figure 3.3C shows a schematic of a custom-made tilt stage that was constructed with wood to enable precise variation of the angle of inclination in 15-degree increments. The imaging chamber was intentionally designed to allow for the incorporation of common household items, as the project had begun in a completely remote setting.
The homemade imaging chamber allowed for basic, initial observations of diffraction patterns from the material. There was no built-in mechanism for the consistent variation of the material’s rotational orientation with respect to the light source. This limitation hindered the precise manipulation of the sample material and prevented the pursuit of quantitative analysis using the homemade imaging setup. However, the combination of consistent lighting and viewing angles, in addition to defined variation in the angle of inclination allowed for a useful avenue of proof-of-concept work. With this setup, it was possible to make exploratory observations of certain sequences that were present in the diffraction patterns.

### 3.2.2 Observations of Angle Dependence

The homemade imaging chamber made it possible to observe consistent sequences in the diffraction pattern, specifically, the angle dependence of the material’s surface structure. Examination of numerous images of the diffraction patterns additionally allowed for the awareness of certain features of the diffraction patterns that changed as the angle of the material’s surface structure changed. Figure 3.4A presents a schematic representation of the surface structure of the material at 0° (top), the observed diffraction pattern (bottom) was a light red color that traveled across the material in a horizontal fashion, having an arc shape at either end of the band. In Figure 3.4B, the sample has been rotated approximately 90°, resulting in a single, smaller diffraction pattern of similar color and straight shape. Figure 3.4C presents the next diffraction pattern once...
the material was rotated an additional 90°, where the band of color now exhibits multiple colors with an arc shape at the edge of the material. In Figure 3.4D, the sample has been rotated a final 90°, and the observed diffraction pattern now has two parallel bands of multiple colors.

![Figure 3.4](image)

**Figure 3.4** The surface structure of the material at the specified angle orientation is represented by the straight lines with the schematics in the top row. The top of the sample is signified by the chevron symbol. A) Diffraction pattern at first orientation, defined as 0°. B) Diffraction pattern at second orientation, 90°. C) Diffraction pattern at third orientation, 180°. D) Diffraction pattern at final orientation, 270°.

This observed sequence of diffraction patterns demonstrated the variability of colors, shapes, and locations of color bands that appeared as the orientation of the surface structure changed with respect to the light source. The phenomenon of varied color reflection at different angles is expected to occur. Since the surface structure of the material is of a dimension comparable to the wavelength of incident light, different wavelengths will be diffracted at different angles as described by equation (11).\(^{39,80}\) Therefore, as the orientation of the periodic surface structure is altered, the position of the points of constructive interference for the different wavelengths of light, described by \(\theta\), will change as well. At some orientations, certain wavelengths will be visible at a \(\theta\) that is captured by the phone’s camera, while at other orientations they won’t. This relationship explains the angle dependence of the observed diffraction patterns.
3.2.3 Limitations with Preliminary Imaging Chamber

To begin the process of examining the difference in diffraction patterns once some contaminant was added to the surface of the material, the imaging chamber had to be tested to ensure differences in observed diffraction patterns were a result of controllable variables, such as the addition of a contaminant, rather than inconsistencies in sample positioning due to the setup. Figure 3.5 presents a sample of material that was pictured in both a clean and contaminated state, where the contaminated sample has been covered in sweat and sebum. Figure 3.5A displays the clean variant of the sample, with one color band that expands from the corner of the material towards the center. The color starts as green, then transitions to yellow, and finally to red. Figure 3.5B displays the contaminated variant of the sample, with one color band that similarly expands from the corner towards the center. From the corner, the color starts as green, transitions to yellow, and finally to red with a faint, disrupted extension of the band. However, throughout the color band there are numerous areas of disruption occurrence, or in other words, areas where no diffraction pattern occurs that otherwise do in the clean variant. Figure 3.5C is an image addition of Figure 3.5A and B. All images in Figure 3.5 have had the backgrounds subtracted to prevent intense light accumulation in the image addition example.

![Figure 3.5](image-url)

**Figure 3.5** A) Clean sample pictured within the imaging chamber. B) Contaminated sample pictured within the imaging chamber. C) Image addition of A and B, with zoomed in section to highlight misalignment of edge features, indicative of misalignment in sample positioning.

Paying close attention to the top right corner of Figure 3.5C allows for a substantial observation. The corners of the samples are visibly offset. If the samples were positioned properly, there would not be any difference in the corners of the samples. Or, in other words, appropriate, consistent positioning of the samples would result in a single line representing both edges of the samples. The presence of the offset gives indication
that the samples were not in the same exact position when pictured. As previously discussed, small orientational differences of the surface structure with respect to the light source results in differences in the observed diffraction pattern. Therefore, visual differences that were observed between the clean and contaminated sample could not be solely attributed to the presence or lack of the contaminant. The observed differences could possibly be a result of the offset that was present in the positioning of the sample. This discovery underlined the major limitation of the homemade imaging chamber, the lack of accurate position manipulation of the sample. With the initial, exploratory observations of the material, it has been shown that the angle dependence of the material is inherently tied to the resulting diffraction patterns. As a result, it was determined a new setup needed to be developed that would allow for more consistency and control with the sample’s position, in addition to the light source and camera angle.

3.3 Development of Triaxially Rotating Apparatus for Diffraction Pattern Investigation

3.3.1 Development Process

To manipulate the position of the sample, light source, and camera more precisely, a new setup was developed. The development and construction of the apparatus was completed by Theo Erikson, an undergraduate mechanical engineering student at the VEMI Lab, a multidisciplinary laboratory at the University of Maine. The triaxial apparatus for the rotation of dependent and independent samples (TARDIS) made it possible to precisely observe diffraction patterns methodically and dynamically. TARDIS enabled the control of previously uncontrollable variables, such as the precise positioning of the samples, which allowed for the isolation and intense scrutiny of diffraction patterns in relation to contamination presence. Additionally, the move to data collection through TARDIS shifted the data collection paradigm. With the previous setup, it was necessary for a researcher to be present and continually take pictures of the samples while changing the position of the samples. Now, the opportunity presented itself to automate the process while also increasing the shear amount of data that was possible to generate. Controlling TARDIS with an Arduino microcontroller and software made it possible to automate data collection. As opposed to images, videos were taken. In this way, TARDIS could vary the angles of the
light source, camera, and samples, while capturing all relevant diffraction patterns at each of the angles of interest. Videos also enabled the team to take data videos of an hour long and cut the video down into thousands of frames. The frames acted as images, and generated thousands of usable, analyzable images of relevant material. Figure 3.6 displays a 3D model of TARDIS with several features highlighted. Figure 3.6A shows the custom printed phone holder that served to stabilize the position of image collection. Figure 3.6B shows the LED holder with a filter to reduce light intensity attached to the light source. Figure 3.6C is the central platform with a custom printed sample holder. The central platform rotates the sample 360° in precise increments. The stencil piece was necessary to prevent any curvature of the material while also securing the position of the sample and preventing unwanted movement in any direction during rotation.

**Figure 3.6 The TARDIS Automated Image Collection Apparatus** A) Cradle for the phone used in data collection, with the ability to change the angle of image collection at a particular angle of interest. B) Light source arm to illuminate the sample. The arm is capable of changing the angle of illumination. Masking tape attached to the end of the arm serves to disperse light. C) Central platform with sample stencil. The central platform precisely rotates the sample and keeps it flat to create a defined area for diffraction pattern observation.
Now that a more precise method of data collection was developed, the next step was to observe the effects of contamination presence upon the resulting diffraction patterns. To better understand the effects of contamination upon the observed diffraction patterns, several contaminants were added to samples of the material and were subsequently observed with TARDIS. It was decided to start the observation process through the use of human sweat, sebum, and saliva as contaminants. In this way, realistic surface contaminants that could be present on a myriad of public surfaces could be examined. The goal in observing diffraction patterns with these contaminants was to identify initial, qualitative features of the color bands that changed in the presence of contamination. Figure 3.7 displays initial differences in observed diffraction patterns once contamination was added. Figure 3.7A is the resulting diffraction pattern of a sample in a clean state, where the diffraction pattern is a strong vertical color band with bright green, orange, and red colors. Figure 3.7B is the resulting diffraction pattern of the same sample covered in saliva, where the diffraction pattern is two bands separated in the center, starting as a dim red and ending as a solid blue color. Figure 3.7C is the diffraction pattern of the same sample with sweat and sebum, where the diffraction pattern is again two bands separated in the center, starting as a dim orange, and ending in a very faint blue.

![Figure 3.7](image)

**Figure 3.7** A) Diffraction pattern of clean sample. B) Diffraction pattern of saliva covered sample. C) Diffraction pattern of sweat and sebum covered sample.
The resulting diffraction patterns gave valuable information, in that there were immediate qualitative differences of the color bands once surface contaminants were added. Several features were specifically noted, including the light intensity, length, and colors of the bands. Each of these features was observed to visually change and were determined to be features the team would focus on for analytical work. Additionally, this initial experiment gave further information about the contaminants used. That is, the sweat and sebum, as well as the saliva, were inconsistent contaminants. In other words, the addition of the contaminants to the surface of the sample and the contaminants themselves were not exactly the same every time. The saliva of one team member is likely different than that of another team member, and the application of saliva to the surface of the material is likely not completely uniform around the sample itself. These same realizations held true for the sweat and sebum. Therefore, several conclusions were made as a result of this experiment. The first being to standardize the contaminant of interest. If consistent differences in diffraction patterns were to be uncovered, the contaminant itself would need to be consistent in itself. To address this, 20 cSt silicone oil was selected as the standardized contaminant. Silicone oil was selected as the standardized contaminant, as it was an inert medium that would be consistent in content and appearance. The development of a standard application procedure via pipettes was also formulated to ensure consistency in contaminant coverage across the sample surface. Furthermore, the features of interest that were visually observed from the diffraction patterns gave the team areas of focus when it came to qualitative analysis. The next steps were to study the diffraction patterns with a consistent contaminant, specifically developing methods to explore quantitative differences in the light intensity, length, and colors of diffraction patterns.

3.4 Development of Analytical Techniques

To begin the process of differentiating between diffraction patterns of clean and contaminated samples of material, it was necessary to devise a method to quantitatively show differences of various aspects of the observed color bands. The following section highlights the methods used to quantitatively investigate the diffraction patterns, where each method specifically focused on one aspect of the diffraction patterns: light intensity, length, and color.
3.4.1 Development of Light Intensity Quantification Method

3.4.1.1 Data Extraction

To analyze light intensity relevant data from the diffraction patterns, the data first had to be refined within ImageJ and subsequently exported to Microsoft Excel for visualization purposes. In the preliminary stages of the development process, the entire image was analyzed to look at the light intensity of all pixels. Analyzing the images in this way proved to be cumbersome, as there were thousands of pixels present in a single image. As a result, a great deal of time and computational power was devoted to analyzing non-relevant pixels of the image. It was decided to specifically focus on the informative area of the images, the color bands. Examining the color band alone both refined the size of the data sets and generated more revealing data. Concentrating on this particular feature of the images was accomplished through the introduction of a user-defined line to select a region of interest. Creating this region discarded the analysis of pixels not included within the region of interest. Next, the team focused on different ways of drawing the region of interest. Initial analysis of the diffraction patterns was completed by drawing the line of interest along the color band. This type of information proved to not be useful, as it only showed the fluctuations in light intensity as the colors of the diffraction patterns changed. However, this finding helped specify what exactly was being studied with this type of light intensity analysis. It became apparent that the line, or region of interest, needed to be drawn in such a way to quantitatively describe the overall shape of the color band. This realization prompted the drawing of the line across the color bands. Figure 3.8A shows an example user-defined line that was drawn across the color band. Figure 3.8B shows the generated light intensity plot of the color band.

**Figure 3.8** A) User-defined line drawn across color band. B) Resulting light intensity plot over color band.
Investigation of the light intensity from the color bands gave good indication that the light intensity profiles would be useful for quantitative analysis of the diffraction patterns. Through the light intensity, it was shown that on either side of the band, there was base noise presence, with a pronounced peak as the line travels over the band itself. The first step of getting the data and visualizing the data was complete. The next step was to further refine the raw data to extract valuable information.

3.4.1.2 Data Filtration

To gain information from the extracted data, the raw data needed to be processed. On several occasions, the resulting raw data plots of light intensity were inundated with noise. The presence of the noise made it difficult to visually discern features of interest. To begin the process of mitigating the noise presence while still maintaining the core characteristics of the data, the data was run through a wavelet denoiser. Although the initial data was not necessarily in the time domain, wavelets were employed as a proof-of-concept to evaluate the feasibility of data filtration with the extracted pixel intensities. The wavelet that was chosen was a coiflet 3 (coif3) that used the Bayes denoising method, soft threshold rule, and level dependent noise assumption. Figure 3.9 displays the effect of the denoising wavelet upon the raw data, where Figure 3.9A shows a graph of the raw data, and Figure 3.9B shows a graph of the processed data.

![Figure 3.9](image.png)

**Figure 3.9** A) Raw light intensity data. B) Processed light intensity data through wavelet denoiser.
The graph of the processed data shows the effect that the wavelet denoiser had on the raw data. Most of the noise present on either side of the peak was decreased, visually pronouncing the peak near the center of the plot around 500 pixels. The denoiser was specifically applied through MATLAB, making it efficient to automatically apply the same wavelet to any desired data sets through code. Other wavelet options were available, however the coif3 wavelet was chosen as it decreased the noise while not completely removing all characteristics of the data. The filtration of the data was used to further the development process of valuable information extraction from the light intensity information.

3.4.1.3 Peak Prominence Identification

Now that the raw data had been extracted and processed, it was time to extract information. As the peaks of the plots were good descriptors of the overall shape of the diffraction patterns, it was decided to focus on a numerical indicator that would describe the peak shape. The peak prominence parameter is used to describe the height of peaks in a signal. It was hypothesized that the addition of contamination would decrease the peak prominence. Therefore, this qualitative change should present itself in the light intensity plots of the diffraction patterns, allowing the use of quantitative differences to explain qualitative observations. In other words, if it was observed that the band shape, represented by light intensity, changed once contaminants were added, then it would be possible to describe this change through the peak prominence values. Figure 3.10 shows a graph of the peak prominence identification of processed light intensity data through MATLAB.

![Figure 3.10 Processed data with marked peak prominence (vertical line).](image-url)
The ability to incorporate the peak prominence identification into code through MATLAB was useful in that it allowed for the automated data generation of multiple data sets. Therefore, data of both clean and contaminated light intensity plots would be analyzed the same exact way each time. A potential limitation of this method is that the processing used to filter the noise from the data had the ability to affect the tip of the peaks. In other words, if a peak of the light intensity data had jagged edges, the smoothing effect of the filter could alter the highest points of the peaks. In this way, it was possible for the peak prominence to be slightly different in the processed data when compared to the raw data. These differences, however, tended to be in the hundreds decimal places and were marginal.

### 3.4.2 Color Band Length Quantification

The next feature that was focused on was the length of the color bands. In this method, the lengths of color bands from both contaminated and clean samples were collected and compared. Similar to the previous method, the development process of this technique began by focusing on regions of interest. User-defined lines, in ImageJ, were drawn along the color bands to measure them in pixels. As these measurements were directly taken manually, it is possible that human error was involved in the data generation. In an attempt to mitigate this fact, the bands were measured three times and subsequently averaged for the most accurate results. Figure 3.8 demonstrates an example length measurement of a diffraction pattern.

### 3.4.3 Color Quantification

#### 3.4.3.1 Defining Color

The third and final feature that was focused on was the color of the bands. The analysis of color proved to be the most complicated of the discussed techniques. The first step in the process of color analysis was to choose a color representation model. As image segmentation and thresholding was of interest, it was decided to use the HSV color space, as it had good segmentation performance in comparison to other color models.
Hues were chosen as the component of choice when indicating color, as this component could be manually analyzed through color thresholding. The hues that were defined as certain colors are contained in Table 3.1.

Table 3.1 Color Definitions via Hues

<table>
<thead>
<tr>
<th>Color</th>
<th>Hue Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>0 – 21, 234 – 255</td>
</tr>
<tr>
<td>Yellow</td>
<td>22 – 63</td>
</tr>
<tr>
<td>Green</td>
<td>64 – 106</td>
</tr>
<tr>
<td>Cyan</td>
<td>107 – 148</td>
</tr>
<tr>
<td>Blue</td>
<td>149 – 191</td>
</tr>
</tbody>
</table>

3.4.1.2 Individual Color Thresholding

Once the colors had been appropriately defined by hue, the next step was to devise a method of systematically gathering data on each color of interest for the color bands. It was decided to manually use the color thresholding window through FIJI to do this. Images were thresholded as described in Section 2.5. Figure 3.12 displays an example of color thresholding where Figure 3.12A shows the original diffraction pattern. Figure 3.12B shows the green portion of the diffraction pattern’s band highlighted. Figure 3.12C shows the blue portion of the diffraction pattern’s band highlighted.

Figure 3.12 A) Original diffraction pattern color band. B) Green thresholding of color bands. C) Blue thresholding of color bands.
3.4.1.3 Color Quantification with Area by Pixels

As previously mentioned, the color thresholding was useful in that it created a region of interest, where the region of interest was a specified color on the color bands. Once the region of interest had been created, it had to be decided what the next step in the analysis process would be. As the goal of the color analysis was to investigate the changes in colors once contamination was present, it was decided to focus on the relative areas of the colors along the color bands. In this way, it could be determined whether certain colors increased or decreased more once there was contamination present on the surface of the sample. This would be accomplished by detecting the total number of pixels that were highlighted back in the previous step. Detecting the total number of pixels would give indication to the area, in pixels, that each color covered along the color band. The generation of these kinds of data from both clean and contaminated samples allowed for the comparison and analysis of how different colors changed.

3.5 Experimental Validation of Analysis Methods

3.5.1 Repeatability of Diffraction Pattern Phenomenon

To investigate the degree of reproducibility and consistency of the observed diffraction patterns, repeatability experiments were performed. Numerous unique samples of the material were extracted and observed at three angles: 0°, 90°, and 180°. Samples were prepared as described in Section 2.7.7.1. Three representative images will be shown at each chosen angle. These three angles were chosen arbitrarily, however they allowed for ease of image collection from data videos as the angles were easy to visually confirm. Figure 3.13A schematically represents the surface structure of the samples at the orientation they were pictured. Figure 3.13B organizes the diffraction patterns of each of the three samples. Figure 3.13B(I) shows sample A’s diffraction pattern at 0°, where the color band starts from the center of the sample and expands up towards the top. At the center of the sample, the color starts as red, then transitions to orange, yellow, and green. Similarly, Figure 3.13B(II) shows sample B’s diffraction pattern at 0°, where the color band starts at the center and expands towards the gold orientation marker. The band starts as a red color that then transitions into green and blue. Figure 3.13B(III) shows sample C’s diffraction pattern at 0°, where
the color band once again starts at the center and expands towards the orientation marker. In a fashion akin to sample B, the band starts as red in the center, but transitions to green and blue as it travels upwards.

![Diagram](image)

**Figure 3.13** A) Schematic representation of surface structure at specified orientation. I) Sample A pictured at 0° with single color band diffraction pattern. II) Sample B pictured at 0° with single color band diffraction pattern. III) Sample C pictured at 0° with single color band diffraction pattern.

The diffraction patterns observed at 0° were generally not repeatable. The diffraction pattern of sample A is visually different in comparison to those of sample B and C. Sample B and C show color bands that are visually similar in color, shape, and location, while sample A differs from both samples in all these categories. For the selected orientation of 0°, the resulting diffraction patterns were not repeatable in terms of color, shape, and location.

Next, all three samples were rotated clockwise 90° and pictured once again in order to establish a position of angle rotation that would give the most reliable, repeatable structural color effects. Figure 3.14A schematically represents the surface structure of the samples at the orientation they were pictured Figure 3.14B(I) shows the resulting diffraction pattern of sample A at 90°. As opposed to the images at 0°, there are now two observable color bands. Both top and bottom bands have a slight arc shape to them, and consist of red, green, and blue colors from the center to outside of the sample. Figure 3.14B(II) shows sample B’s diffraction pattern at 90°. Like Sample A, there are two noticeable bands at the top and bottom of the sample. Traveling from the center to outside of the sample, the colors of the top band transition from red, green, and finally blue. The bottom band, on the other hand, only reflects green and red, with blue being
cut off at the edge of the sample. Figure 3.13B(III) shows the diffraction pattern of Sample C at 90°. Once again, two bands are present, where the top consists of red, green, and blue, while the bottom mainly consists of red and green.

**Figure 3.14** A) Schematic representation of surface structure at specified orientation. I) Sample A pictured at 90° with single color band diffraction pattern. II) Sample B pictured at 90° with single color band diffraction pattern. III) Sample C pictured at 90° with single color band diffraction pattern.

The diffraction patterns observed at 90° were observed to be repeatable. In all three samples that were tested, the resulting diffraction patterns are visually consistent. In color, shape, and location, criteria that were markedly different at the previous orientation, the three samples visually appear to be the same. In terms of repeatability, these observations gave indication that 90° was an orientation of image capture that would give repeatable results across independent samples.

The samples were rotated an additional 90°, resulting in a total of 180° of clockwise rotation, and were observed yet again to determine the repeatability of observed diffraction patterns at various orientations. Figure 3.15A schematically represents the surface structure of the samples at the orientation they were pictured Figure 3.15B(I) shows the resulting diffraction pattern of sample A at 180°. There is a single color band traveling in a longitudinal fashion through the sample, consisting of blue, green, and red. Figure 3.15B(II) shows the resulting diffraction pattern of sample B at 180°. There is a single band that only consists of a dark orange color. Figure 3.15B(III) shows the diffraction pattern of sample C at 180°. Similar to sample B, there is a single color band of a dark orange color.
The diffraction patterns observed at 180° were not repeatable. Sample B and C visually appear to be similar in color, shape, and location of their color bands, while sample A differs in all these categories. This difference is similar to the one noted in the first orientation tested, 0°. At both these orientations the surface structure of the material is positioned in a longitudinal fashion. The position of this surface structure promotes the production of vertical color bands, which were observed to be more variable. Regardless, at 90° the surface structure of the material is positioned in a lateral fashion. Here, the diffraction patterns were consistent on all three of the tested samples.

These observations of consistency were further quantitatively verified. When the process of analytical development was initially started, the images at each of the selected orientations were analyzed to test the feasibility of quantitatively differentiating between clean and contaminated diffraction patterns with the discussed techniques. The full width at half max (FWHM) parameter was tested for use as an indication of cleanliness, in addition to the length measurement. This parameter gave information that helped further the process on narrowing down the tools that could be used for analysis. Figure 3.16 displays two graphs that organize information on the angles tested for repeatability. In Figure 3.16A, the length of diffraction patterns at each angle are compared between clean and contaminated samples. At both 0° and 90° a paired t - test found the differences in length measurements to be statistically significant with $p <$
0.001 for both. At 180°, however, there were not any significant differences in the measured lengths. Figure 3.16B compares the measured FWHM values between clean and contaminated samples at each tested angle. With a paired t-test, it was found that there were statistically significant differences at 90° and 180°, with p = 0.03 for both data sets. This time, though, there were not significant differences at 0°.

**Figure 3.16** A) Measured lengths of diffraction patterns of clean and contaminated samples at specified angles of sample orientations. B) FWHM values of clean and contaminated samples at specified angles of sample orientations.

These findings were important to the process of the project, as it gave insight as to what positions of the sample gave the most consistent, statistically significant results. In both tests, 90° was found to be consistent in that it gave statistically significant data. For both 0° and 180°, on the other hand, one of the angles was significant in one test, while the other was significant in a different test. In other words, they varied in consistency. These findings underline the qualitative observations that were made during the repeatability experiments. As a result of these observations and data, it was decided to focus on data collection at a sample orientation of 90° to prevent the detection of unique color band observations that were a result of typical variation, as observed at 0° and 180°, rather than the presence of contamination.

### 3.5.2 Silicone Oil as Contamination

Once the analytical techniques, setup, and standardized contaminant had been determined, focus was turned to examination of diffraction patterns once the silicone oil contaminant was added. The goal with these experiments were to quantitatively assess the changes in diffraction patterns with contaminant on the
surface in comparison to clean samples. In other words, the purpose was to show quantitative differences of visual changes in the structural color effects of the material. This was accomplished by specifically focusing on each of the previously discussed features of interest: light intensity, length, and color of the diffraction patterns. In the experiments that were subjected to each of the analytical processes, five unique samples were used. For each discussed technique, the data were collected from the samples first in a clean state, then in a contaminated state, where contaminated samples were covered in 5 μL of silicone oil spread evenly across the surface, as described in Section 2.7.1.2. Images were analyzed where the samples were pictured at 90° as discussed in Section 3.5.1 to obtain the most reliable data. To minimize the possibility of edge effects, samples were selected in such a way that the diffraction patterns occurred away from the edges of the sample holder and towards the center of the sample.

3.5.2.1 Light Intensity

The first feature of interest was light intensity. To numerically describe and detect changes in the color band shape, the light intensity pixel values were extracted from each image. From this, the peak prominences would be gathered and analyzed. The goal with this experiment was to show that the light intensity peak values, and by association the peak shape, are different when comparing clean and contaminated diffraction patterns. Figure 3.17 shows the resulting light intensity and peak prominence data from this particular experiment. Figure 3.17A(I) and (II) show representative images of the resulting diffraction pattern of a clean and contaminated sample. It can be visually noted that there is a difference in light intensity of the color band. Figure 3.17B shows a graph of one sample’s resulting light intensity profile, where the gap in peak prominence is clearly pronounced between the clean and oil contaminated sample. Figure 3.17C shows the resulting peak prominence data from all tested samples, where a paired t-test found the difference in peak prominences between clean and oil contaminated samples statistically significant with \( p < 0.001 \).
The two graphs underline the main observation with this experiment, there is a significant difference in the light intensity peaks of clean and contaminated samples. The decrease in light intensity once the contaminant was added to the surface was qualitatively observed, but furthering this concept, the quantitative confidence level underscores the reliability of light intensity as an indication of contamination presence. This finding was expected, as it numerically describes a visual change that was observed when initially examining the qualitative differences in the color bands.

**Figure 3.17** A) Representative image of clean sample diffraction pattern. II) Representative image of contaminated sample diffraction pattern. B) Example light intensity plot of a particular sample that was measured in both clean and contaminated states. C) Average peak prominences of clean and
3.5.2.2 Diffraction Pattern Length

The second feature of interest was the length of diffraction pattern. Again, since this was an observable difference that was initially noted, the goal of the experiment was to specifically look at this aspect and show quantitative differences between clean and contaminated diffraction pattern lengths. The lengths of both clean and contaminated diffraction patterns from all the samples were measured to compare how this feature changed once the contaminant was present. Figure 3.18 displays results of the length analysis from the experiment. Figure 3.18A(I) and (II) show representative images of a clean and contaminated diffraction pattern, respectively. Figure 3.17B shows a graph of the length data from the experiment. The lengths of the clean diffraction patterns were markedly longer than those of the oil contaminated samples. The difference in these measurements were found to be statistically significant with $p < 0.01$.

![Figure 3.18](image)

**Figure 3.18** A) Representative image with clean diffraction pattern. II) Representative image with contaminated diffraction pattern. B) Averaged measured lengths of all tested samples.
The gap in measured diffraction pattern lengths was statistically significant, and further verified the use of length as an indication of contamination presence. Furthermore, the size of the error bars of the oil contaminated data sets highlights an additional point. That is, the overall, increased variability that tended to occur once the contaminant was added to the surface. This variability could be explained by the fact that it was generally more difficult to accurately measure the length of the diffraction patterns once the light intensity was lower. Therefore, this increase in variability is expected to occur.

3.5.2.3 Color

The third and final feature of interest was the color of the diffraction patterns. The goal with analyzing the colors of the diffraction patterns were to show, quantitatively, that the colors change once contamination is added to the surface of the material. However, previously in the repeatability experiments, it was shown that there existed variability in observed colors among clean samples themselves at two of the three tested angles. That is to say, two clean samples at 0° or 180° were observed to exhibit different colors. While at 90°, this same effect was not observed. Therefore, this further verifies the selection of 90° as the sample orientation for data collection and analysis. Figure 3.18 demonstrates an example of the color-specific analysis that is capable through the color thresholding analysis.

![Figure 3.19 A) Original color band. B) Color-specific highlight on color band.](image)
Figure 3.20 displays the detected pixels by color. The colors green, blue, and yellow were found to be very similar in the number of pixels detected when comparing clean and oil contaminated samples. On the other hand, two colors showed significant differences through paired t-tests, red and cyan.

![Bar chart showing detected pixels by color.](image)

**Figure 3.20** A) Detected pixels by in sample images by color.

The significant differences found with red and cyan are not a surprise. This finding validates the observations in the preliminary stages of the project, where the diffraction patterns were observed to appear different colors once various contaminants were added to the surface. However, as previously discussed, some of these changes at the time may have been due to certain inconsistencies in sample positioning or other uncontrollable factors with the initial imaging chamber. With this experiment, the images were obtained from a very reliable angle of sample orientation, where the kinds of color changes were not observed to happen solely due to variation, as was observed at 0° or 180°. Therefore, the statistically significant differences that were detected in this experiment were likely due to the one factor that was being controllably changed, the contaminant.
3.5.3 Limit of Detection

As the goal of the project was to develop a sensor system of contamination detection, it was understood that an important criterion regarding the system would be the limit of detection. Regardless of the application, it would be wise to understand the lowest volume of contaminant that could be detected using the material of interest. Therefore, to achieve this information, limit of detection tests were conducted. In these tests, the volume of silicone oil added to the samples was varied. The setup for this experiment was described in Section 2.7.2.3. Ethanol was used as a control in addition to the typical clean material control. The volumes tested ranged from $10^{-5} - 1 \mu L/64\ cm^2$. It should be noted that these reported volumes were spread across a 64 cm² surface. In testing these volumes, the goal was to apply the same analytical techniques previously discussed to see what the lowest volume would be where statistically significant results would be achieved. This way, it would be possible to understand the lower limit of detection that the sensor system, in conjunction with the developed analytical techniques, were capable of. Figure 3.21 shows representative images of a sample at each treatment level, beginning with clean and ethanol and then organized by least to greatest volume of contaminant in order. The first great visual change is observed at $1 \times 10^1 \mu L/64\ cm^2$ in terms of light intensity and color presence.

![Figure 3.21](image_url)

**Figure 3.21** Representative images of diffraction patterns at each treatment level. I) Clean II) Ethanol III) $1 \times 10^{-5}\ \mu L/64\ cm^2$ IV) $1 \times 10^{-4}\ \mu L/64\ cm^2$ V) $1 \times 10^{-3}\ \mu L/64\ cm^2$ VI) $1 \times 10^{-2}\ \mu L/64\ cm^2$ VII) $1 \times 10^{-1}\ \mu L/64\ cm^2$ VIII) $1\ \mu L/64\ cm^2$
As before, the first technique that was used to analyze the data was the peak prominence light intensity data. Figure 3.22 displays the light intensity and peak prominence data from the limit of detection experiment. Figure 3.22A is a graph of the resulting peak prominences, while Figure 3.22B is the light intensity data itself that were used to obtain the prominences. The data are offset successively by 100. As the volume of oil contaminant is increased, the peak heights decrease, where successive plots of higher contaminant concentration display a flattening of the peak. With the peak prominences, the first volume that showed statistically significant differences with an ANOVA test was the treatment of $1 \times 10^{-1}$ μL/64 cm$^2$, with $p < 0.001$.

\[ \begin{align*}
A & \quad B
\end{align*} \]

**Figure 3.22** A) Resulting peak prominences from each treatment. B) Average light intensity plots from each treatment.

The treatment of $1 \times 10^{-1}$ μL/64 cm$^2$ of the oil contaminant being the first treatment where significant differences were detected, gives indication that with light intensity and peak prominences, the lowest limit of detection was $1 \times 10^{-1}$ μL/64 cm$^2$. This detection is impressive, as this is not something that was easily detectable through only visible means. This first indication of the potential of detection to $1 \times 10^{-1}$ μL/64 cm$^2$ is promising. Now that a tentative limit of detection was uncovered with one method, the next step was to verify this limit with the remaining two analytical methods.

The next tested method was the length analysis. Figure 3.23 displays the resulting length measurements of the observed diffraction patterns by treatment. Similar to the previous technique, the
lengths for the successive treatments following the clean and ethanol measurement are quite similar. However, $1 \times 10^{-1} \mu L/64 \text{ cm}^2$ is the first treatment where a distinct difference is observed. This difference is further underlined by its statistical significance, having $p < 0.001$ from the ANOVA test.

![Figure 3.23 Resulting length measurements organized by treatment.](image)

Similar to the analysis that occurred with the light intensity and peak prominence data, it was found that the lowest volume where significant differences occur between clean and contaminated samples occurs at $1 \times 10^{-1} \mu L/64 \text{ cm}^2$. This means that two out of the three analytical methods tested have both verified the lowest limit of detection as $1 \times 10^{-1} \mu L/64 \text{ cm}^2$. Finally, the images were analyzed with the third and final analytical technique, the color analysis.
The same images were analyzed again, but this time, with the color analysis technique. As it was discussed previously of the colors that were found to be indicative of contamination, red and cyan, other colors were not analyzed in this experiment. The rationale behind this was if other colors were not detectable to be indicative of contamination at a level of 5 μL, then it was likely that they would not be detectable at a lower, limit of detection volume. Figure 3.24 displays graphs of the red and cyan pixel detection data. In Figure 3.24A, the red pixel data is displayed. Following the trend of the previous techniques, significant differences were detected at the $1 \times 10^{-1} \mu L/64 \text{ cm}^2$ treatment point, with an ANOVA test producing $p < 0.001$. Figure 3.24B shows the cyan pixel data. Once again, the same trend follows. With $p < 0.001$ for differences in comparison to detected cyan pixels for the clean and $1 \times 10^{-1} \mu L/64 \text{ cm}^2$ treatment.

![Graphs of red and cyan pixel detection data.](image)

**Figure 3.24** A) Detected red pixels by treatment. B) Detected cyan pixels by treatment.

For the third and final technique, the lowest detectable volume of contamination on the material was determined to be $1 \times 10^{-1} \mu L/64 \text{ cm}^2$. All three of the developed techniques verified the selected volume as the lowest limit of detection. Therefore, from the limit of detection experiments it was shown that the material with associated analytical techniques can detect the silicone oil contaminant down to $1 \times 10^{-1} \mu L/64 \text{ cm}^2$. 

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3.6 Machine Learning

Through the development of quantitative, analytical techniques and the identification of a limit of detection, the work that constituted the bulk of the project had promising results for the use of Sappi’s rainbow coated 947 film and a smartphone as a sensor of surface contamination. But to truly empower this concept, it would be wise to incorporate a more automated method of recognition of clean or contaminated diffraction patterns. As the discussed results were positive, it was decided that it could serve as a proof-of-concept in the use of machine learning. To have a computer, as opposed to a human, quantitatively uncover differences between clean and contaminated images of this material, would allow for the rapid and contactless identification of surface contamination only using an image. Dr. Salimeh Sekeh and her PhD student Josh Andle, from the School of Computing and Information Science at the University of Maine, led this effort to incorporate machine learning technology into the project. The basic goal of this concept would be to train a network to be capable of identifying between clean and contaminated diffraction patterns from the material of interest. In doing this, it would then be possible to incorporate this algorithm into an application that could be downloaded onto mobile devices. Essentially placing the power of surface contamination identification into the pockets of everyone.

3.6.1 Data Generation

To begin the process of machine learning integration into the project, preparations were made to train the machine learning network. The key concept with machine learning was the fact that the resulting algorithm was only as powerful as the data set used to train the network. In other words, it was of paramount importance to efficiently and appropriately train the network to learn the difference between clean and contaminated diffraction patterns. The network’s understanding and identification capability is solely based on the training data. To accomplish this, it was necessary to generate thousands of images of these diffraction patterns. The use of very few images, less than a thousand for example, would not be appropriate. Data generation of this scale was possible through the use of TARDIS. As previously discussed earlier in the chapter, the data collection through the automated image capturing system was completed through
videos. During video collection, several features were controllably changed, such as the position of the sample, the position of the light source, and the position of the phone itself. Then, these videos would be cut down into frames, serving as images. In this way, it was possible to generate massive amounts of images for use in training of the network.

3.6.2 Data Extraction

Now that the data had been generated on a massive scale, the next step in the process was to extract relevant data. Now that images on a massive scale were being worked with, the monitoring of computer resources became an important item to keep track of. In the images discussed, the resulting diffraction patterns do not cover a great portion of the sample surface. Therefore, in analyzing thousands and thousands of these images pixel by pixel, a great deal of computational power and time would be wasted analyzing non-relevant space of the sample, or space that had no diffraction pattern. To mitigate this, Dr. Sekeh’s machine learning team incorporated feature extraction and selection. Now, before analyzing images, the images were processed in such a way to only select the color band that was present in the image, if any. In this way, the thousands of images that would be fed to the network were only the color band itself with no dead space. The introduction of this feature allowed for more rapid training and testing of the machine learning network.

3.6.3 Preliminary Results

Once the procedure for data collection and extraction were finalized, it was time for the machine learning network to be ran on the produced data set. The goal of testing the machine learning network with these images was to understand the ability of the network to classify between clean and contaminated samples. The first set of images that were fed to the network were images where the samples pictured were either clean or contaminated with 5 μL/64 cm² of silicone oil. This volume of silicone oil was selected to initially teach the network to simply differentiate between completely clean or completely contaminated samples. At 5 μL/64 cm² it was possible to view the diffraction patterns with the naked eye and observe differences in light intensity, length, and color. Therefore, the goal of feeding the network with these two kinds of images was not to push the network to its limits but was to firstly establish the ability of the network
to correctly classify samples as clean or contaminated. Samples for this experiment were prepared as described in Section 2.4. With around 3,000 images generated for the network, a portion of these images were used strictly as training data, and the remaining images were used to test the network and arrive at classification results. For this particular experiment, the machine learning network was found to be adept at classifying between clean and contaminated samples with 5 μL/64 cm² of the silicone oil contaminant. The network was found to correctly classify the raw data with a 91 +/- 1% accuracy, and a 99% accuracy when images that are blurry or black due to darkness are removed from the set. This first test confirmed the ability of a machine learning network to successfully classify samples. The next step was to further test the network and push it to see what more it was capable of.

To further push the network, it was decided to use the limit of detection images to test the capability of the network to correctly classify samples as the volume of the contaminant was decreased. This experiment mimics the same purpose and goals of the limit of detection tests completed to verify the developed analytical techniques but were now being used to verify the potential capabilities of the network. Instead of only being fed contaminated samples with a volume of 5 μL/64 cm², the network would be fed contaminated samples ranging from volumes of $1 \times 10^{-5} – 1 \mu L/64 \text{ cm}^2$. It should be reaffirmed that at these volumes, it was increasingly difficult to visually note contamination present on the surface, therefore it would be of interest to understand the machine learning network’s ability to correctly classify samples that did not easily give the appearance of contamination. Table 3.2 organizes the data from the machine learning experiment, specifically highlighting the accuracy of classification by treatment level. The network performed extremely at higher concentrations of contamination, as expected. At these volumes, it is more visually apparent that contamination is present. However, as the volume of the contaminant decreases, so too does the classification accuracies.
Table 3.2 Machine Learning Classification Results by Treatment

<table>
<thead>
<tr>
<th>Treatment (μL/64 cm²):</th>
<th>Clean</th>
<th>Ethanol</th>
<th>1</th>
<th>1 x 10⁻¹</th>
<th>1 x 10⁻²</th>
<th>1 x 10⁻³</th>
<th>1 x 10⁻⁴</th>
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<td>100</td>
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<tr>
<td>Avg. Accuracy (%)</td>
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<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>90</td>
<td>100</td>
<td>55</td>
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The main trend that is observed with these results is the decrease in classification accuracy as the volume of contamination decreases. At the lowest volume of $1 \times 10^{-5} \mu$L/64 cm² the average accuracy in classification was only 55%. This trend is expected, because at this low of a volume the visual cues that indicate the presence of contamination are fairly hard to pick up. Furthermore, it should be noted that the screenshots of extracted images were being analyzed. This means that the overall resolution quality of the analyzed images were lower than expected. With this in mind, it is possible that the increase in resolution that would be afforded by analyzing true extracted images would result in an increase in classification accuracy at even lower volumes of contamination. The features that are harder to detect at lower volumes would likely be more apparent with higher quality of images. In general, though, the machine learning results reaffirm the ability of the network to correctly classify samples as clean or contaminated at almost all the tested treatment levels.
3.7 Summary

In this section, Sappi’s Rainbow Coated 947 film was first characterized. These findings reaffirmed the diffraction grating surface structure that is responsible for the structural color effects observed with a smartphone. Through the development of a homemade imaging chamber, preliminary observations regarding the angle dependence of the material were made, or in other words, the variation of the diffraction patterns as the material’s position is changed. Following this observation, the need for a new experimental setup was understood due to the uncontrollable inconsistencies in positioning that plagued the homemade setup.

To rectify this issue, an automated sample collection system was used, and allowed a great deal of control and precision in dynamically observing the structural color effects of the material. As a result of the increased control and precision, it was possible to finally observe diffraction pattern differences once contamination was added to the material. To start this process, sweat, sebum, and saliva were used. Although these contaminants were not consistent in application and content, it allowed for the observation of diffraction patterns in the presence of real contaminants present on public surfaces. Following these experiments, it was decided to choose a consistent and standardized contaminant. The 20 cSt silicone oil was chosen as this contaminant as it was the most consistent in content and application to the sample itself.

Next, the development of analytical techniques capable of showing quantitative differences in features that were qualitatively observed to change in the diffraction patterns once contamination was present was prioritized. Three features were focused on, light intensity, length, and color presence. Through the development of these techniques, it was found that all three of them were able to show statistically significant data that identified contamination presence, starting at 5 μL. With color, red and cyan specifically were found to be indicative of contamination presence.

To further push the limits of the material, the limit of detectable contamination was identified. This limit was found to be $1 \times 10^{-1} \text{μL}/64 \text{cm}^2$. The main takeaway from these experiments was the quantitative differences shown between clean and contaminated samples, with high statistical probability.
Finally, the promising results of analysis were used for a proof-of-concept for machine learning introduction into the project. Thousands of images were generated to train and test the network. The machine learning network performed well in correctly classifying between clean and contaminated samples, especially when the volume of contaminant was 5 μL/64 cm². When different volumes of contamination were tested, the network continued to perform at a high level until the volume of contamination was lowered around $1 \times 10^{-5}$ μL/64 cm². Even at this low concentration, however, the average accuracy was 55%. These preliminary results with machine learning integration show the potential of this technology for a rapid, automated, and contactless method of contamination detection on surfaces.
CHAPTER 4

CONCLUSIONS AND OUTLOOK

4.1 Overview

The work completed in this project has laid the foundations for the use of this diffraction grating material as a rapid, low-cost method of contamination detection. Current solutions to the complex issue of continuous monitoring of surfaces can be timely, costly, and inaccessible to the general public. The application of a platform that uses Sappi’s mass-produced rainbow coated 947 film and smartphone into the public sphere has great implications for human health, especially following the SARS-CoV-2 pandemic. The developed platform is rapid, low-cost, and easily accessible. Several imaging chambers were designed and constructed with varying degrees of precision and success, with the automated sample collection system proving to undoubtedly be the most useful experimental setup for data collection. Three techniques were developed to quantitatively detect changes in diffraction patterns once contaminants were present on the sample surface. These techniques focused on three features: light intensity, length, and color of diffraction pattern. In each of the developed techniques, there were statistically significant differences when comparing data from clean diffraction patterns to those from contaminated samples. To take this concept a step further, the limit of detection was identified. Identification was accomplished through the use of each of the analytical techniques. In all the techniques, the lowest volume of statistically significant detection was achieved at $1 \times 10^{-1} \mu\text{L}/64 \text{ cm}^2$. The encouraging results allowed for the introduction of machine learning into the project. The preliminary results generated from machine learning established a proof-of-concept in using this type of technology with this particular application. The network performed well in differentiating between clean and various volumes of contamination $1 \times 10^{-1} \mu\text{L}/64 \text{ cm}^2$. These findings offer an optimistic view for the introduction of this concept into mobile applications. In doing so, the team will have achieved its goal of putting the power of rapid surface assessment into everyone’s hands, keeping people safe amongst the unknown of the public surfaces they found themselves in contact with.
4.2 Future Directions

As the quantitative differences in select features of diffraction patterns has been established, the next steps will be to further this analysis. A more robust method of image analysis is necessary for more complex mathematical analyses. One example of this is the introduction of curve fitting into the data analysis procedure. To additionally enhance image analysis, it may be wise to use a different method of image collection. Although the specifications of the phone camera that was used were acceptable, it is possible that certain features are more pronounced or detectable only in higher image quality. Along the same idea, the analysis of the true images will offer a higher resolution of image rather than screenshots. In this way, certain features that were lost with the lower resolution could offer additional features of analysis with higher resolution. Furthermore, the addition of wavelength analysis could further add features of interest when differentiating between clean and contaminated samples, especially wavelengths not in the visible range.

Machine learning also offers another exciting opportunity for the project. The integration of this concept, in conjunction with a machine learning network, has great potential. A network adept at differentiating between clean and contaminated diffraction patterns would do a great deal to pushing the project forward. Adding a mobile application that uses this algorithm would additionally get closer to the goal of empowering those around the world with this type of technology.
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**BIOGRAPHY OF THE AUTHOR**

Oisin Biswas was born in Galway, Ireland in June of 1998. He was raised in Brewer, Maine and graduated from Brewer High School in 2016. He went on to receive his Bachelor of Science in biomedical engineering from the University of Maine in 2020. He worked as a research assistant to Dr. Caitlin Howell starting in 2018, when he joined Dr. Howell and Dr. Richard Corey at the VEMI Lab the following fall. Oisin is a candidate for the Master of Science degree in Biomedical Engineering from the University of Maine in December 2021.