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The Application of Dispersion Staining and Infrared Microspectroscopy to Analyze Physical Evidence in Developing Countries

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By

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Abstract

The Application of Dispersion Staining and Infrared Microspectroscopy to Analyze Physical Evidence in Developing Countries

By

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In developing countries like Thailand and in remote forensic laboratories around the world, scientific investigations of crimes are limited by the shortage of trained personnel and financial resources. The premise of this research is that polarized light microscope and dispersion staining methods will be developed which allow investigators with limited training to analyze physical evidence at a minimal cost. This research identifies specific liquids for the analysis of trace evidence using the dispersion staining technique. The development of dispersion staining technique and identification of specific liquid will extend the application of forensic science to remote laboratories and in the field to improve criminal investigations and justice. The methods developed in this research are fast, inexpensive and require minimum training; meeting the needs of developing countries and laboratories in the remote area. Dispersion staining is a non-destructive microscopical method that creates a uniquely colored image of a transparent sample when it is mounted in a specific liquid. This color is produced by differences between the refraction of light by the sample and surrounding liquid. The scientific principles of these techniques are well-established, but when applied to forensic science, they advance the capacity of developing countries to achieve high standards of evidentiary proof at lower cost. The collection of evidence, documentation of a crime scene and scientific

examination of physical evidence are foundations of criminal investigation which both strengthen the prosecution and prevent wrongful convictions.

This technique can be used to detect, compare and identify very small particles. In this study, three classes of trace evidence were selected to demonstrate the practicality and advantages of this technique. The three classes are fibers, controlled drugs and soil minerals. Samples of each evidence class were analyzed in two steps: first, the Becke line method was used to observe and record the relative refractive index between a sample and mounting liquid, the second step was to put each sample in a specific refractive index liquid to generate distinct colors of the sample. The dispersion staining technique can differentiate and identify an unknown sample by the color produced in a liquid of a specific refractive index value. To validate the composition of exemplar evidence material, FTIR microprobe analysis was used. The rapid and reliable results of this method will aid criminal investigation in a remote area, improve law enforcement and reduce demands on central laboratory facilities.

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1. The Problems

This research identifies, develops and validates practical methods for analyzing physical evidence that are consistent with the needs and resources of a developing country. Developing countries may recognize the essential role forensic science plays in the criminal justice system, but implementation is a problem. The application of forensic science encounters many obstacles including limited access to experts in specialized examinations, limited understanding of the importance of forensic science by individuals working cases, and limitations on financial resources. In addition, education and training are needed to foster knowledge and professionalism. Forensic science in developing countries requires an approach to solve the problem that addresses these obstacles.

Criminalistics is the application of techniques and methods of natural science to the collection and analysis of various pieces of evidence relevant to crimes. It provides answers to crucial questions such as whether a crime has been committed, how and when the crime was committed and who committed the crime. There are many analytical techniques applied in laboratories in places like the United States and Europe, but these methods frequently require significant funding to provide the equipment, maintenance, and expertly trained personnel necessary for operation and interpretation of results. It is impractical for developing countries to invest their limited resources in expensive methods with limited specialized forensic applications. Low-cost analytical methods that provide high value to forensic science are needed in these situations. Light microscopy methods such as stereomicroscopy, polarized light microscopy (PLM) and dispersion staining microscopy are low cost analytical methods that provide high value to forensic science.

In the past, dispersion staining was not commonly applied in major laboratories where funding allows for expensive equipment and training; however, their value as useful techniques that provide accurate results is not diminished. In places like Thailand, where resources are significantly limited, this method and others like it are sufficient to meet standards of evidentiary proof in most cases, and therefore they offer the potential for improving the speedy acquisition and application of evidence to the entire criminal caseload. This method is also valuable because it can be applied to many other different types of physical evidence. The technique is not limited to drugs, fibers and soils. As laboratory personnel become more familiar with the method, the type of evidence they will be able to analyze will increase. In addition, they can naturally expand their education and training to other types of light microscopy evidence including phase-contrast microscopy, microcrystal tests and other methods. Forensic scientists who perform these light microscopy methods are a valued resource. This method will improve the role of the forensic scientist and improve the criminal justice system.

Microscopy is a technique used to examine samples and details of structure that cannot be seen with the unaided eye. Microscopes are versatile and can be applied to a variety of evidence types. Although the use of these methods are low cost, they maximize the information obtained from samples. This fundamental technique is considered valuable and affordable. A large budget is not required to acquire and maintain a microscope. With proper training, scientists in developing countries may successfully apply microscopical methods to many different types of physical evidence.

It is the goal of this dissertation to identify, develop, and validate microscopical methods to analyze physical evidence recovered in criminal investigations. Stereomicroscope, Polarized Light Microscope (PLM) and dispersion-staining methods were applied to drugs, fibers and soil

mineral samples. This research develops a protocol and methods for scientists working within developing countries to successfully apply forensic science to aid in investigations, prosecution and adjudication of criminal cases. The contribution this protocol and methods will provide to forensic scientists in Thailand is significant. It will now be possible for forensic scientists to successfully classify, identify and even individualize evidence. It will provide much needed guidance using methods that can ultimately be applied to many other types of physical evidence. It will greatly increase the role of the forensic scientist in the criminal justice system.

2. The Significance of the Problems

Thailand is a good example of a developing country that would benefit from affordable forensic science techniques. King Ananda Mahidol was murdered in Thailand in 1946, and the investigation of his death established forensic science in this country. Shooting tests to examine bullet trajectories and autopsy were performed. Since that time, forensic science has gradually developed and is currently studied in the major educational institutions in Thailand. There is, however, minimal funding allocated to support forensic science. The need to develop efficient, low-cost, high-value methods is critical to improve criminal justice in Thailand.

This research developed and validated affordable techniques so that countries with limited resources will be able to perform reliable forensic examinations. Without development and training of personnel to use this method, criminals may remain free to continue to commit crimes, or innocent people will be falsely accused or imprisoned.

Criminal justice in Thailand and in other developing countries should be able to rely on good forensic science to help successfully adjudicate cases. In fact, without good forensic science methods, it is easily argued that justice is not possible. Within these countries, specific dispersion staining methods can be successfully developed. It is critical that this method provide value and affordability. There are number of forensic science laboratories in the provincial regions of Thailand that support the criminal justice system. These laboratories need equipment and trained personnel to investigate crime and reduce the number of cases coming to the central investigation unit in Bangkok. If equipment and training is not multidisciplinary and affordable across these laboratories, good forensic science is not possible. These laboratories will benefit from having affordable, multidisciplinary methods to analyze physical evidence.

Most techniques applied in crime laboratories in countries like the United States and Europe depend on a large financial commitment for instrumentation and for training of personnel. Allocation of these types of resources will not happen in countries like Thailand where most of the population still needs food and basic means for living. However, effective criminal investigations rely heavily on well conducted forensic examinations. The forensic scientist in this country and others like it must use methods that can be applied to many different types of physical evidence. This will allow them to increase their role in the criminal justice system, and help bring criminals to justice.

Microscopical methods provide the forensic scientist in developing countries the best chance for successful investigation of crime because these methods can be applied to many different types of physical evidence. Although this project is applied to drugs, fibers and minerals, there is no reason the method cannot be successfully applied to many other types of physical evidence as laboratory personnel become more familiar with the method.

Microscopical methods are very effective and relatively quick. The forensic microscopists, by nature, is cross trained because the evidence in crime crosses many disciplines. The knowledge gained from analyzing one type of evidence may be applied to the analysis of other types of evidence. This multidisciplinary approach to the analysis of physical evidence coupled with the minimal budget requirements to purchase and maintain light microscopes is a significant benefit of microscopical method.

3. Literature Review

3.1 Light Microscopy

Light microscopes have been used by scientists to examine the physical world for hundreds of years. Anton van Leeuwenhoek (1632-1723) is credited as the first person to build and use a microscope to observe bacteria. Since that time, light microscopes have been continually improved and successfully applied to many different scientific disciplines including botany, chemistry, geology and medicine (Dobell, 1960). Forensic scientists have also used light microscopes and many different microscopic methods to analyze all types of physical evidence (DeForest, 2002).

PLM and dispersion-staining are microscopical methods that have been successfully applied to analyze physical evidence. These methods meet the needs of forensic scientist in developing countries (McCrone & Julian, 1970). McCrone studied the identification of asbestos fibers using PLM and dispersion staining. Dispersion staining is the method using modified optics and a specific refractive index (RI) of a liquid medium to produce distinctive identification color that would be able to identify the sample. The samples of amosite and crocidolite were studied, and these two fibers were differentiated by their dispersion staining color. The wavelength where the refractive index of the sample and immersion liquid are the same is called the matching wavelength and is represented by λ_m. Samples were mounted in Cargille liquid having a refractive index (n_D) at 25 °C= 1.680. Using the central stop objective, amosite exhibits blue and gold color (λ_m=550-650 nm), and crocidolite exhibits a yellow color (λ_m = 400-500nm).

Diamond J.A. (1972) used dispersion staining to identify crystalline antibiotic drugs. The crystalline drug was mounted in liquid with a refractive index that matched the drugs' at one

wavelength of the visible region. The arrangement of mounting liquids of closely graduated refractive index is commercially available (Cargille Laboratories, Cedar Grove, NJ). This author stated that the method may be used for drug identification as well as for cross contamination, airborne dust control application as well as identifying glass fragments.

McCrone (1977) stated that dispersion staining has become an increasingly valuable tool for the microscopists and described some of the challenges to successfully apply the technique. He mentioned the difficulty in determining how to assign a valid wavelength to be observed and how this is the major problem with the dispersion staining method. He stated that most parameters can be controlled by microscopist, but some variables cannot. The microscopist can control an illumination system, condenser aperture stop size and the color temperature of the light source to the degree of the dispersion of the liquid. He can also control the immersion liquid to be applied. Consequently, the spectrum generated by refraction at the particle-liquid interface changes in size relative to the stop in the objective back focal plane and then affects the colors observed. The major problem to apply dispersion staining quantitatively is to identify the observed color to the matching wavelength (λ_m). The central stop dispersion staining color is the complimentary color of the λ_m (Delly, 2007).

McCrone also studied a number of tools to be applied to characterize and identify asbestos such as infrared absorption, x-ray diffraction, differential thermal analysis (DTA), scanning electron microscopy (SEM), transmission electron microscopy (TEM), and the light microscope. He stated that each has advantages and limitations. He found that polarized light microscopy has many advantages with high sensitivity (ppm), and it has an extremely fast analysis, within five to fifteen minutes to identify all major and minor trace components of most samples. TEM is most useful for the detection and identification of asbestos fibers smaller than

the resolving power limit of the PLM. SEM has similar advantage to TEM but it may fail to see the smallest fibers and it cannot identify some fibers especially in a crowded sample. XRD (X-Ray Diffraction) is an applicable technique for quantitative measurement but it cannot give the information about size or shape (fiber or not) and it is not a high sensitivity technique.

Resua & Petraco (1980) studied the fiber optics illuminator used in dispersion staining. The fiber optics illuminator has a collection lens, which has an adjustable sleeve and field diaphragm that enables it to gain a high intensity of beam of Kohler illumination. This type of light source is required for dispersion staining. They showed that this system allows the microscopist to obtain bright, detectable detail with small colorless particles. An EPOI (Ehrenreich Photo-Optical Industries) fiber optics source with a quarter-inch diameter cable and a 750-watt, 21-volt quartz-iodine lamp were used. A wooden box was employed to furnish the fibers, clamp, collecting lens and field diaphragm. A Leitz PLM without the mirror, and 8-times ocular and a McCrone Associates 10-times dispersion staining objective with built-in annular and central stops were used in this study.

Wanagho et al. (1989) studied particle size distribution of soil by using microscopic method. The microscope for particle size analysis requires skill and can be time consuming. By applying image analyzers to assist an examination of particles makes it more convenient and reliable. This technique consists of two parts: a unit for the conversion of the optical image to electrical pulses and a unit which analyzes the electrical pulse to generate a quantitative image. The instrument can examine particle size in the range of 0.4- 150 µm.

Fraysier & Hoven (1992) studied the application of dispersion staining to soil. Since forensic scientists do not always have a strong foundation in mineralogy, soil samples are not

widely examined in the forensic crime laboratory. The investigators normally base their examinations on sieve size and the color for the exclusionary objective. Dispersion staining was introduced for the soil examination as well as color, relief, and birefringence. The authors applied a six-step procedure with the polarized light microscope. Soil was washed, dried, and mounted in xylene. This examination was used as a screening step based on color, birefringence and the presence of magnetic particles or isotropic minerals with a refractive index less than xylene. The following step was to count mineral distribution by using a micrometer eyepiece. Then a dispersion staining method was applied (central stop). The majority of light minerals were tested in 1.540 liquid, while micas and talc were tested in 1.585 liquid. The Cargille series E high dispersion liquids were used to enhance the color. Amphiboles and pyroxenes were examined in 1.660 and 1.690 liquids. The heavy minerals such as garnet and epidole were examined in 1.780 liquid. About thirty minerals were identified by this technique followed by a comparison with standard reference minerals so that the soil examination can be conducted with microscopical skill.

Houck (2003) studied and performed an inter-comparison of unrelated fiber evidence recovered from one item of evidence from each of 20 unrelated crimes in three groups (bank robbery, homicide and kidnapping). The fibers to be examined were removed from the sample and analyzed by light microscopy, fluorescence microscopy and microspectrometer. The fibers were categorized into natural and manufactured groups and then divided into color and polymer class. He found that there is no two fibers showed same microscopic characteristics so this result leads to the individual differentiation of two unrelated items.

This review shows that light microscopy with dispersion staining methods have been successfully applied to many different types of common physical evidence.

3.2 Infrared Microspectroscopy

Infrared microspectroscopy was used as a validation technique for exemplar samples used in this study. Infrared spectroscopy is a well-established technique for the chemical identification of physical evidence and is considered a confirmatory technique in the field of forensic science. The successful application of this method is established in the literature and is summarized in this research.

Koulis et al. (2001) studied the comparison of the infrared transmission spectrum of cocaine HCl to its attenuated total reflection (ATR) spectrum. Transmission is traditional method for retrieving infrared spectra. However, ATR methods have gained favor and play an important role to their rapidity, reproducibility in spectral features and convenience of sample preparation. The authors found that when infrared spectra are collected using different modes or sample preparation methods, there are small variations of peak intensity ratio or peak position. These have a minor impact on qualitative measurement. For the quantitative analysis, these authors state that only spectra collected by the same technique should be conducted.

Reffner (2005) studied the application of internal reflection spectroscopy as a preferred method for infrared microprobe analysis. This author established that there are many good reasons to apply this method. For example, sample preparation is reduced or eliminated, and can be used on uncommon samples such as rock or trace evidence on bullets or burglary tools. Reffner also states that FT-IR attachments are available for Raman microprobes enabling same-spot Raman and infrared spectral measurements. In addition, he supports that the ATR objective is a main reason for the increased use of reflection methods for infrared microbeam analysis. The depth that the infrared beam penetrates into a sample is determined by the refractive index

difference between the sample and the internal reflection element (IRE) and, therefore, sample preparation issues are no longer significant. Both thin and thick sample can be analyzed by contacting with the IRE. Sample preparation is a main concern for microscopy and microprobe analysis. Preparing a thin sample can distort the sample's morphology such as crushing the sample by mechanical rolling. Since standard cover glasses and mounting media absorb mid-infrared radiation, mounting samples by conventional method cannot be applied for infrared microprobe analysis. Barium fluoride cover glasses and deuterated oils are used instead.

Reffner & Leary (2006) illustrated the advances in the field of infrared microprobe analysis applied rapidly and reliably to identify seized drug samples. This type of sample analysis is crucial when considering that over the past few years, about 2.5 million narcotics cases were submitted to crime laboratories in the United States for analytical testing. Decreasing sample turn-around time for these analyses is an expected outcome of any drug analysis in crime laboratory. The use of infrared microprobe analysis for the identification of seized drug samples enables performance of quick and reliable analysis of the sample. The analytical scheme proposed results in identification of the unknown samples using methods that follow industrial guidelines established for the analysis of this type of sample. The ability to integrate polarized light microscope with infrared microprobe analysis to almost all seized drug samples is unprecedented.

Weinger (2007) studied soil mineral analysis by using the IlluminatIR® (Smith Detection, Danbury, CT) infrared (IR) microprobe analysis. She established a reviewable record for forensic soil analysis as a useful tool for forensic investigation. The diamond Attenuated Total Reflection (ATR) is applied for an IR analysis by mounting the sample in immersion liquid to prevent the shattering of a mineral. The infrared microprobe analysis is reproducible and also

sensitive enough to distinguish between mineral polymorphs (minerals having same chemical formula but difference in structure). The limitation of this technique is that this technique cannot be applied to halide minerals, metal oxide and sulfide minerals.

Kerstin et al. (2012) studied the application of attenuated total reflection infrared spectroscopy (ATR-IR) as an on-site drug testing tool as a reliable and portable method. They developed an optical sensor platform based on infrared spectroscopy focused on detection of cocaine in saliva using a dried sample and ATR spectroscopy. They also developed the extraction technique of cocaine in saliva to a transparent solvent and recorded ATR spectra with a commercially Fourier Transform-infrared spectrometer. It demonstrated to be a quantitative technique and the limit of detection is about 10 µg/ml.

The principle of dispersion staining has been established but the application of dispersion staining to forensic investigation is limited. However, the application of dispersion staining to trace evidence in criminal cases is minimal. The analysis of fibrous asbestos material has been the major forensic application of this technique. Asbestos litigation and federal regulation are testimonial to the acceptance of dispersion staining analysis by the courts (Crane, 1992).

In this research, dispersion staining was improved and extended to variety of evidence materials. This study extended and applied dispersion staining to a variety of forensic evidence and demonstrates the practicality of its use in investigating criminal cases. The molecular compositions of the exemplar samples were validated by recording their infrared ATR spectra and comparing with standard reference data.

The research challenges are

1. The identification of low cost refractive index liquid that covers the useful range of refractive index of samples
2. Selection of exemplar samples and validation of their molecular composition
3. Determination of the preferred liquid for each exemplar evidence sample to retrieve the distinctive dispersion staining colors
4. The evaluation of the microscope for dispersion staining analysis

These research questions serve as guidelines and all are summarized in the conclusion of this dissertations.

4. Material and Methods

The goal of this research is to develop practical methods for analyzing commonly found physical evidence that are consistent with the needs and resources of a developing country. Three different classes of trace evidence samples were analyzed to illustrate the practical application of polarized light microscopy and dispersion staining methods. The first class is commonly found synthetic and natural fibers: acrylic, cotton, diacetate, modacrylic, polyamide, polyester, rayon, silk and triacetate. The second class is commonly illegal drugs; cocaine, codeine, flunitrazepam, ketamine, methamphetamine, morphine, oxycodone and phencyclidine. The third class is common soil surface minerals: aragonite, calcite, fluorite, gypsum, kaolinite, quartz, talc, topaz and tremolite. Most of samples selected are based upon the trace evidence commonly found in Thailand. All samples are transparent because dispersion staining cannot be applied to the opaque materials.

4.1 Light Microscope

Light microscopy has many advantages for forensic investigations. Basic morphological identification of the forensic evidence is performed using light microscopy. The analysis is quick and requires little sample preparation. This type of evaluation may be even performed on site if a microscope is available (Crane, 1992).

Most microscopical evaluations begin by using a stereo microscope. The stereo microscope (stereoscopic binocular microscope) is widely used in the crime laboratory and other areas of criminalistics such as firearms analysis, toolmark analysis, trace evidence, document examinations and drug chemistry. The stereomicroscope is typically used for preliminary examination of evidence. It is used to detect and examine many types of trace evidence such as

fibers, drugs, minerals or other crystalline aggregates. The lens system of the stereo microscope corresponds with the lens of the regular compound microscope (Kerr, 1977).

The stereo microscope is composed of two low-to moderate- power compound microscope mounted side by side in a common housing so that the two are aligned to the same area of the sample at slightly different angles. Image erecting prisms are included in a stereo microscope to make three dimensional images more realistic. These prisms are also used to eliminate the image reversal typical of most other compound microscope design (Wheeler & Wilson, 2008).

There are two types of illumination that are used when observing samples with the stereo microscope, reflected light and transmitted light. Reflected light is used when examining objects that are opaque. If the sample is transparent it may be observed using transmitted light. Many samples are best examined by both reflected and transmitted light. There are two major designs of stereomicroscopes. The first is the Greenough stereo microscope and the second is the Common Main Objective (CMO). The Greenough uses two identical optical systems within twin body tubes. The CMO stereo microscope uses a single objective that is shared between a pair of ocular tubes and lens. Most of stereomicroscopes are CMO type. For this study a stereo microscope was used for sample preparation (Wheeler & Wilson, 2008).

After observation using a stereo microscope, samples were analyzed using the PLM. The PLM is a basic tool for identification and very valuable when analyzing crystalline or other materials like drugs, fibers and minerals. The optical properties of these materials can be investigated by the effect that the material has on transmitted light through the sample. The relative speed of light (refractive index) as it travels through a birefringent material can be

measured and compared with known value for identification of the sample. PLM is used to measure optical properties.

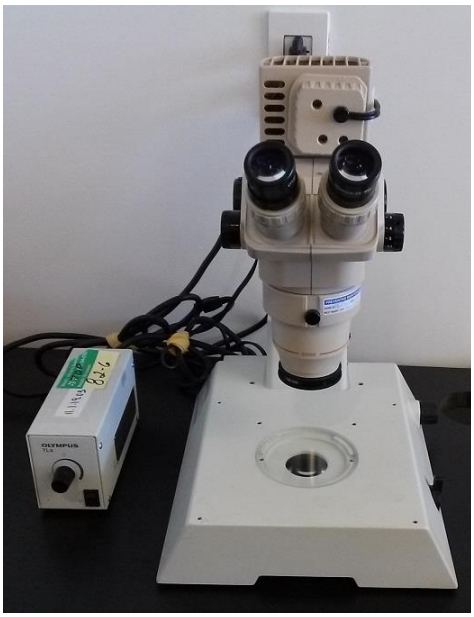



Figure 1. CMO Stereo Microscope

PLM contains typical parts found in compound microscopes such as a light source, condenser, sample stage, objective and ocular lenses. It is converted to a PLM by the addition of a polarizer, analyzer and rotating stage. PLM may be used to analyze many optical properties of the sample such as refractive index, birefringence, extinction and pleochroism. A polarizer is placed in the light path before a sample and an analyzer is placed in the light path above the sample, normally at the back of the objective lens. A polarizer is an optical element that converts ordinary light to linearly polarized light. In a PLM, the light originates from the illuminator passes through the polarizer and is focused by the condenser onto the sample. When the light passes through the polarizer, its electric field vector is oriented in a certain vibrational direction, normally in modern microscope, left to right (E-W). The plane polarized light interacts with the sample. The light is

collected by the objective and it transmits to form the primary image. The analyzer is a polarizer inserted in the light path with its polarization direction most often at ninety degrees to the sub-stage polarizer, usually top-bottom (N-S). The ocular receives this image and refocus onto the viewer's eye. When polarizer and analyzer are in this crossed (90°) position with no sample in the field of view, no light can pass through and the field of view is dark. Some samples remain black while others appear brightly colored or appear to emit light. The samples that transmit light under crossed polars are called anisotropic. These samples have more than one refractive index and show extinction periodically on rotation of ninety degree intervals. The samples that only appear black are isotropic and have a single value of refractive index (Wheeler & Wilson, 2008).

The PLM may be configured for orthoscopic or conoscopic observation. The orthoscopic arrangement provides the eye with an image of the object on the microscope stage. The sample may be observed with a single polarizing device or with crossed polars. Conoscopic observation provides an interference figure which represents an optical pattern caused by the behavior of light in individual crystal. These interference figures are observed in the back focal plane of the objective. They may be observed with the unaided eye if the eyepiece lens is removed, with crossed polars and full aperture illumination. When a Bertrand lens is inserted, the eyepiece can remain and an enlarge image of the interference figure can be observed (Kerr, 1977).

PLM can be used to collect data of physical properties of the sample such as color, size, thickness, width, length, shape, surface texture or opacity. This information can be used to benefit the investigation by comparing a questioned sample found at the crime scene to evidence recovered from the victim or offender. In general, any PLM can be adapted for dispersion staining but in this study OLYMPUS BX51 and LEICA ICC40HD were used. The LEICA ICC40HD microscope employs a Light Emitting Diode (LED) white light source compared to

the traditional tungsten-halogen light source. The better spectral quality and higher intensity of an LED light source improves the performance this microscope for dispersion staining analysis. The Polarized light microscope used in this study are shown below.

OLYMPUS BX51

LEICA ICC50HD

Figure 2. Polarized Light Microscope (PLM) with dispersion staining objective

When using the PLM, many methods using refractive index have been shown to be useful to forensic scientists (Petraco & Kubic, 2004).

These methods include, but are not limited to:

- Immersion methods
- The Becke Line method
- Oblique illumination method
- Dispersion staining method

All of these methods may be used to analyze forensic evidence, but the dispersion staining method was used in this study because it is fast, efficient and cost effective. It best fits the needs of developing countries to improve their forensic science capability.

4.2 Dispersion Staining Method

Dispersion staining is the production of color fringes generated by using a combination of aperture stops and mounting the sample in a liquid. The liquid must have a specific refractive index that matches the sample's refractive index at some wavelength. This wavelength is defined as matching wavelength (λ_m). Dispersion Staining refers to the visual coloring observed at the interface between the liquid and sample. The application of objective aperture and condenser stops is called aperture masking. There are two modes of operation. The first one is annular stop and the other is central stop. The annular stop in the objective blocks a scattered or refracted light and allows an undeviated light (λ_m) to pass to the image plane. The central stop blocks the undeviated light and permits refracted or scattered light to pass to the image plane. With the central stop, the condenser aperture is restricted to limit the incident illumination from the condenser to have the same aperture as the central stop to produce a darkfield.

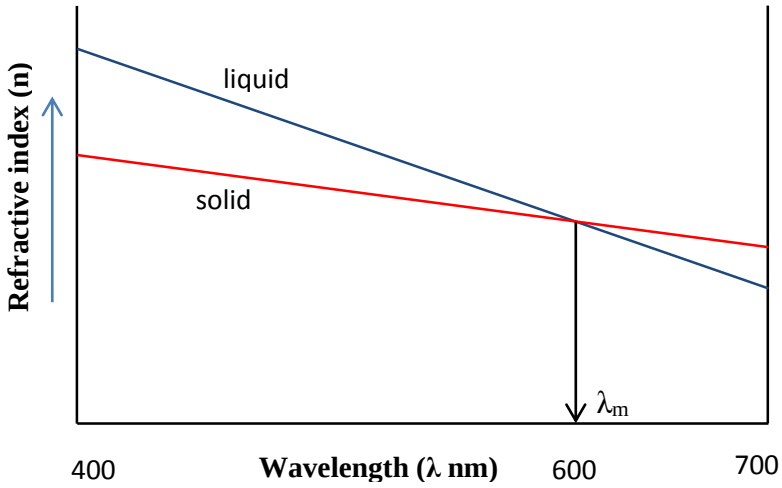


Figure 3. The dispersion plot on a Hartmann net paper for a solid and liquid is shown to cross at 600 nm (matching wavelength, λ_m)

Figure 3 is a graph of refractive index of a solid sample and liquid plotted versus wavelength. Normally the dispersion plot is curved but if the plot is done on Hartmann dispersion net paper, the plot yields straight line. A Hartmann plot of refractive index vs. wavelength, normally slopes downward at the longer wavelengths. Hartmann plot is retrieved from equation below

$$n = A + \frac{10^5 \text{ nm}}{(\lambda - 200) \text{ nm}}$$

A is an empirical constant

The immersion liquid normally has a higher slope of dispersion line than that of a solid sample. The dispersion line of the liquid and solid sample intersects at a λ_m. At λ_m, the liquid and solid sample has the same refractive index (Bloss, 1999).

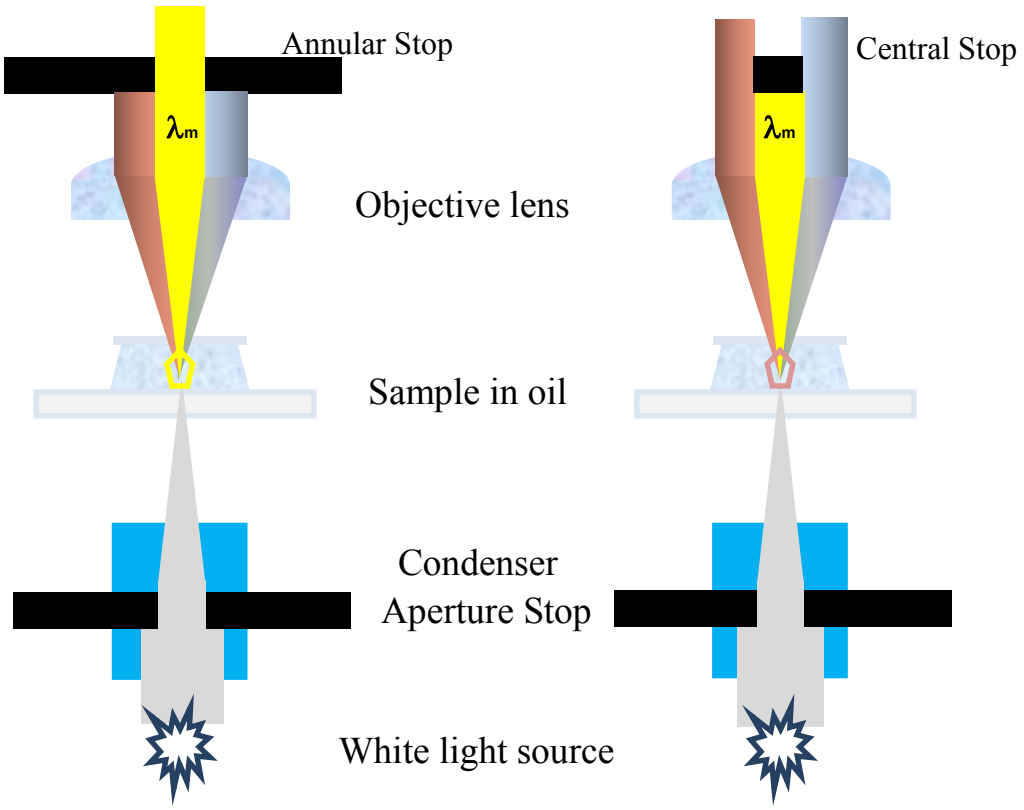


Figure 4. Dispersion staining color formation with annular stop and central stop

Dispersion staining can be achieved using two types of aperture stops; the first is annular stop and the second is central stop. The annular stop reduces the microscope's objective aperture to exclude scattered and refracted light from the sample. To obtain dispersion staining color using the annular stop requires the following steps:

- The sample is mounted in a specific liquid which has a refractive index matching the sample at some wavelength (λ_m).
- The sample is illuminated with a small aperture illumination.
- The sample is viewed using the annular stop dispersion staining objective.
- A brightfield image is formed with colored fringes, the color of λ_m, around the entire border of the microscopic particle.

The annular stop has a restricting aperture, allows only parallel rays to pass through the objective and the grain edges display a color consistent with λ_m. At the liquid and solid interface, all non- λ_m radiations are scattered or refracted.

The central stop blocks low angle incident radiation and images are formed with scattered light, creating a darkfield and colored images of the solid sample. To obtain dispersion staining color using the central stop requires the following steps:

- The sample is mounted in a specific liquid which has a refractive index matching the sample's refractive index at some wavelength (λ_m).
- The sample is illuminated with a small aperture illumination.
- The sample is viewed using the central stop dispersion staining objective.
- A darkfield image is formed with colored fringes, complimentary to λ_m, around the entire border of the microscopic particle.

The matching wavelength is the wavelength where the refractive index of a liquid medium is equal to the refractive index of a solid sample. When using the central stop, the grain's edge color is composed of the non-matching wavelengths, which is white light minus λ_m. This produces the complimentary color to λ_m (see Figure 5). Normally, a central stop is preferred to an annular stop because it blocks out not only the λ_m wavelength but also all rays of white light that do not pass through grain edge at all. The grain edges observed are seen against a dark background (darkfield) while annular stop grain-edge colors appear against a bright (brightfield) background, which is more difficult to observe and judge (Bloss, 1999).

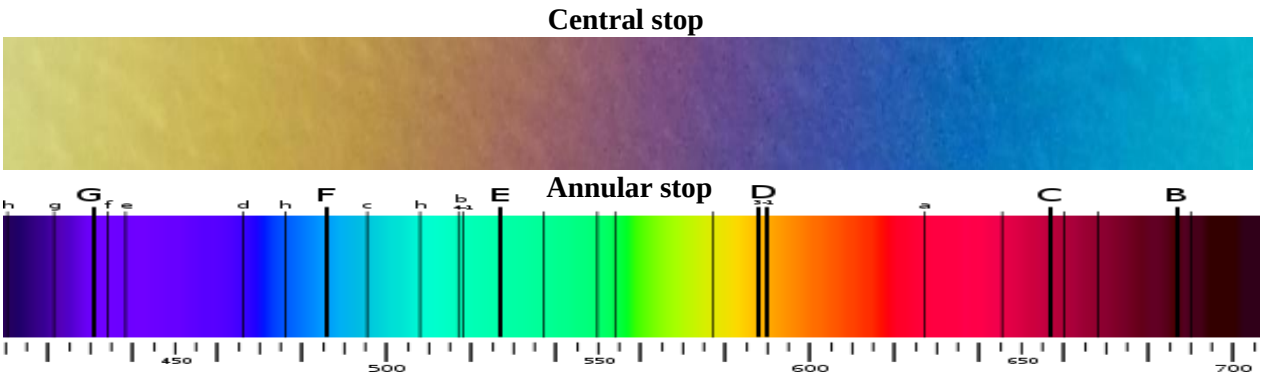


Figure 5. The complimentary color chart of annular stop (λ_m) and central stop dispersion staining color

Dispersion staining technique is a sensitive and relatively fast method without requiring extensive sample preparation. The image of the sample is seen as a bright dispersion staining color against a dark background using central stop objective. This technique is straightforward and does not require a high-skilled microscopist to conduct an analysis. The cost of the analysis per sample is relatively low compared to other techniques.

4.3 Becke Line Observation and Dispersion Staining Plot

The Becke line was used to determine relative refractive index of the sample. The relative refractive index of the sample to the immersion liquid can be determined quickly by observing the halo of light around the sample in the mounting medium. This halo is named Becke line. When the sample slide on a stage of the microscope is lower, the Becke line will move into the medium having a higher refractive index (Gaudette, 1988).

Each evidence item was determined by relative refractive index compared to a mounting liquid and dispersion staining color of each sample was recorded. The further analysis is to find

the suitable refractive index of a mounting liquid for a specific sample to produce a unique central stop dispersion staining color. If the sample and mounting liquid' s refractive indices are known, the Hartmann plot was used to predict the matching wavelength color. By using Hartmann plot (Figure 6), the color appearing with the dispersion staining technique can be determined. The three major refractive indices of a sample and mounting liquid were plotted at wave length 486 nm (F line), 589 nm (D line) and 656 nm (C line). The matching wavelength is where the two lines cross and the dispersion staining color can be identified. The example of plotting is shown in Figure 6 by using quartz with three refractive indices of Cargille oil (1.540, 1.544 and 1.546).

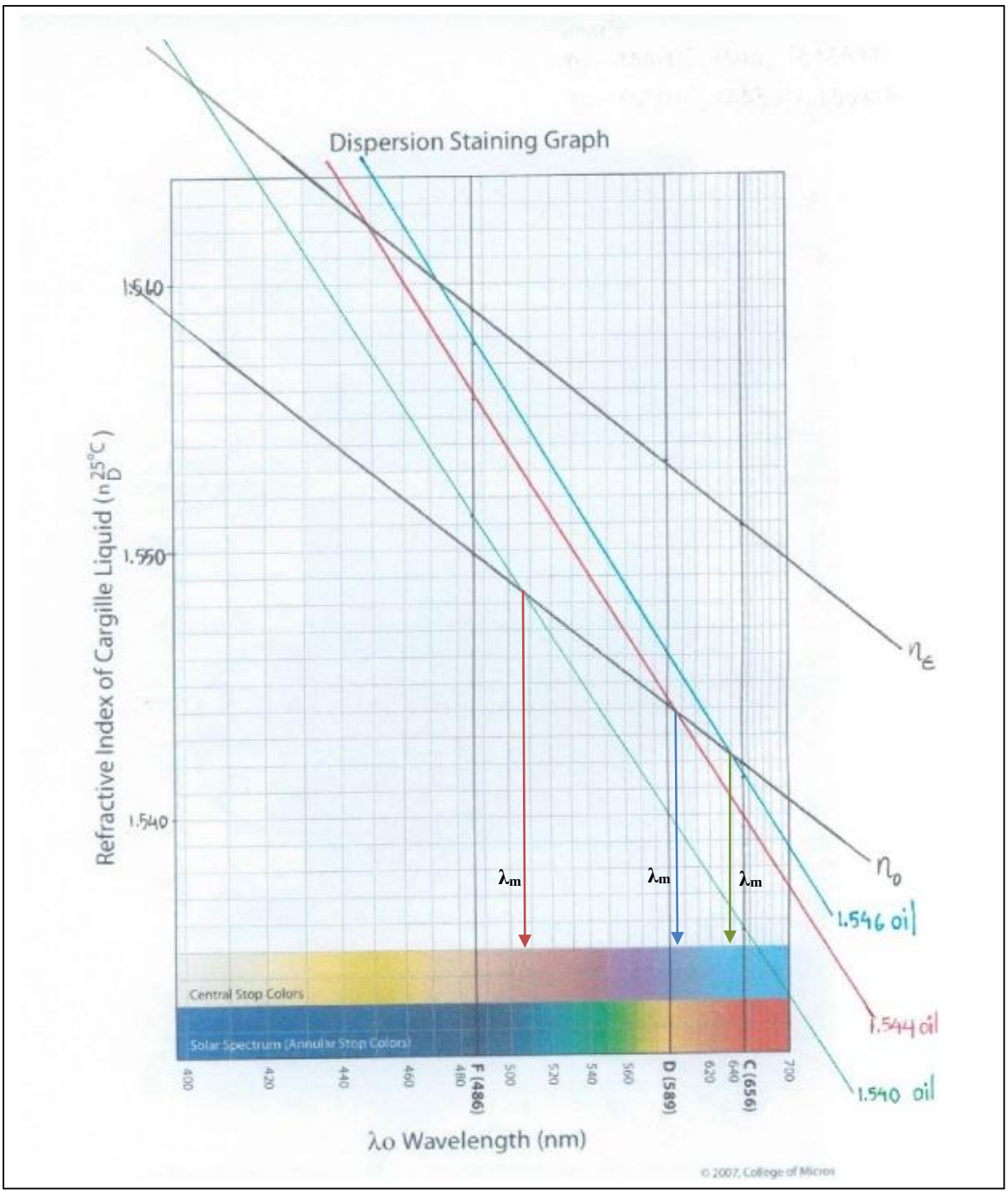


Figure 6. Hartmann plot of quartz in Cargille liquids to identify dispersion staining color (Delly, 2007)

The classes of trace evidence examined were divided into three groups. The first group is common synthetic and natural undyed fibers, the second group is commonly abused drugs and the last group is commonly found soil minerals. The instrumentation used in this research is a polarized light microscope with central stop dispersion staining objective and an ATR microspectrometer with diamond ATR objective. All analytical testing was repeated to assure the repeatability of these techniques. An infrared spectrum was collected from each sample to validate the identity of these samples used in this study (Weinger, 2007).

4.4 Exemplar Materials

Three classes of trace evidence samples were studied to illustrate the practical forensic application of polarized light microscopy and dispersion staining methods. Fibers, soil minerals and illicit drugs were selected as exemplars to illustrate the versatility, utility and practicality of polarized light microscopy and dispersion staining for use in remote laboratories in developing countries. The molecular composition of the exemplar samples were validated by recording their infrared ATR spectra and comparing with standard reference data.

Class 1, *Fiber evidence:* Textile Fiber evidence is common trace evidence recovered at crime scenes. Fibers are used and may be found everywhere; therefore, fibers have a crucial role in crime investigation to link the suspect, crime scene and victim. When viewed with the microscope, many textile fibers look similar to each other. Traditionally, morphology and optical properties are used to differentiate textile fibers. Equipped with a polarized light microscope and a suitable set of refractive index liquids, it is possible to determine the refractive index and other optical properties of a fiber. The optical properties that are most useful for fiber identification are: sign of elongation, refractive index (n_{90}) for light vibrating in a plane

perpendicular to the length of the fiber, refractive index (n_0) for linear polarized light vibrating in a plane parallel to the length of the fiber and quantitative birefringence. Refractive index alone is insufficient because certain types of fiber such as flax and silk may have similar refractive index values, and individual samples of a fiber, especially plant fibers, are prone to show some variation in refractive index (Eyring & Gaudette, 2005).

Generally, fibers behave like uniaxial crystals; most of them have a positive sign of elongation (n_0 is greater than n_{90}), but a few of them such as Orlon has a negative sign of elongation (n_{90} is greater than n_0). Due to the fact that most fibers are anisotropic, each of refractive indices and dispersion staining colors should be measured with linear polarized light in both parallel and perpendicular to the fiber axis. Dispersion staining, when applied to fibers, leads to many advantages. This technique is inexpensive, fast and nondestructive to the sample. The dispersion staining objective is used to observe the illumination of color in both parallel and perpendicular direction (Forlini, 1971).

Although analyzing asbestos is not a part of this study, asbestos analysis is one of the major examinations for which, dispersion staining is broadly applied. Dispersion staining for asbestos analysis has been applied and accepted by both court and administrative agencies such as Occupational Safety and Health Administrations (OSHA) and Environmental Protection Agency (EPA) as a valid method (Crane, 1992).

Asbestos is a term for naturally occurring fibrous minerals including chrysotile, amosite, anthophyllite, tremolite, crocidolite, actinolite and any of these minerals which have been chemically treated or altered. Asbestos minerals belong to two mineral families: the serpentines, and the amphiboles. In the serpentine family, the only common fibrous mineral is chrysotile.

Sometimes, the mineral antigorite, a polymorph of serpentine, occurs in a fibril habit with morphology similar to the amphiboles. Asbestos analysis consists of three parts: The determination of whether there is asbestos present, what type is present and the determination of how much is present. The general flow of the analysis is gross examination, examination with PLM and determination of species by dispersion staining (Dyar et al., 2008).

Most fragments of asbestos minerals are differentiated from true asbestos fibers because the true cleavage fragments normally have larger diameters than 1 µm. Internal structure of particles larger than this usually shows to have no internal fibrous structure. The cleavage fragments of the monoclinic amphiboles show extinction under crossed polars with no compensator. Examination the sample by unaided eye and estimate whether any sample preparation is necessary to identify physical characteristic of each fiber. The sample slide is prepared and observed with PLM with total magnification of 100X and 400X. The morphology of each fiber is noted such as long, thin, or straight with little curvature which are the indications of fibers from the amphibole family. Curved, wavy fibers are usually indicative of chrysotile. Once the birefringent fibers have been identified to be present, microscope is adjusted for dispersion staining mode. The dispersion staining central stop objective is inserted and the color of scattered light is observed. The colors must be consistent with the colors generated by standard asbestos reference materials for a positive identification. For example, for a liquid (n = 1.550 HD), chrysotile shows blue and magenta color. In certain cases, the liquid does not give a good dispersion color. For example, in n= 1.605 HD liquid, chrysotile and amosite appear white in all directions so they cannot be distinguished one from another. Some fibers have a coating on them which makes dispersion microscopy very difficult or impossible. Becke line analysis or electron microscopy may be performed in those cases (Crane, 1992).

For this study, all nine fiber standards were purchased from Testafabrics, INC, West Pittston, Pennsylvania. These exemplar fibers contain acrylic, cotton, diacetate, modacrylic, polyamide, polyester, rayon, silk and triacetate.

Class 2, Soil minerals: Forensic soil examination is well known and used because of its ability to match a perpetrator or victim to the crime location (Raymond & Tedrow, 1991). Soil evidence is valuable because there are an unlimited number of soil varieties and soil compositions change relatively quickly from each location, even if the separation is a short distance. The discriminating power of soil examination depends on the mineral components in soil. The purpose of the examination is to identify the minerals contained in a sample of soil from a certain location. Soils are complex mixtures both natural and artificial substances that can vary greatly within short geographic distances. A proper association requires multiple samples to be correlated. Theoretically, soil may contain unique particles, a characteristic property or artifact that will increase the value for comparison. For instance, if a rare mineral in soil from the scene where the body was found is matched with a sample soil from a suspect's vehicle, this would be strong incriminating evidence. Polarized light microscopy examination requires the characterization of the particles' optical properties. Microscopist can identify many particles based on properties such as color, transparency, refractive index, pleochroism, birefringence, surface texture and morphology (De Forest, 2002).

This research evaluated the use of the dispersion staining as an efficient means for mineral identification and as a complementary technique to classical polarized light microscopy. Soil evidence has been used in many ways in both criminal and civil cases. Notwithstanding, the value of soil evidence, it is not used to its fullest potency by many forensic science laboratories due in large part to the lack of criminalists trained in forensic geology and the time required for

soil analysis. To rectify these problems, there is a necessity to find supplementary methods to PLM analysis that decreases the time and increase the precision of mineral identification (Murray & Solebello, 2002).

For soil minerals, this study focuses on the major soil minerals found in the surface soil. Ms. Krittaya Pattamalai, senior geologist at Bureau of Mineral Resources, Department of Mineral Resources of Thailand identified the following nine minerals as the most common soil surface minerals found in Thailand. These are kaolinite, bentonite, diatomite, talc, marl, quartz, illite, smectite and pyrophylite. Not all these standard minerals were available or suitable for this research. Therefore, nine standard minerals that are transparent and colorless were used instead as exemplars. The list of transparent mineral standards is aragonite, calcite, fluorite, gypsum, kaolinite, quartz, talc, topaz and tremolite. The standard minerals were purchased from R.P. Cargille Laboratories, INC., Cedar Grove, New Jersey, USA. The identity of mineral samples was validated by infrared microspectral analysis.

Class 3, Illicit Drugs: in developing countries, identification of illicit drugs is one of the major problems where the drug analysis must be efficient and inexpensive. Drug detection is a major problem in a provincial area due to the limited budget and personnel. With the high number of cases related to illicit drugs, the analyst in a remote laboratory needs to have a method that can detect and analyzed drugs rapidly and reliably. Drug analysis usually involves analysis of drugs in "street form". A classification scheme is dependent on how the drugs occur. Drugs could be naturally occurring such as cocaine morphine codeine and marijuana; semi- synthetic (prepared from naturally occurring material) such as heroin and LSD, or synthetic such as phencyclidine, amphetamines and barbiturate. In major well-funded laboratories, identification

of the drug is performed using costly instrumentation such as GC/MS or HPLC requiring highly trained analyst (Siegel, 1988).

Due to the large number of cases in developing countries, the simple and effective method is required for these countries to achieve their goal to provide better justice. The drug analysis is also a major crime in many developing countries. Dispersion staining is a simple method that meets this need. The samples of prevalent drugs of abuse were used as an exemplars for dispersion staining technique is shown in Table 1.

Table 1. List of standard drugs used in this study

Drug name	source
1. Cocaine hydrochloride c-II	Sigma Life Science-C5776-5G; Lot #SLBB5746V
2. Codeine C-II	Sigma/Fluka Analytical-C5901-50 MG; Lot #SLBF7351V
3. Flunitrazepam (C-IV) (aka Rohypnol)	Sigma Life Science-F9261-100MG; Lot 056F0685V
4. Ketamine hydrochloride (C-IIIN)	Sigma Life Science-H159-25MG; Lot #091M4628V
5. Methamphetamine hydrochloride C-IIN	Sigma Life Science-M8750-5G;Lot # SLBG3762V
6. Morphine sulfate salt pentahydrate C-II	Sigma Life Science-M8777-50MG; Lot# SLBF7353V
7. Oxycodone hydrochloride	Spectrum-03269,1G; Lot#qK2163
8. Phencyclidine hydrochloride C-II (PCP)	Sigma life science-P3029-100MG; Lot #081M4033V

The value of the microscope is well established. It is the goal of this dissertation to determine the best approach to be taken so that a workflow to identify trace evidence using the

light microscope was created. As previously mentioned, infrared spectroscopy was used to validate the chemical identity of exemplar samples.

4.5 Infrared Spectroscopy Validation Method

Infrared microspectroscopy was used to validate identifications made using light microscopical methods. Samples were analyzed using an attenuated total reflection infrared microspectrometer (IlluminatIR®, Smith Detection. Danbury, CT) equipped with a diamond ATR objective, see Figure 7.

Figure 7. The ATR microscpectroscopy unit

High quality spectra are obtained when good contact is made between the ATR objective and the sample. One of the few factors that must be considered is the hardness of the sample. A soft sample usually makes good contact with the internal reflection element and no sample preparation required. Brittle solids do not usually compress without shattering when force is applied. To solve this problem, the brittle solid sample is mounted in oil (mineral or Nujol). The mounting of a sample in oil has three major advantages: the highest quality image is obtained due to the reduction in visible light scattering, optical properties, such as relative refractive index, are able to be determined, and the viscosity of the oil prevents the scattering of the shattered fragments. Nevertheless, IR microscopists are concerned with the effect of the oil on the resulting spectra, since oils are organic liquids with some IR absorption. However, while contact is increased, the oil is squeezed from between the diamond and the sample. When sufficient contact is reached, there is no interference by the oil with the sample's IR spectrum (Reffner et al., 2007).

Infrared spectroscopy works by monitoring vibrational energy changes that occur during the absorption of the infrared radiation. Atoms in the molecule are vibrated relatively to each other. This vibration has a certain energy level or specific frequency. Nearly all molecules absorb infrared radiation except homonuclear diatomic molecule such as N_2 and H_2. The IR spectrum of polyatomic molecules can be complicated because of many possible vibrational transitions and the existence of overtones, sum and difference bands. However, IR absorption bands are characteristic of specific groups within the molecule. IR spectrum is useful for the identification of the structure of compound (Ingle & Crouch, 1988).

Infrared reflection methods, especially the Attenuated Total Reflection (ATR) method, are widely applicable because of fewer requirements for sample preparation and it can be applied

to many types of samples. In ATR spectroscopy, the sample is placed tightly against the surface of an internal reflection element (IRE). Attenuation due to absorption occurs on an internal reflection at the interface between the sample and the IRE. The reflecting crystal should be chemically inert. One of the main advantages of ATR spectroscopy is that absorption spectra can be derived from many types of sample requiring a minimum of sample preparation. Samples of fabrics, threads, yarns, or fibers can be pressed against the ATR crystal. The mid-ATR spectrum of liquids can also be obtained (Reffner, 2005).

Infrared spectroscopy has proven invaluable in the analysis of organic and inorganic covalent materials because the infrared spectrum of a material is a physical constant. Materials that differ in molecular structure give rise to different IR absorption spectra, thus an IR spectrum can be used to identify an unknown by comparison with known standard spectra. Minerals are naturally occurring inorganic solids, and thus have characteristic spectra that correlate with their chemical formula and their crystal lattice. Infrared microprobe analysis unites light microscopy with IR spectroscopy to create system capable of correlating microstructure with chemistry (Weinger, 2008).

Attenuation in ATR takes place in a very thin surface layer. If the sample thickness is more than the penetration depth of the beam, the variation of thickness will not affect the spectrum. However, the band intensity is proportional to the concentration; therefore the quantitative measurement can be made (Reffner, 2005).

4.6 Analytical Methods

Polarized Light microscopy and dispersion staining were evaluated to determine their usefulness to forensic scientists working in developing countries. These methods are inexpensive and have been shown to be successful when applied to many types of physical evidence. This makes these methods potentially valuable to forensic scientists working in countries with limited resources. The goal is to develop a method that may be used by scientists to accurately and rapidly characterize and identify physical evidence.

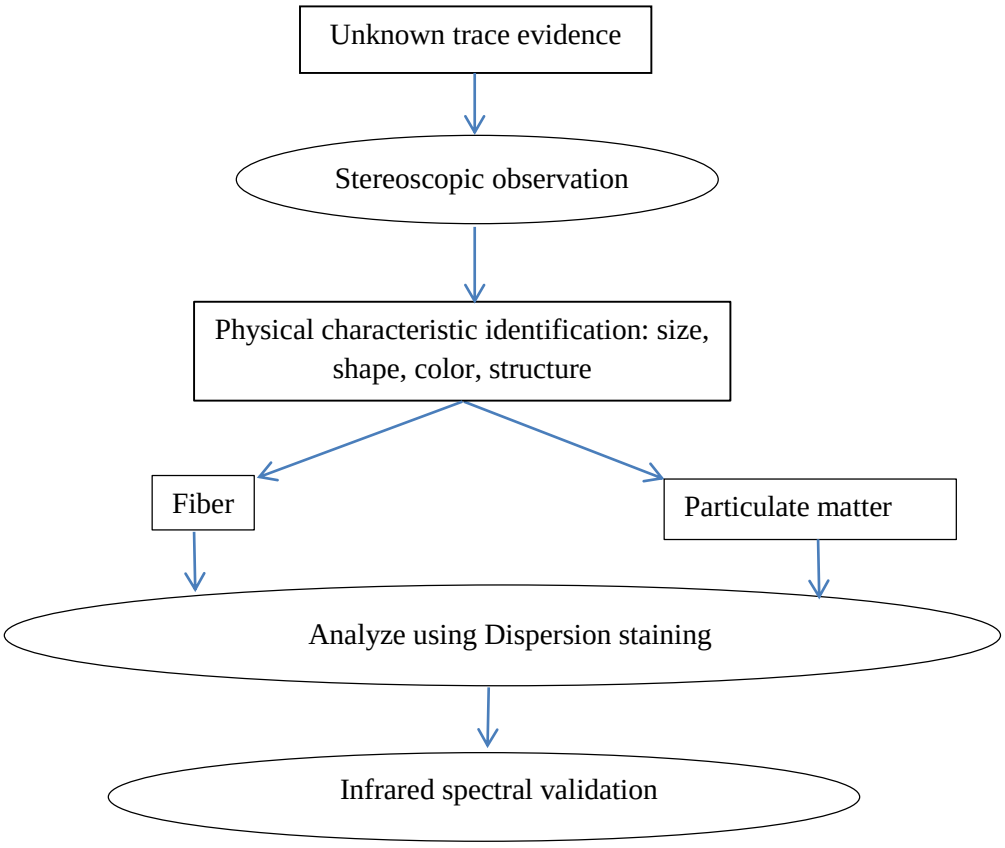
An initial step is to use stereo microscope to examine and prepare samples for PLM and dispersion staining analysis. The sample was mounted on a glass slide with a known refractive index liquid and observed using the PLM at 200X magnification and linearly polarized light to determine the relative refractive index by using the Becke line method. A comparative refractive index was noted and each sample was observed using central stop dispersion staining objective and the color was noted. Then each sample is analyzed by using an appropriate refractive index of a liquid to retrieve the good dispersion staining color (the region between blue to red and the combination of blue, red and yellow region). The appropriate refractive index of an immersion liquid was noted for each sample.

For an ATR analysis, each strand of fiber was separated on to the same slide and the diamond ATR objective was used to touch the sample, an ATR spectrum of each strand of fiber was retrieved. For a mineral sample, each mineral sample was prepared by using a drop of mineral oil as a media to hold the sample on a slide while moving down an ATR objective to touch the sample. The use mineral oil is to hold the mineral sample in place and prevent the sample from scattering away from contact with diamond ATR objective. An ATR spectrum of

each mineral sample was collected. For the drug sample, each small drug sample was put onto a slide and diamond ATR objective was moved down to touch the sample and ATR spectrum was collected. All ATR spectra for all standard samples are shown in an appendix D, E and F in this research.

The flow chart, Chart 1 below, represents the path to analyze an unknown sample by using dispersion staining technique and validation with ATR. It shows how unknown trace evidence can be categorized and analyzed by using by dispersion staining and infrared spectroscopic techniques.

Chart 1. The analytical flow chart of unknown physical evidence



Stereomicroscopic examination is used to identify the sample's morphology, color and structure. At this stage, the differentiation between a strand of fiber and particulate matter is accomplished by observing their obvious morphological differences. Fibers and particulate are further identified using the polarized light microscope with dispersion staining. Dispersion staining can differentiate chemical composition by using a specific refractive index liquid for each component (Palenik, 1974).

The immersion liquid used in this application should have stronger dispersion than the sample to produce unique colors with greater color contrast. Most commercially available immersion liquids of index beyond 1.52 have strong dispersion, but lower index of refraction liquids have lower dispersion than that of the solid sample, so the color effects become less striking. For the routine application of dispersion staining, greater color contrast is useful. The quality of the sample's color is enhanced and the sensitivity is increased (Willcox, 1964).

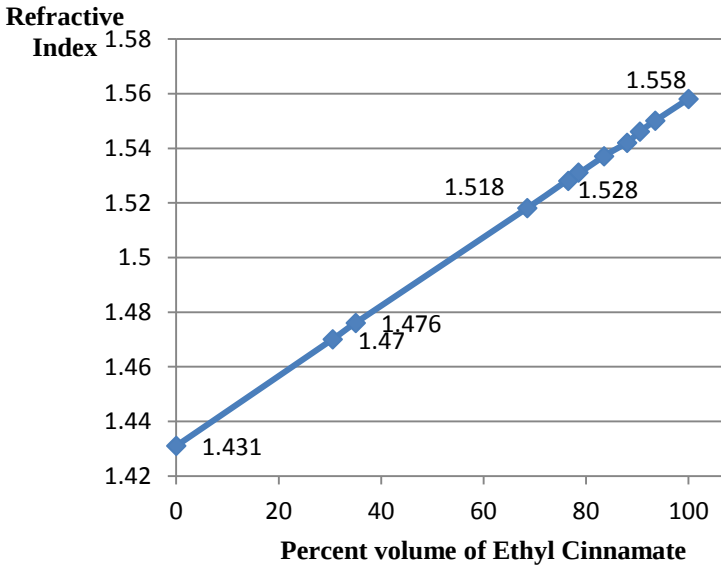
For this study, the high dispersion liquids were prepared by mixing two pure liquids having moderate refractive index and high dispersion. A set of liquid are made from ethyl cinnamate (n_D =1.558) and glycerol triacetate (n_D=1.431). The index range between them was made by mixing the two liquids at a series of volumetric ratios. The desired refractive index can be estimated by plotting the volumetric ratio versus refractive index of the two liquids. This mixture is suitable for the test because it can be easily made, the refractive index of the mixture is stable for an extended period of time and the cost is low compared to the set of Cargille refractive index liquids. For the refractive index outside this range, a Cargille set was used to conduct an experiment (Wilcox, 1964). Table 2 shows values of refractive index and the volume percent of the ethyl cinnamate in mixture with glycerol triacetate. The refractive indices were measured at 25 °C with a calibrated Abbe refractometer.

Table 2. The volumetric ratio between ethyl cinnamate and glycerol triacetate to make a high dispersion immersion liquid

refractive index of a mixture	percent volume of Ethyl Cinnamate
1.431	0.0
1.470	30.5
1.476	35.0
1.518	68.5
1.528	76.5
1.531	78.5
1.537	83.5
1.542	88.0
1.546	90.5
1.550	93.5
1.558	100.0

The plot below illustrates how to make a mixture having a desired refractive index.

Figure 8. Percent volume of ethyl cinnamate and glycerol triacetate



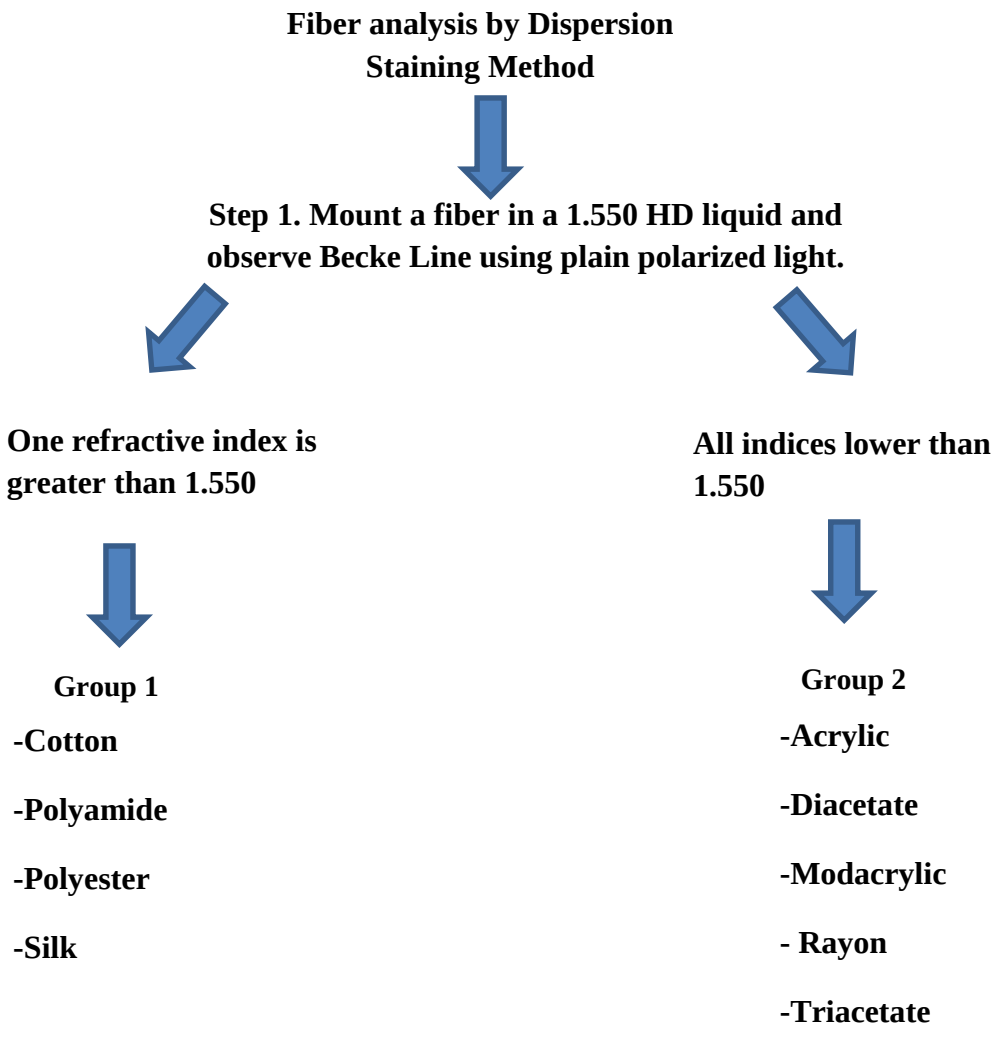
The refractive of any mixture (N) of glycerol triacetate and ethyl cinnamate can be calculated by using the following formula: N=0.00127(%volume ethyl cinnamate) + 1.431.

5. Result and Interpretation

Exemplar samples of all three classes of evidence were studied using the central stop dispersion staining technique. Every sample was mounted in an immersion liquid of known refractive index to observe Becke line (Johannson, 1914) and determine the relative refractive index of the solid sample compared to the immersion liquid. This mounting liquid was selected to separate sample into subgroups. The central stop objective was used to observe the dispersion staining color for this immersion liquid. This color can facilitate differentiation of some evidence but not all. Samples may display similar color so that a more specific immersion liquid is required to uniquely differentiate each sample.

Fiber evidence is readily distinguishable from particulates and is treated as a unique class. A fiber sample is mounted in a 1.550 high dispersion (HD) liquid (Wilcox, 1964). The first immersion liquid was selected based on the average value of refractive indices among all exemplar of fibers. From this step, the exemplar fibers can be divided into two subgroups based on their relative refractive index to the 1.550 liquid (Becke line observation). The first subgroup contains fibers that have one value of principle of refractive index (either parallel or perpendicular to the fiber axis) higher than 1.550. The first group of fibers contains cotton, polyamide, polyester and silk. The second subgroup contains fibers with all refractive indices lower than 1.550. The second group of fibers contains acrylic, diacetate, modacrylic rayon and triacetate.

Chart 2. Diagram illustrated dispersion staining method applied to fibers.



The next step is to observe a dispersion staining color with the central stop objective using the first immersion liquid with refractive index 1.550 HD. Some fibers show different colors but some show the same color in this liquid. The color was observed in two directions which are parallel (n_0) and perpendicular (n_{90}) to the fiber axis. The refractive index parallel to the fiber axis is represented by n_0 and the refractive index perpendicular to the fiber axis is represented by n_{90}.

The central stop dispersion staining color of each fiber mounted in a 1.550 HD liquid was noted and is shown in Table 3. From this data, certain fibers show the same color. In order to differentiate all fibers, each fiber was mounted in a series of refractive index liquid to determine a unique central stop dispersion staining color for each fiber. Each liquid was selected based on a unique dispersion staining color it produced for each fiber composition. Most desired dispersion staining colors when using central stop are red, blue, green, yellow, orange or magenta because these colors are the most distinct and can be easily differentiated. The appearance of white light indicates there is a large different refractive index and all wavelengths are being scattered. The specific refractive index of each fiber and the desired color is shown in Table 4.

Table 3. The central stop dispersion staining color of exemplar fibers in 1.550 high dispersion liquid

Fiber group1	**Color1(n_0)**	**Color2(n_{90})**
Cotton	yellow	purple
Polyamide	yellow	green
Polyester	blue	yellow
Silk	yellow	blue

Fiber group2	**Color1(n_0)**	**Color2(n_{90})**
Acrylic	blue	red-blue
Diacetate	yellow	yellow
Modacrylic	blue	blue
Rayon	purple	blue
Triacetate	yellow	yellow

In this research, two sets of refractive index liquids were used. The high dispersion liquid is made from ethyl cinnamate and glycerol triacetate and is designated as HD. The other set is a commercial set from Cargille and is designated as C in Tables 4, 6, 8 and 9.

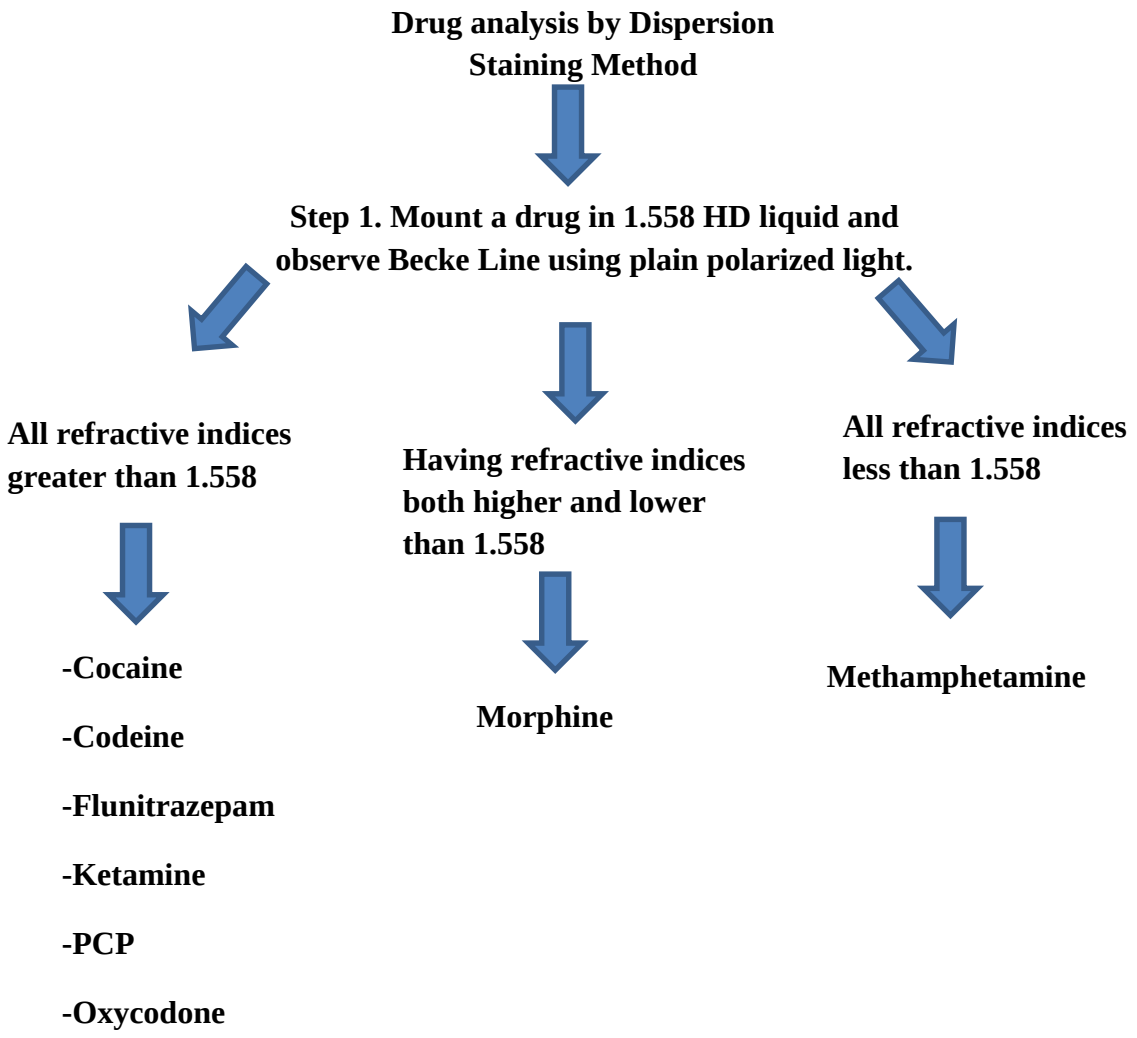
Table 4. A list of preferred refractive index values of mounting liquids for each fiber that produce a unique central stop dispersion staining color

Fiber type	Preferred liquid RI at λ=589 nm	Color1(n_{90})	Color2(n_0)
Acrylic	1.517 HD 1.527 HD	dark-blue blue-green	dark-blue blue-green
Cotton	1.544 HD 1.558 HD	dark-blue bright blue	yellow-white yellow
Diacetate	1.475 HD 1.469 HD	dark-blue orange	dark-blue yellow
Modacrylic	1.558 HD 1.500 HD	bright- blue blue	bright- blue blue
Polyamide	1.541 HD 1.550 HD	blue green	yellow yellow
Polyester	1.558 HD	blue	yellow
Rayon	1.527 HD 1.517 HD	blue red	yellow yellow
Silk	1.558 HD 1.550 HD	blue blue	yellow yellow
Triacetate	1.475 HD 1.517 HD	bright blue green	bright blue blue-green

The dispersion staining method for drug samples is similar to that used for fibers with minor modifications, see Chart 3. In the first step of drug analysis, a sample of drug was mounted in a 1.558 HD mounting liquid. The refractive index of the mounting liquid was selected because the exemplar has relatively high refractive index value (Winchell, 1954). In this step, the exemplar drugs can be divided into three subgroups based on a relative refractive

index to the 1.558 HD liquid (Becke line was observed at extinction position). The first subgroup contains drugs having all values of principle of refractive indices higher than 1.558, the second subgroup contains drugs with principle refractive indices both higher and lower than 1.558 at extinction position and the third subgroup contains drugs with principle of refractive index lower than immersion liquid at extinction position. The first group of drugs contains cocaine, codeine, flunitrazepam, ketamine, phencyclidine (PCP) and oxycodone. The second group of drugs contains morphine and the last group contains methamphetamine. From this step, morphine and methamphetamine can be determined by the central stop dispersion staining colors in this mounting liquid.

Chart 3. Diagram illustrated dispersion staining method applied to drugs.



The next step is to observe a dispersion staining color with the central stop objective with the first immersion liquid with refractive index 1.558 HD. Some drugs show different colors but some show the same color in this liquid. The color was observed in an extinction position of the drug particle because it is the direction of a principle refractive index. The color of each drug was noted and show in Table 5. From the data, certain drugs show the same color. In order to differentiate one drug from another, each drug was immersed in a series of refractive index liquid

to determine a unique dispersion staining color for each drug. Each liquid was selected based on a unique dispersion staining color produced for each fiber. The desired dispersion staining colors are the same colors as described for fibers.

Table 5. Dispersion staining color of drugs using central stop objective in 1.558 HD liquid at extinction position

Drug	Color1	Color2
Cocaine	yellow	yellow
Codeine	blue	blue
Flunitrazepam	white	purple
Ketamine	yellow	dark yellow
Methamphetamine	blue-green	blue-green
Morphine	white	blue
Oxycodone	yellow	yellow
PCP	orange	purple

Codeine, flunitrazepam, methamphetamine, morphine and PCP have distinguished colors but ketamine, oxycodone and cocaine display poor colors and cannot be differentiated, see Table 5.

The next step was to find a preferred mounting liquid that produced a unique central stop dispersion staining color for each drug. Table 6 shows a preferred mounting liquid with its central stop dispersion staining colors. The refractive index of drugs and mounting liquids are represented by n_d and n_l consecutively.

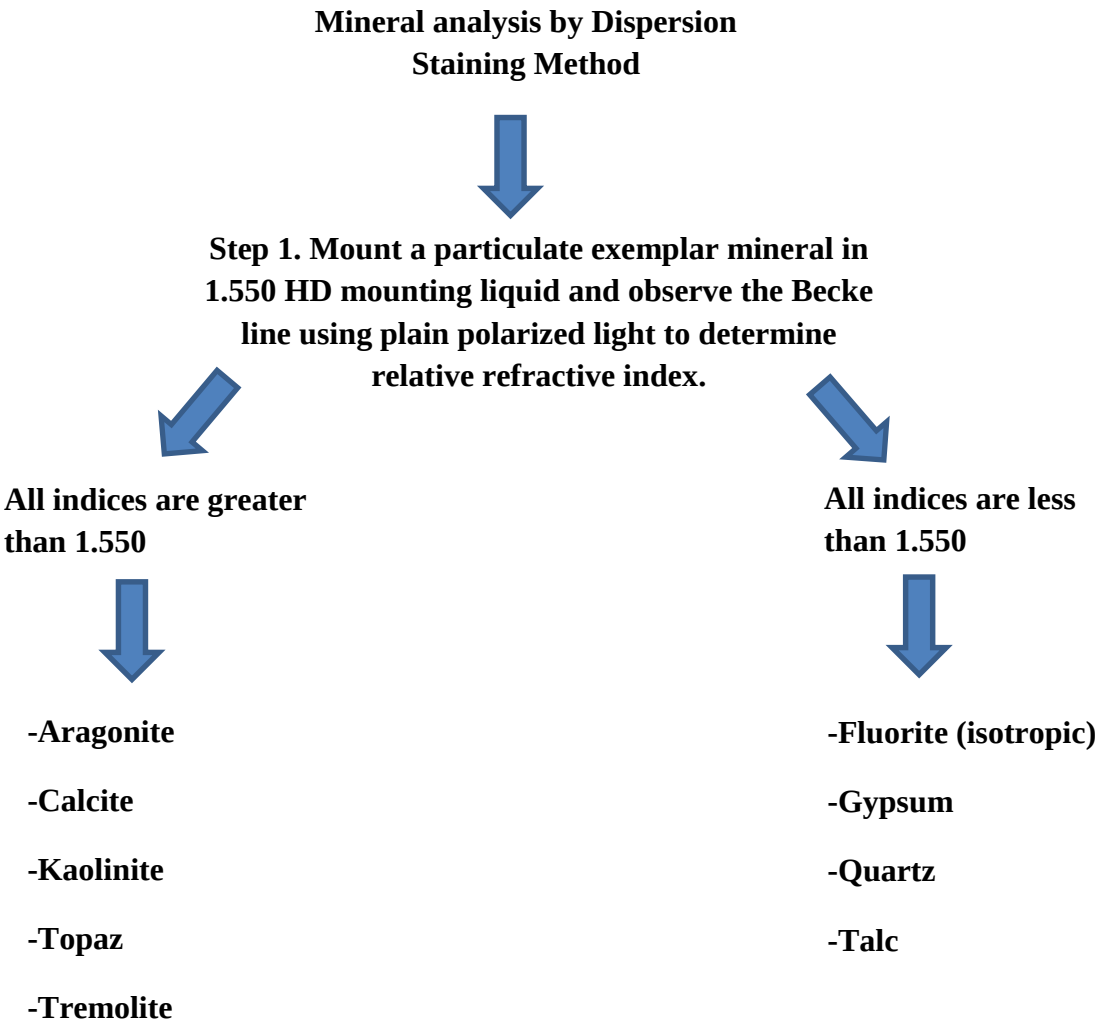
Table 6. Dispersion staining color of drug sample in a specific liquid to confirm the test

Drug name	Preferred refractive index of liquid	Color 1	Color 2
Cocaine	1.624 C 1.630 C	purple($n_d<n_l$) blue ($n_d<n_l$)	purple($n_d<n_l$) blue ($n_d<n_l$)
Codeine	1.469 HD 1.558 HD	blue-green ($n_d>n_l$) blue($n_d<n_l$)	yellow ($n_d>n_l$) blue ($n_d<n_l$)
Flunitrazepam	1.650 C 1.694 C	blue ($n_d>n_l$) purple ($n_d>n_l$)	white ($n_d>n_l$) yellow ($n_d>n_l$)
Ketamine	1.650 C 1.652 C	yellow($n_d\sim n_l$) blue-green ($n_d<n_l$)	purple($n_d\sim n_l$) blue($n_d<n_l$)
Methamphetamine	1.541 HD 1.544 HD	blue ($n_d<n_l$) blue-green ($n_d<n_l$)	blue ($n_d<n_l$) blue ($n_d<n_l$)
Morphine	1.620 C 1.624 C	blue ($n_d<n_l$) purple ($n_d<n_l$)	yellow ($n_d<n_l$) yellow ($n_d<n_l$)
Oxycodone	1.594 C 1.630 C	purple ($n_d<n_l$) blue ($n_d<n_l$)	yellow ($n_d>n_l$) blue ($n_d<n_l$)
PCP	1.630 C 1.650 C	blue ($n_d<n_l$) blue ($n_d<n_l$)	orange ($n_d<n_l$) yellow ($n_d<n_l$)

The first step in mineral analysis, a sample of each exemplar mineral was mounted in a 1.550 HD liquid. From this step, the exemplar mineral can be divided into two subgroups based on their relative refractive index (Becke line was observed at extinction position). The first subgroup contains minerals with refractive indices higher than 1.550, the second subgroup

contains minerals with refractive indices lower than 1.550. The first immersion liquid was selected based on the average value of refractive indices among a group of minerals (Winchell, 1964). The first group of minerals contains aragonite, calcite, kaolinite, topaz and tremolite. The second group of minerals contains fluorite, gypsum, quartz and talc. From this step, fluorite is also separated from other minerals by its isotropic property (when rotating the stage with crossed polars, the sample remains dark in all direction). The next step is to observe a dispersion staining color with the central stop objective with the first immersion liquid (1.550 HD). Some minerals show different colors but some show the same color in this liquid. The color was observed in two extinction positions of the mineral particle. The color of each mineral was noted and show in Table 7. From this data, certain minerals show the same color or a white color at this stage. In order to differentiate one mineral from another, each mineral was immersed into a series of refractive index liquid to determine a unique dispersion staining color produced for each mineral. Each liquid was selected based on a unique color shown with each mineral. The desired colors are similar to that of the colors of fibers and drugs (blue to red region or combination of these two regions and yellow). Chart 4 shows the diagram of the technique applied to the minerals.

Chart 4. Diagram illustrating the dispersion staining method applied to mineral analysis.



All particulate minerals in Chart 4 are anisotropic unless noted.

Table 7. Dispersion staining color of minerals in immersion liquid of N= 1.550 using central stop objective at extinction position

Mineral group 1	Color1	Color2
Aragonite	yellow-white	yellow-white
Calcite	yellow-white	white
Kaolinite	white	blue
Topaz	yellow	dark yellow
Tremolite	Blue-green	blue-green

Mineral group 2	Color1	Color2
Fluorite	yellow-white	yellow-white
Gypsum	yellow-white	white
Quartz	white	blue
Talc	yellow	dark yellow

All particulate minerals produce central stop dispersion staining colors that are indistinguishable with the exception of tremolite showing blue-green color in both orientations. Aragonite, fluorite and talc show yellow-white dispersion staining color in both direction which indicates that λ_m does not fall in a region producing preferred dispersion staining colors. Kaolinite and quartz show white and blue central stop dispersion staining color so these two minerals cannot be differentiated with this liquid. The next step is to find the unique index of a mounting liquid for each drug to confirm the test and can differentiate among the minerals that have the same dispersion color from Table 7. The table 8 displays the specific set of mounting liquid for each mineral with its central stop dispersion staining color. The comparative refractive index of each drug was also recorded in the table. The differentiation between drugs and minerals in a specific mounting liquid is shown in table 9.

Table 8. Dispersion staining color of mineral samples mounted in a specific liquid to confirm the identification.

Name	Liquid index	Color 1	Color2
Aragonite	1.682 C	blue($n_m<n_{oil}$)	blue($n_m<n_{oil}$)
	1.684 C	blue ($n_m<n_{oil}$)	blue ($n_{m\sim}n_{oil}$)
Calcite	1.558 HD	orange ($n_m<n_{oil}$)	purple($n_m<n_{oil}$)
	1.654 C	purple ($n_m<n_{oil}$)	yellow($n_m<n_{oil}$)
Gypsum	1.530 HD	blue($n_m<n_{oil}$)	blue($n_m<n_{oil}$)
	1.536 HD	bright blue($n_m<n_{oil}$)	bright blue($n_m<n_{oil}$)
Fluorite (isotropic)	1.431 HD	orange($n_m>n_{oil}$)	yellow($n_m>n_{oil}$)
	1.436 HD	bright blue($n_m<n_{oil}$)	bright blue($n_m<n_{oil}$)
Kaolinite	1.550 HD	orange($n_m>n_{oil}$)	orange($n_m>n_{oil}$)
	1.558 HD	blue-Green($n_m<n_{oil}$)	blue-green($n_m<n_{oil}$)
Quartz	1.544 HD	orange($n_m>n_{oil}$)	blue($n_m>n_{oil}$)
	1.550 HD	blue-green($n_m<n_{oil}$)	blue-green($n_m<n_{oil}$)
Talc	1.541 HD	yellow-purple($n_m<n_{oil}$)	yellow-purple($n_m<n_{oil}$)
	1.544HD	yellow-purple($n_m<n_{oil}$)	green-purple($n_m<n_{oil}$)
Topaz	1.550 HD	yellow-magenta ($n_m>n_{oil}$)	yellow-magenta ($n_m>n_{oil}$)
	1.620 C	blue-green($n_m<n_{oil}$)	blue-green($n_m<n_{oil}$)
Tremolite	1.590 C	yellow($n_m>n_{oil}$)	yellow($n_m>n_{oil}$)
	1.606 C	yellow($n_m>n_{oil}$)	blue($n_m>n_{oil}$)

Table 9. Differentiation between particulate samples (drugs and mineral) in each specific refractive index liquid

refractive index	mineral (colors1), (color2)	drugs (color1), (color2)
1.431 HD	fluorite (orange),(yellow)	
1.436 HD	fluorite (bright blue),(bright blue)	
1.469 HD		codeine (blue-green),(yellow)
1.530 HD	gypsum (blue),(blue)	
1.536 HD	gypsum (bright blue), (bright blue)	
1.541 HD	talc (yellow-purple), (yellow-purple)	methamphetamine (blue),(blue)
1.544 HD	quartz (orange), (blue)	methamphetamine (blue-green), (blue)
1.544 HD	talc (yellow-purple, green-purple)	
1.550 HD	kaolinte (orange), (orange)	
1.550 HD	quartz (blue-green), (blue-green)	
1.550 HD	topaz (yellow-margenta), (yellow-margenta)	
1.558 HD	calcite (orange), (purple)	
1.558 HD	kaolinte (blue-green), (blue-green)	codeine (blue), (blue)
1.594 C		oxycodone (purple), (yellow)
1.606 C	tremolite (yellow-blue)	
1.620 C	topaz (blue-green), (blue-green)	morphine (blue), (yellow)
1.624 C		cocaine (purple), (purple)
1.624 C		morphine (purple), (yellow)
1.630 C		cocaine (blue), (blue)
1.630 C		oxycodone (blue),(blue)
1.630 C		PCP (blue),(orange)
1.650 C		ketamine (yellow), (purple)
1.650 C		PCP (blue), (yellow)
1.650 C		flunitrazepam (blue),(white)
1.652 C		ketamine (blue-green), (blue)
1.654 C	calcite (purple),(yellow)	
1.682 C	aragonite (blue), (blue)	
1.684 C	aragonite (blue), (blue)	
1.694 C		flunitrazepam (purple), (yellow)

Colored regions represent the sample with the same refractive index of a mounting liquid.

The established dispersion staining method was confirmed by this study to be a valid, reliable and practical method to analyze three classes of commonly found trace evidence. The techniques to be employed are light microscopy with dispersion staining and infrared microscpectroscopy. The infrared spectra of samples were collected to validate the identity of the samples used as exemplar in developing this dispersion staining method.

The ATR spectrum for each sample was collected by using IlluminatIR®. The IlluminatIR® is an infrared microspectrometer combined with a polarized light microscope. The diamond ATR objective is used to touch the sample and collect the ATR spectra. The system also provided the magnified digital image of the sample seen through an infrared objective and can control an area the beam impinges on. The diamond ATR objective is a unique diamond internal reflection objective that operates like a standard objective but it can also collect the ATR spectrum. One of the advantages of this objective is that it is easy to clean, just wipe with an alcohol solution and it is ready to use for the next sample. The ATR spectrum for each class of samples is shown in Appendix D, E and F.

6. Contribution to the Fields of Forensic Science and Criminal Justice

This research advances forensic science and criminal justice by developing and extending the dispersion staining method to the examination of physical evidence in criminal investigations. This advanced method is intended to be used to examine the physical evidence in developing countries and laboratories with limited funding. The dispersion staining method is straightforward, fast, reliable and inexpensive. It is also easy to train personnel to apply this method to the analysis of physical evidence. Dispersion staining was advanced by the availability of high intensity of LED light source, refined optics and the development of inexpensive high dispersion refractive index liquid systems. Because of the relatively low cost of microscopes, their minimal maintenance costs and ease of use, it is possible to implement dispersion staining techniques in many laboratories. Thereby, equipping forensic scientist with a valuable tool to investigate crimes in the field where rapid and reliable information is needed to investigate crimes. In criminal investigations, timely response is important to insure that justice is served. In remote areas, justice is not always served because there is limited access to scientific evidence examination. Improving and extending the dispersion staining method for the examination of physical evidence advances forensic science and criminal justice.

7. Conclusion

The dispersion staining technique is a solution for analysis of trace evidence in laboratories with limited funding and trained personnel. This research shows that dispersion staining can be applied to create a unique staining color for individual evidence sample. Using polarized light microscope with central stop dispersion staining objective and specific refractive index liquids, each sample is identifiable by its unique dispersion staining color. Tables of dispersion staining colors produced by a specific refractive index liquids were established for each evidence class as shown in Table 4, 6 and 8. This data can be used as a reference for routine work. The technique is simple, inexpensive and easy to train personnel to use. These advantages will encourage and broaden the application of the dispersion staining method to laboratories in a remote area around the world (Shieh & Chen, 2013). This study also demonstrate that the technique can be applied to variety of evidence.

Trace evidence found at a crime scene can be identified by its dispersion staining color. The method developed in this research for analyzing trace evidence is effective and efficient. The method is best applied to rapidly answer the question of identity. Is this white powder methamphetamine? This question is readily answered by applying this dispersion staining method. The powder sample is placed on a microscope slide and with a drop of the 1.544 HD liquid and observed the central stop dispersion staining color. If the color is blue-green in both extinction positions, then the material is methamphetamine. No other common drugs produce this color under this condition. If other colors are observed, then the material is not methamphetamine. If the question then become what is it? The method is less efficient and further testing would be required. In practical use, where the evidence sample belongs to a limited group of material, the dispersion staining method is very efficient.

The outcomes of this research are summarized below

1. The low cost of immersion liquid was made by using a mixture of ethyl cinnamate and glycerol triacetate. The refractive index of a mixture was determined by a linear relationship between the refractive index and the volume ratio of the two liquids. This mixture can be applied to the majority of the samples in this study. High quality dispersion staining colors are generated using these mixtures. These mixtures can be applied to the range of refractive index value between 1.431 and 1.558.

The reason this outcome is important to forensic scientists in Thailand is because without the availability of a low-cost refractive index liquid, the price to perform the dispersion staining method would be more expensive. A set of commercial refractive index oils costs approximately 20-times more than this proposed refractive index liquid. This is especially important when analysis will be performed in remote laboratories and funding is even more strained than in Bangkok's central laboratory.

2. The exemplar samples analyzed in this study were selected to represent the commonly found materials for each evidence class. The samples analyzed with dispersion staining must be transparent in order to produce dispersion staining color. The chemical identity of exemplar samples was verified by using FT-IR microprobe analysis. The ATR spectra were collected for each exemplar material. The spectrum of each sample represents chemical composition in the molecule and is shown in Appendix D, E and F.

Verification of the exemplar samples analyzed in this studying using FT-IR was important. FT-IR is a well-accepted method in the field of forensic science. The infrared spectrum of a material is sometimes referred to as a chemical fingerprint. Using the FT-IR, the

protocol and methods proposed were validated in this research and results are accurate and reliable.

3. To show a distinctive color or colors using the dispersion staining technique, every sample was mounted in a liquid having a refractive index that matches the refractive index of the sample. The refractive index of each liquid was selected to provide a unique dispersion staining color for each sample. These colors are used to differentiate one sample from another. Visual observations using the microscope were used to classify each piece of physical evidence. The preparation of these unique liquids was used to confirm identification. This is important when comparing evidence samples to include or exclude them as coming from a common source. This determination is essential investigative information

4. The light source of a microscope affects an image quality of dispersion staining color. A microscope using LED light source produces better quality images and more intense dispersion staining colors than the traditional PLM using tungsten-halogen lamp. LED has a high intensity and low power consumption compared to the tungsten-halogen lamp in a traditional PLM. Dispersion staining technique requires an intense light source to generate higher quality images and more discriminating dispersion staining colors. The documentation of this observation is important because it allows the analyst to observe the dispersion staining colors more easily, making the method more user friendly.

Conclusively, the technique using PLM meets the need of the forensic investigators in crime laboratories in developing countries or in remote areas. The cost efficiency of a microscope, compared to other spectroscopical instruments is shown in Table 10. Investigators can learn to apply the techniques developed in this research with a minimal training. The

dispersion staining method provides information that has immediate impact on criminal investigations. The results of dispersion staining analysis are readily recorded and have been qualified and accepted by courts.

Table 10. Comparison of cost efficiency among spectroscopical techniques and PLM (Wilbur, 2005)

Technique	**System cost**	**Sample analyzed**	**Frequency of use**
Flame AA	15K-25K	single element	few
GFAA	30K-60K	few elements	few
ICP-OES	60K-100K	many elements	rarely
ICP-MS	130K-200K	many elements	rarely
GC/MS	50K-200K	drugs, explosives, chemicals	frequently
SEM	300-1000K	GSR, paint, soil, inorganic materials	frequently
PLM	**3K-10K**	**many types of evidence**	**frequently**

Flame AA = Flame Atomic Absorption

GFAA = Graphite Furnace Atomic Absorption

ICP-OES = Inductively Coupled Plasma- Optical Emission Spectroscopy

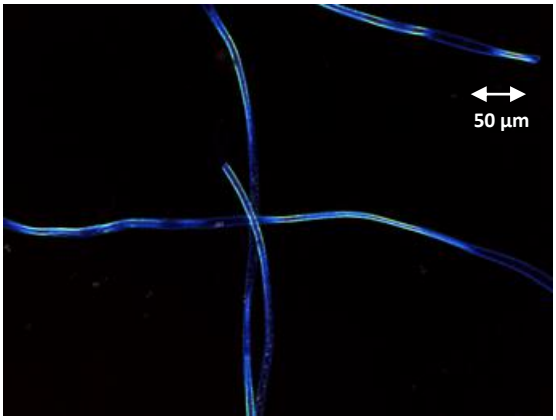
ICP-MS = Inductively Coupled Plasma- Mass Spectrometry

GC/MS = Gas Chromatography/ Mass Spectrometry

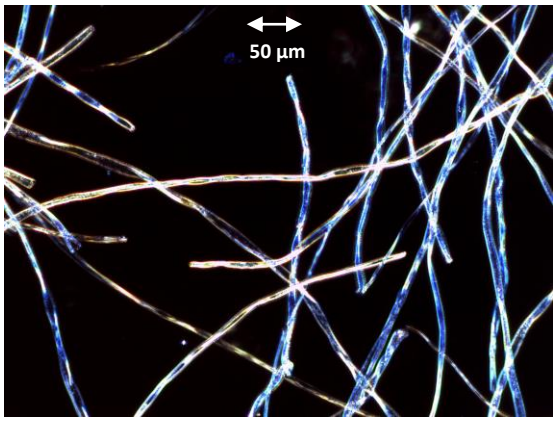
SEM = Scanning Electron Microscope

From the table above, the PLM is relatively inexpensive for the initial cost and can be applied to analyze many types of samples. It also does not require high cost of maintenance and intensive training. PLM and dispersion staining is suitable for many laboratories with limited resources to conduct physical evidence examination and better serve justice to the community.

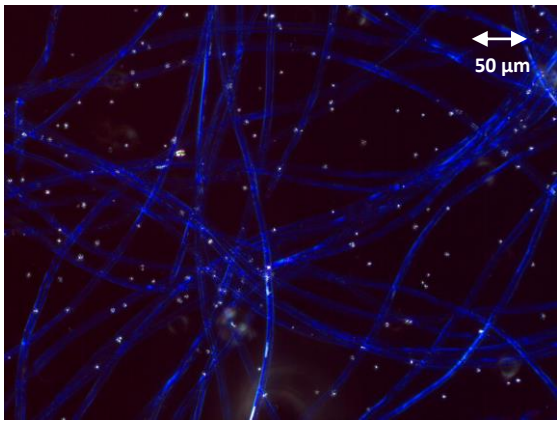
Appendix A. Central Stop Dispersion Staining Colors of Fibers



Acrylic fibers mounted in 1.527 HD liquid



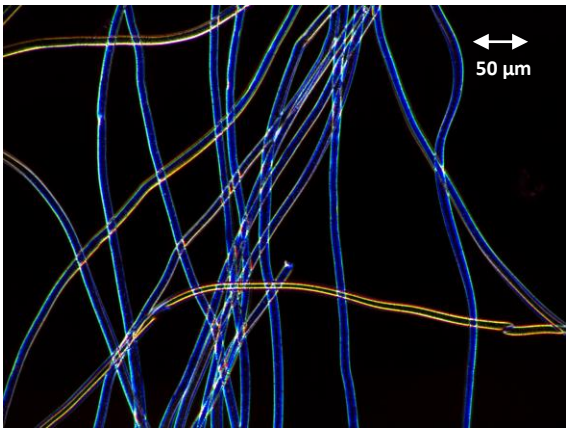
Cotton fibers mounted in 1.544 HD liquid



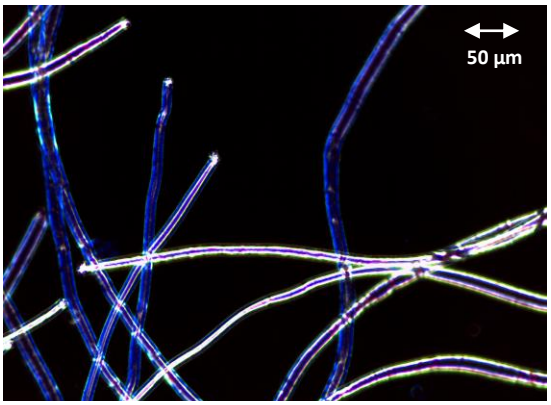
Diacetate fibers mounted in 1.527 HD liquid

Appendix A. Central Stop Dispersion Staining colors of Fibers (cont.)

Modacrylic fibers mounted in 1.550 HD liquid



Polyamide fibers mounted in 1.541 HD liquid

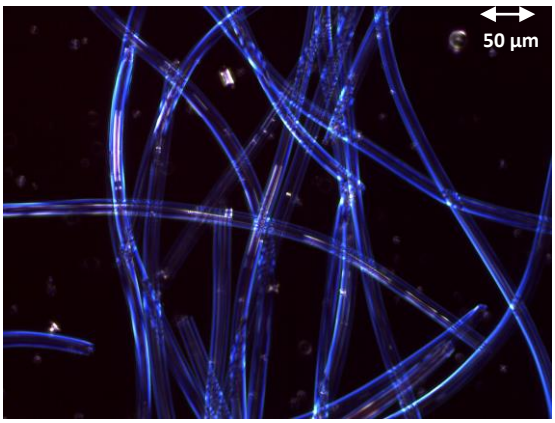


Polyester fibers mounted in 1.558 HD liquid

Appendix A. Central Stop Dispersion Staining colors of Fibers (cont.)

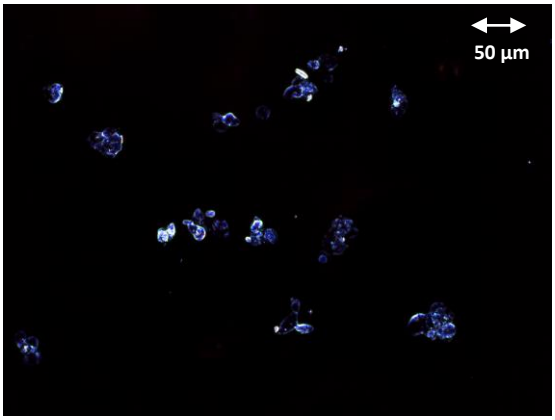
Rayon fibers mounted in 1.527 HD liquid

Silk fibers mounted in 1.550 HD liquid

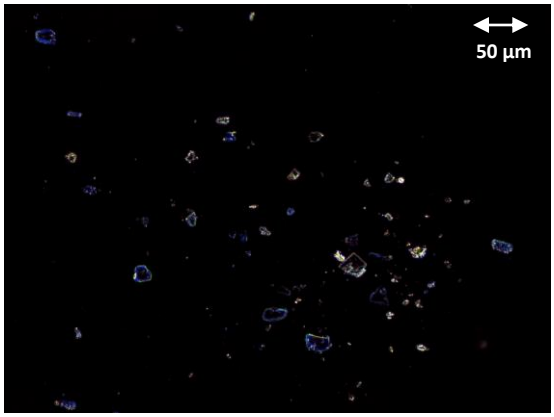


Triacetate fibers mounted in 1.475 HD liquid

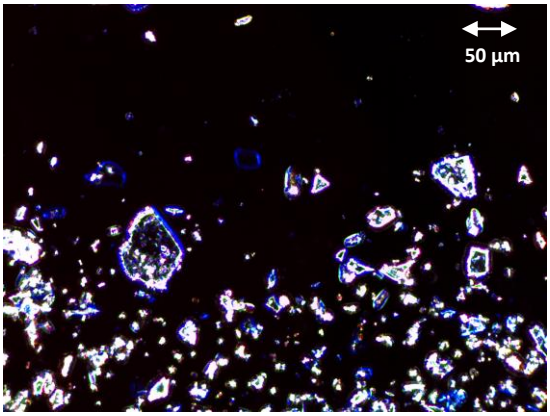
Appendix B. Central Stop Dispersion Staining Colors of Drugs



Cocaine mounted in 1.63 C liquid

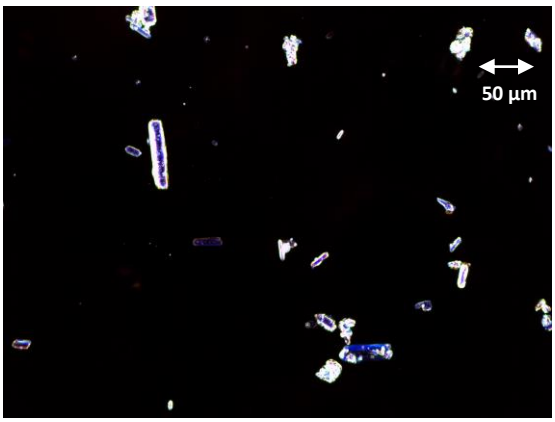


Codeine mounted in 1.469 HD liquid

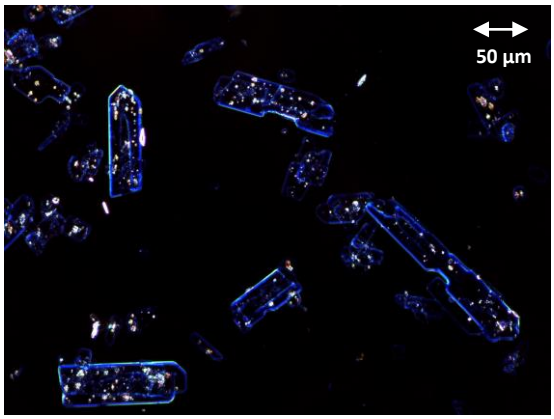


Flunitrazepam mounted in 1.650 C liquid

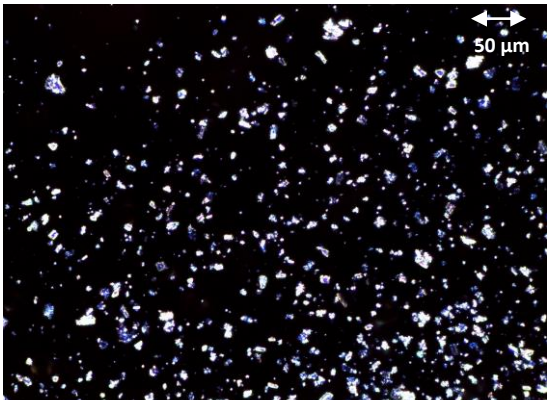
Appendix B. Central Stop Dispersion Staining colors of drugs (cont.)



Ketamine mounted in 1.65 C liquid

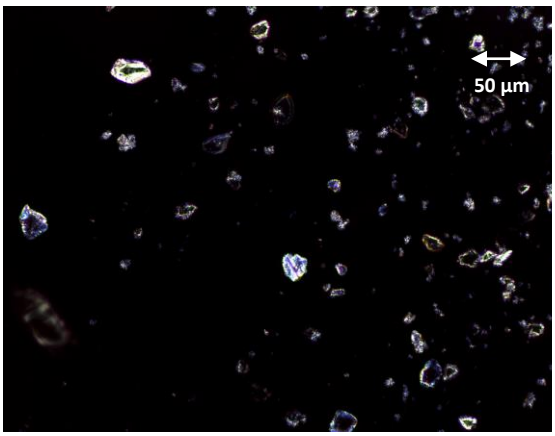


Methamphetamine mounted in 1.544 HD liquid

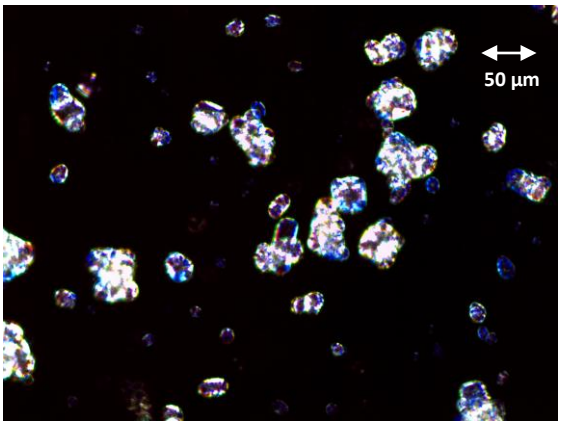


Morphine mounted in 1.624 C liquid

Appendix B. Central Stop Dispersion Staining colors of drugs (cont.)

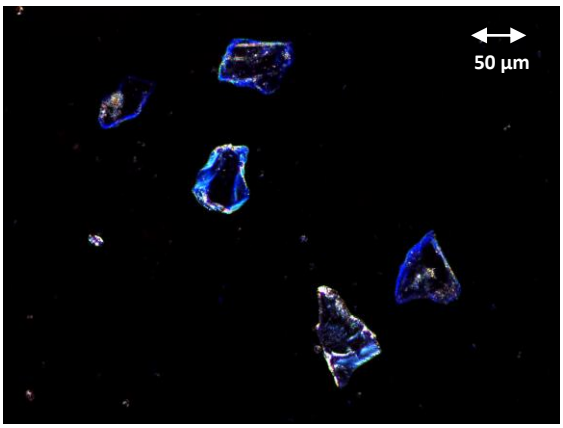


Oxycodone mounted in 1.594 C liquid

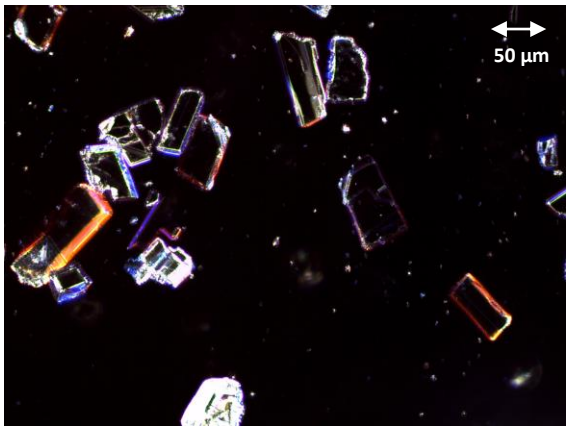


PCP mounted in 1.650 C liquid

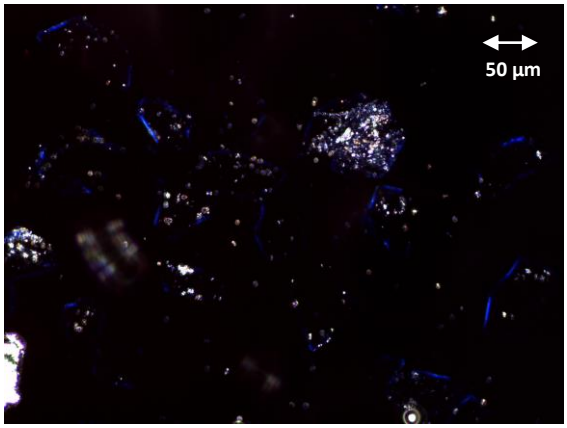
Appendix C. Central Stop Dispersion Staining Colors of Minerals



Aragonite mounted in 1.682 C liquid

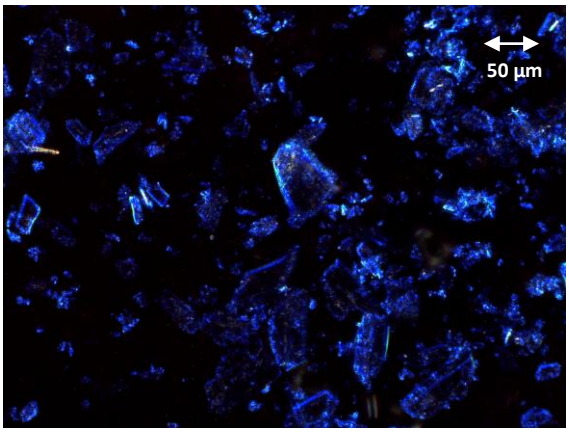


Calcite mounted in 1.558 HD liquid

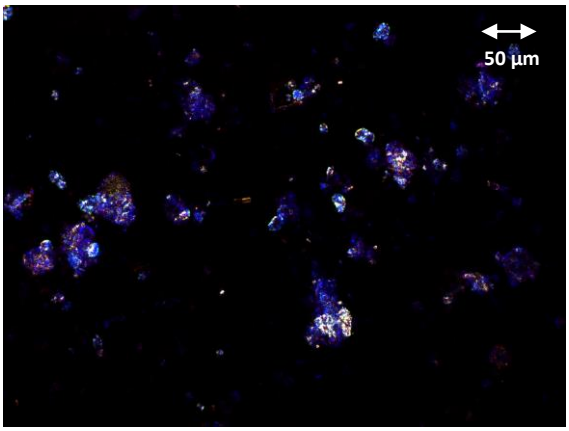


Fluorite mounted in 1.436 HD liquid

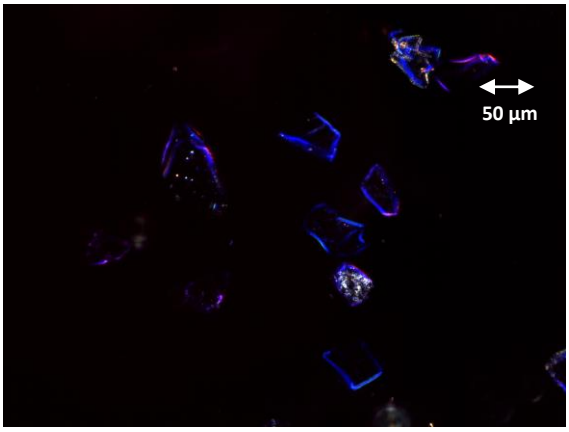
Appendix C. Central Stop Dispersion Staining Colors of Minerals (cont.)



Gypsum mounted in 1.530 HD liquid

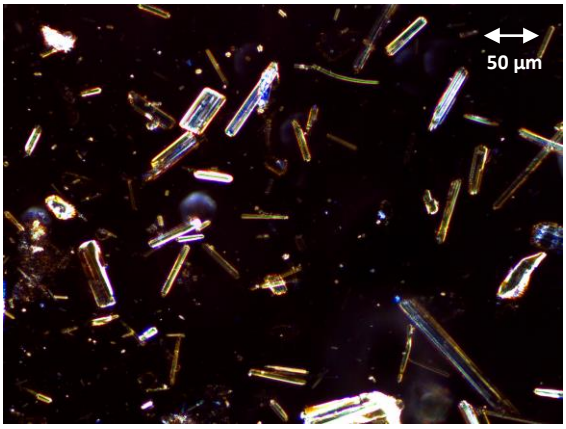


Kaolinite mounted in 1.558 HD liquid

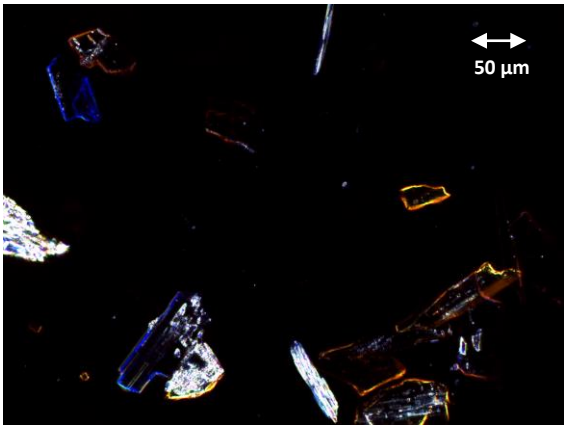


Quartz mounted in 1.544 HD liquid

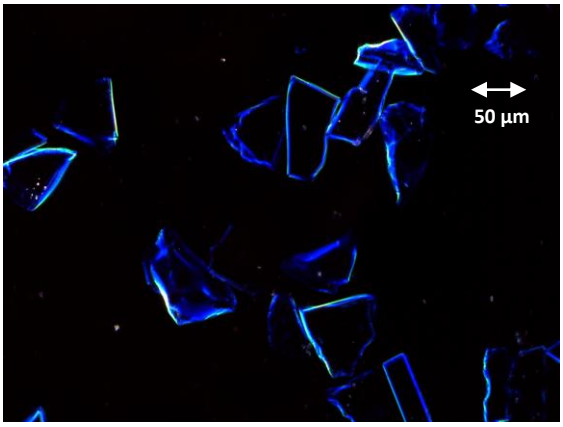
Appendix C. Central Stop Dispersion Staining Colors of Minerals (cont.)



Talc mounted in 1.550 HD liquid



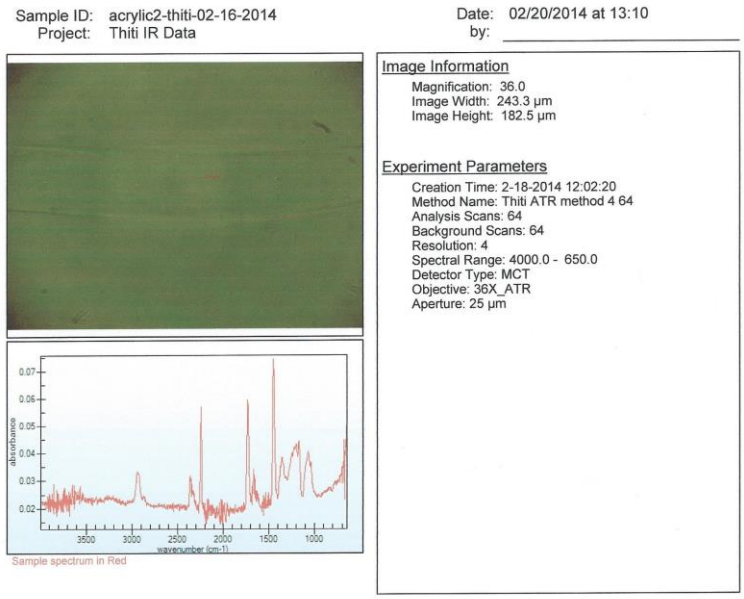
Tremolite mounted in 1.606 C liquid



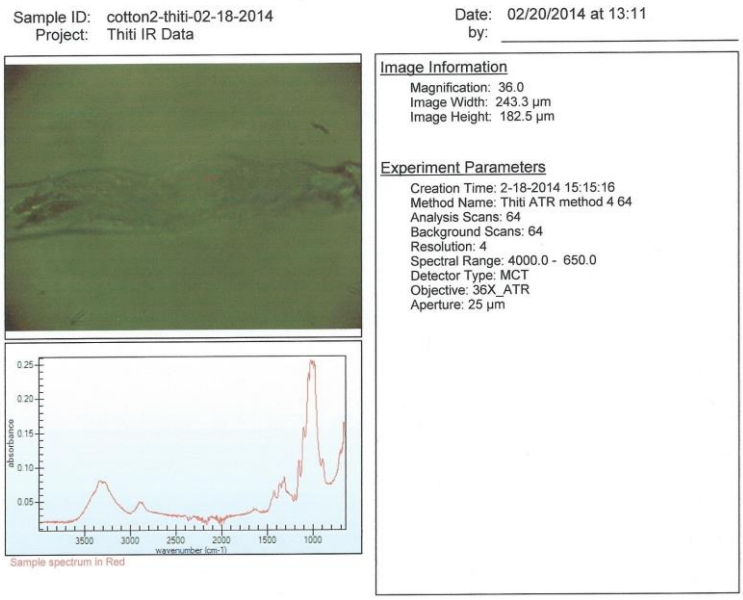
Topaz mounted in 1.620 C liquid

Appendix D. ATR Spectrum of Fibers

Acrylic Fiber

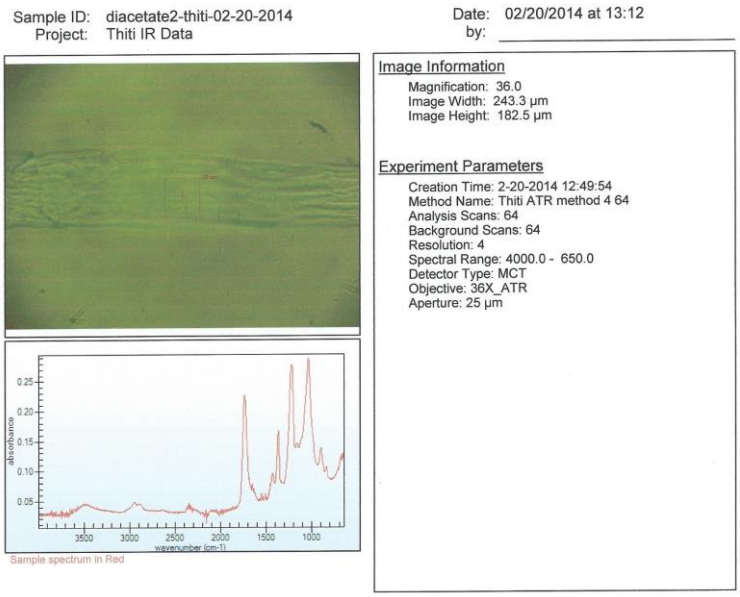


Cotton Fiber

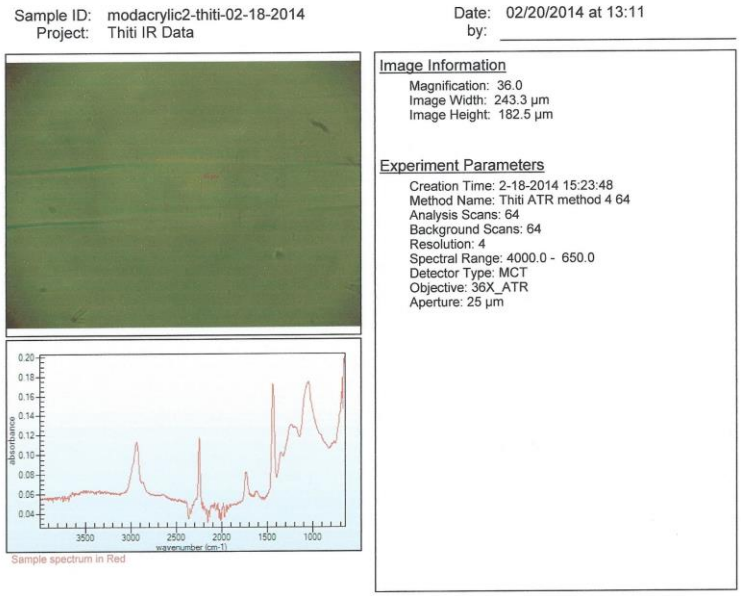


Appendix D. ATR Spectrum of Fibers (cont.)

Diacetate Fiber

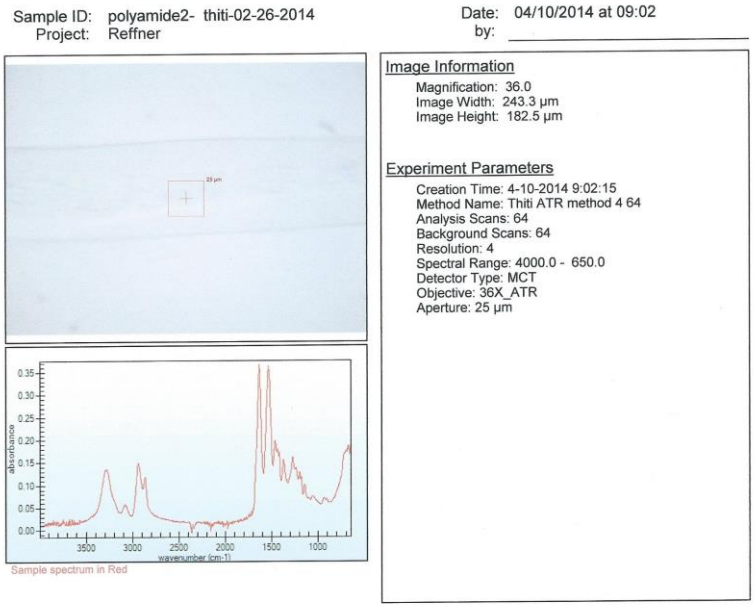


Modacrylic Fiber

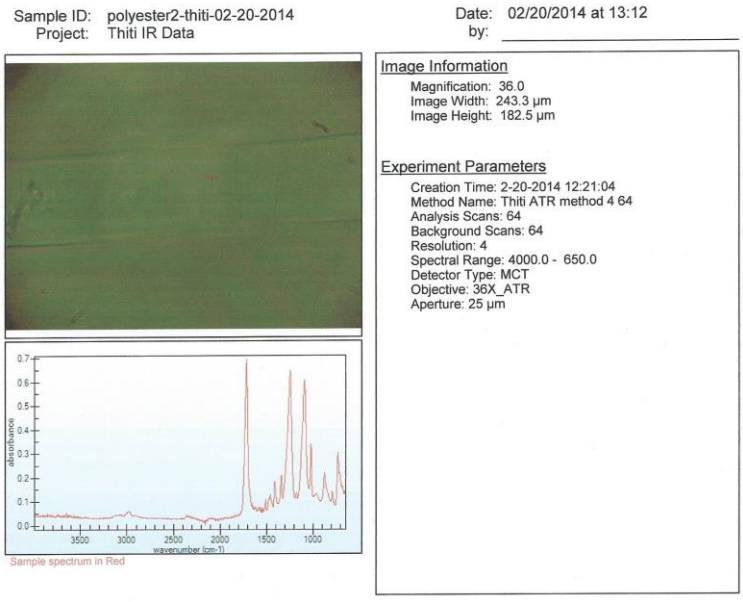


Appendix D. An ATR Spectrum of Fibers (cont.)

Polyamide Fiber

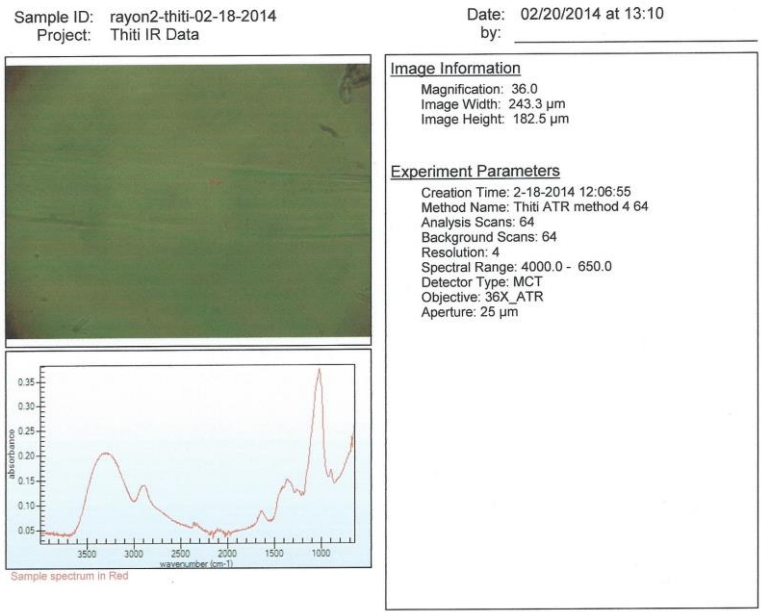


Polyester Fiber

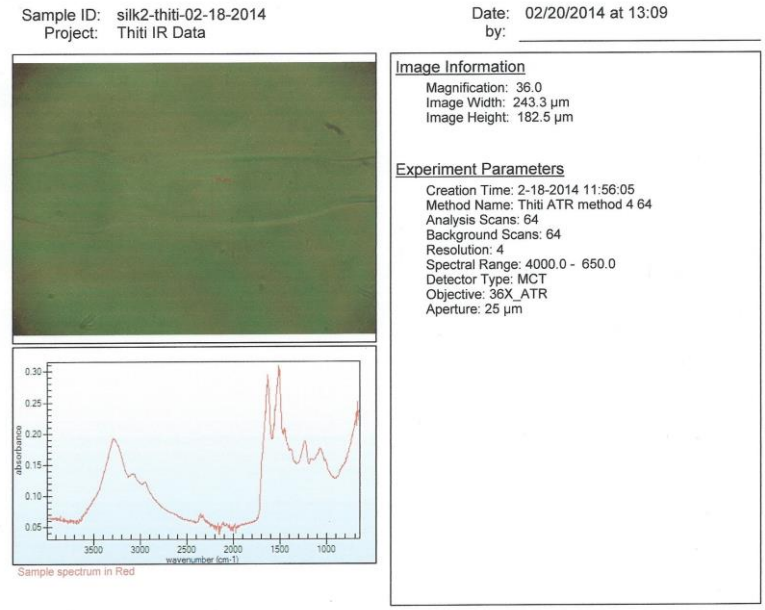


Appendix D. An ATR Spectrum of Fibers (cont.)

Rayon Fiber

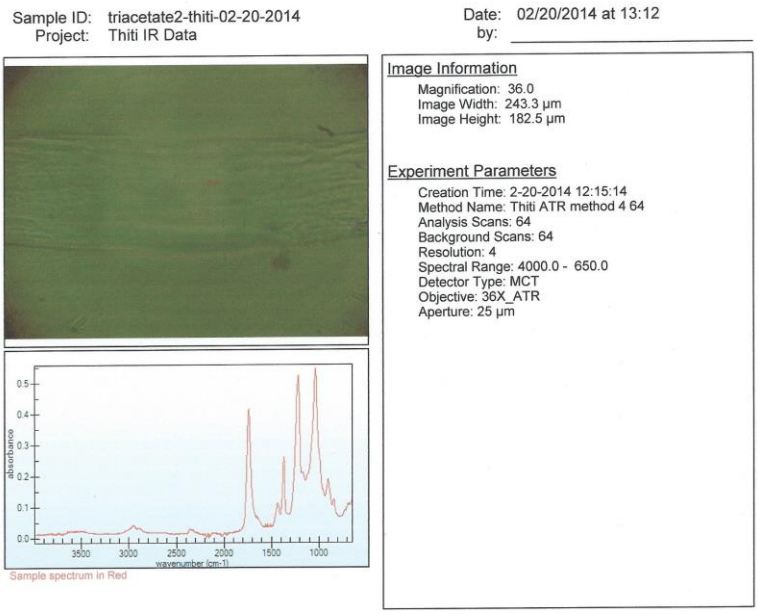


Silk Fiber



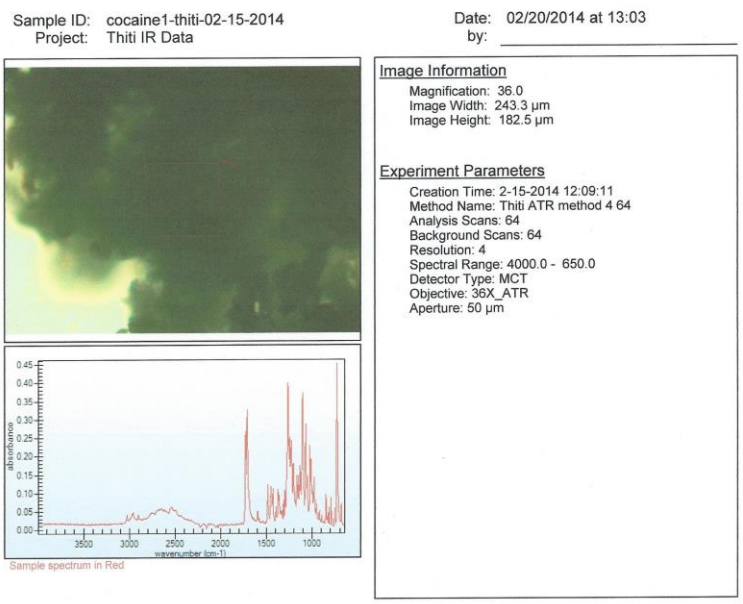
Appendix D. An ATR Spectrum of Fibers (cont.)

Triacetate Fiber

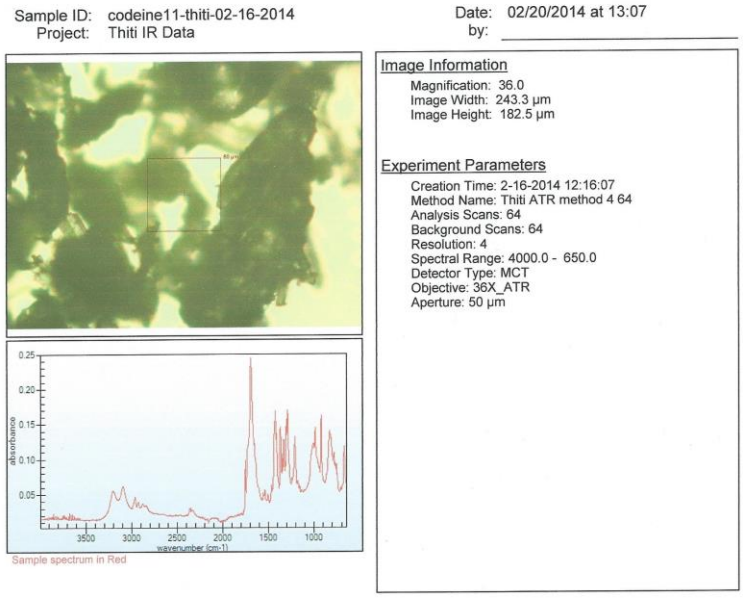


Appendix E. An ATR Spectrum of Drugs

Cocaine hydrochloride C-II Sigma Life Science-C5776-5G

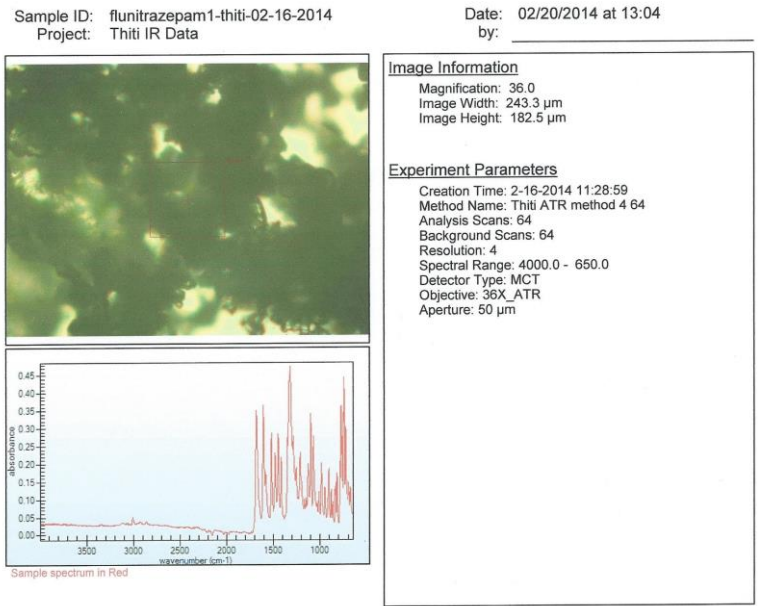


Codeine C-II Sigma/Fluka Analytical-C5901-50 MG

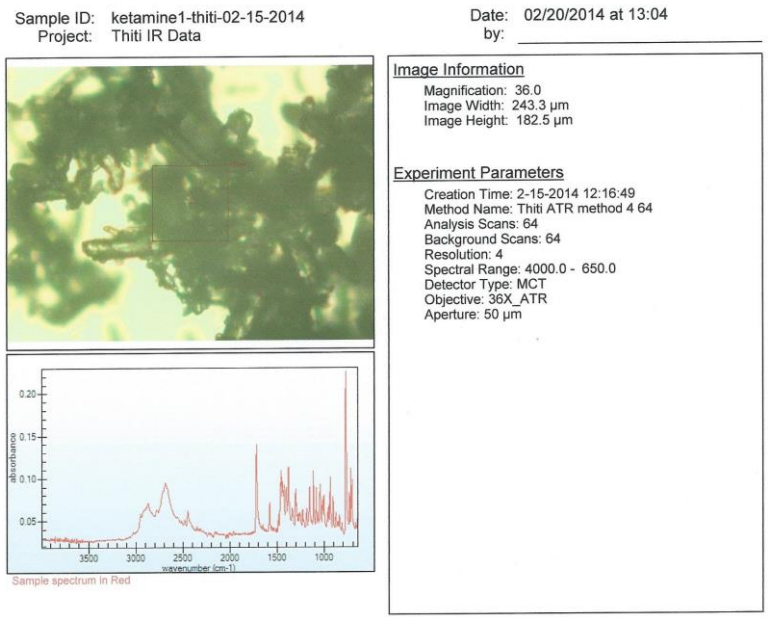


Appendix E. An ATR Spectrum of Drugs (cont.)

Flunitrazepam (C-IV) Sigma Life Science-F9261-100MG

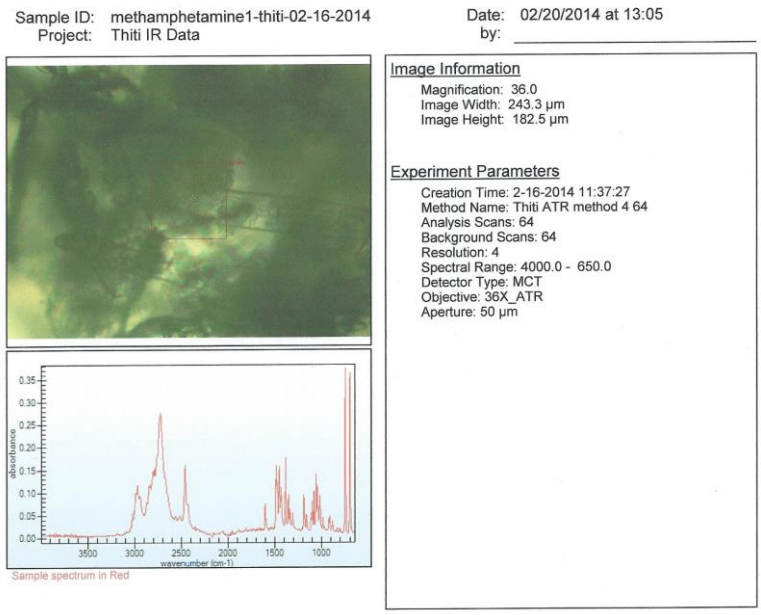


Ketamine hydrochloride (C-IIIN) Sigma Life Science-H159-25MG

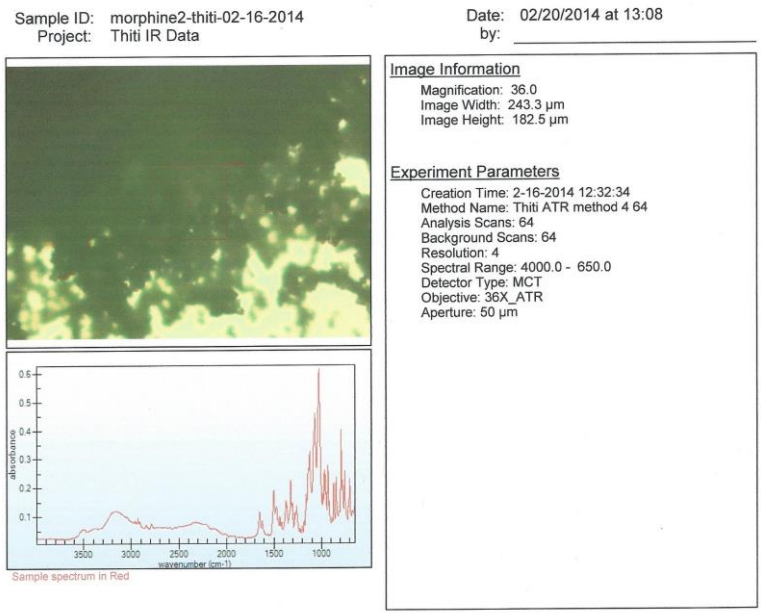


Appendix E. An ATR Spectrum of Drugs (cont.)

Methamphetamine hydrochloride C-II Sigma Life Science M8750-5G

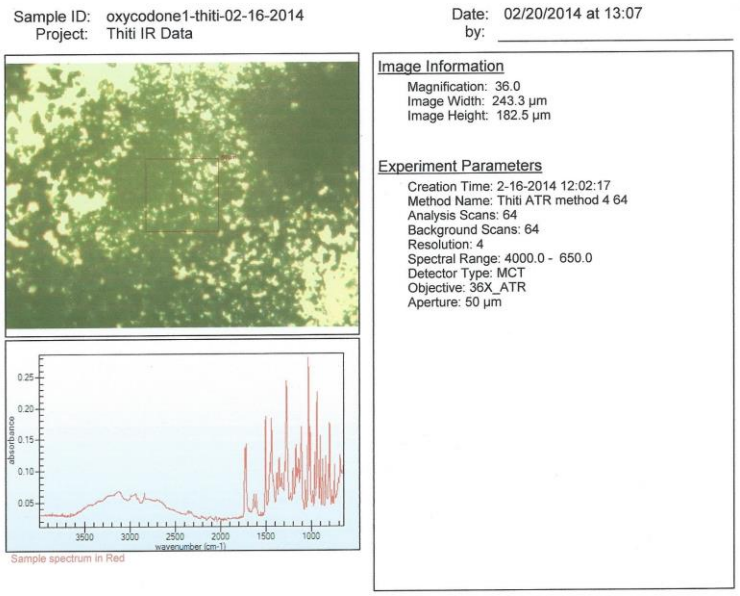


Morphine sulfate salt pentahydrate C-II Sigma Life Science M8777-50 MG

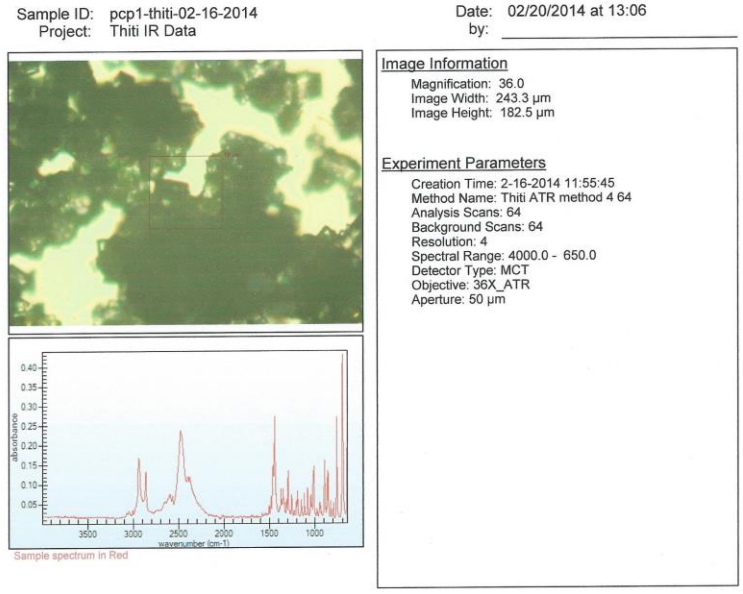


Appendix E. An ATR Spectrum of Drugs (cont.)

Oxycodone hydrochloride Spectrum-03269, 1G



Phencyclidine hydrochloride C-II (PCP) Sigma Life Scinece-p3029-100 MG



Appendix F. An ATR Spectrum of Minerals

Aragonite

Sample ID: aragonite2-thiti-02-11-2014
Project: Thiti IR Data

Date: 02/20/2014 at 12:55
by: _____

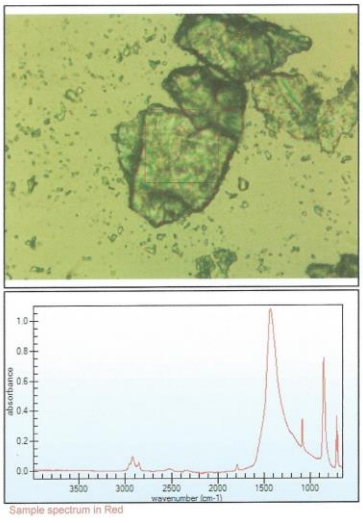


Image Information
Magnification: 36.0
Image Width: 243.3 µm
Image Height: 182.5 µm

Experiment Parameters
Creation Time: 2-11-2014 14:21:55
Method Name: Thiti ATR method 4 64
Analysis Scans: 64
Background Scans: 64
Resolution: 4
Spectral Range: 4000.0 - 650.0
Detector Type: MCT
Objective: 36X_ATR
Aperture: 50 µm

Calcite

Sample ID: calcite1 thiti 02-11-2014
Project: Thiti IR Data

Date: 02/20/2014 at 12:54
by: _____

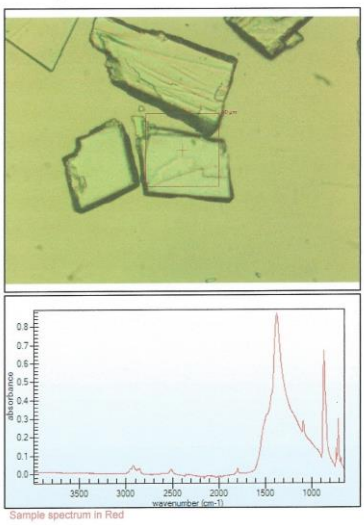
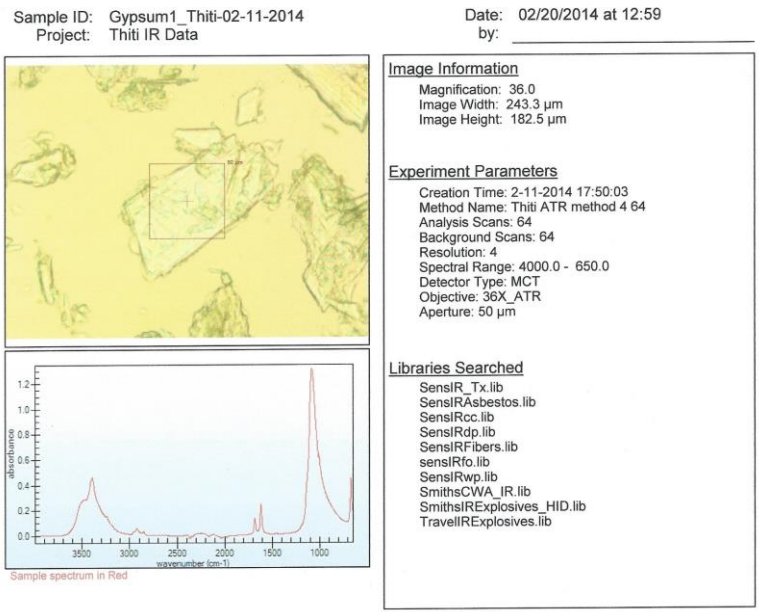


Image Information
Magnification: 36.0
Image Width: 243.3 µm
Image Height: 182.5 µm

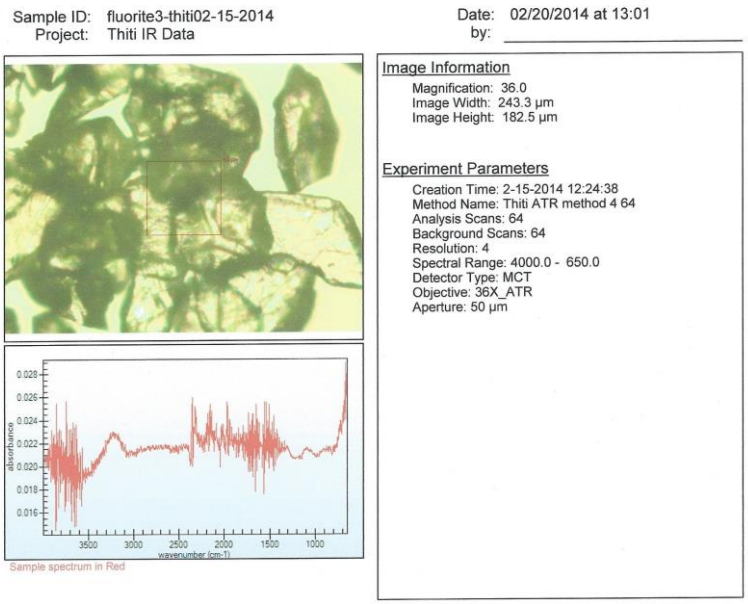
Experiment Parameters
Creation Time: 2-11-2014 14:04:56
Method Name: Thiti ATR method 4 64
Analysis Scans: 64
Background Scans: 64
Resolution: 4
Spectral Range: 4000.0 - 650.0
Detector Type: MCT
Objective: 36X_ATR
Aperture: 50 µm

Appendix F. An ATR Spectrum of Minerals (cont.)

Gypsum

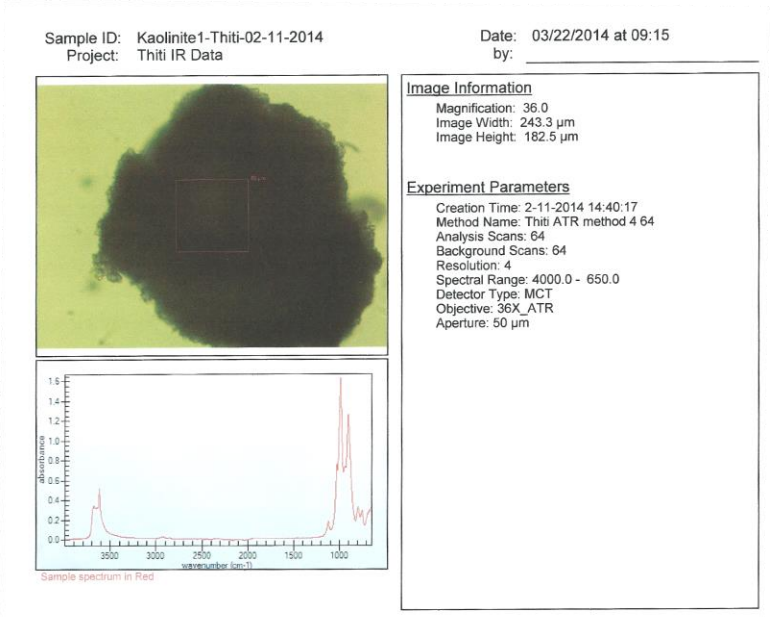


Fluorite

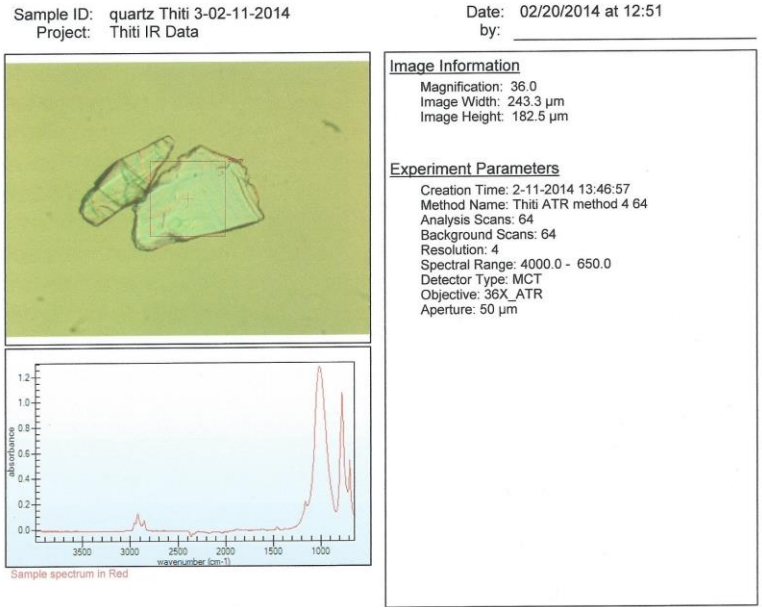


Appendix F. An ATR Spectrum of Minerals (cont.)

Kaolinite

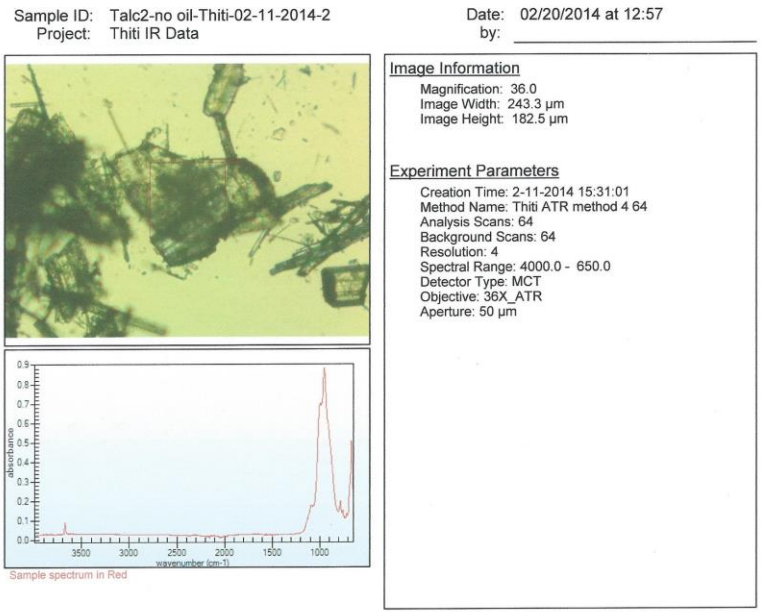


Quartz

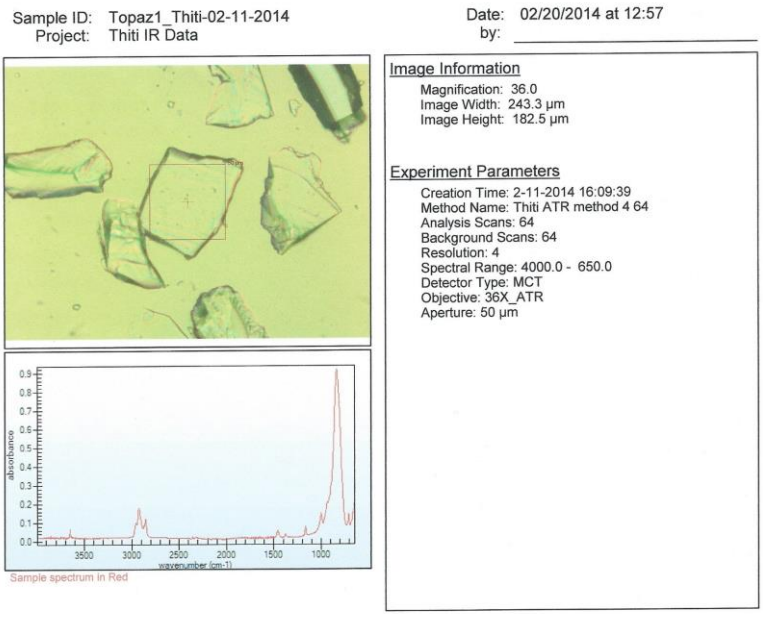


Appendix F. An ATR Spectrum of Minerals (cont.)

Talc

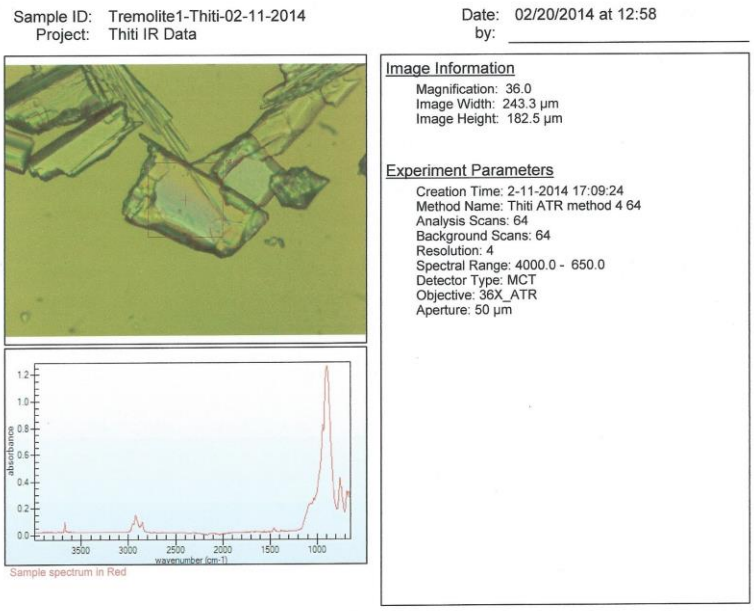


Topaz



Appendix F. An ATR Spectrum of Minerals (cont.)

Tremolite



Appendix G. Cargille Refractive Indices

No.	n_C	n_D	n_F
1	1.3978	1.4	1.4054
2	1.3998	1.402	1.4074
3	1.4018	1.404	1.4094
4	1.4037	1.406	1.4114
5	1.4057	1.408	1.4134
6	1.4077	1.41	1.4154
7	1.4097	1.412	1.4175
8	1.4117	1.414	1.4195
9	1.4137	1.416	1.4215
10	1.4156	1.418	1.4235
11	1.4177	1.42	1.4256
12	1.4197	1.422	1.4275
13	1.4217	1.424	1.4296
14	1.4237	1.426	1.4315
15	1.4257	1.428	1.4366
16	1.4277	1.43	1.4356
17	1.4297	1.432	1.4376
18	1.4316	1.434	1.4396
19	1.4337	1.436	1.4416
20	1.4356	1.438	1.4436
21	1.4377	1.44	1.4456
22	1.4397	1.442	1.4476
23	1.4417	1.444	1.4496
24	1.4436	1.446	1.4517
25	1.4457	1.448	1.4537
26	1.4477	1.45	1.4557
27	1.4496	1.452	1.4577
28	1.4516	1.454	1.4596
29	1.4536	1.456	1.4617
30	1.4557	1.458	1.4638
31	1.4577	1.46	1.4658
32	1.4596	1.462	1.4679
33	1.4616	1.464	1.4701
34	1.4635	1.466	1.4722
35	1.4655	1.468	1.4743
36	1.4674	1.47	1.4765
37	1.4693	1.472	1.4786
38	1.4713	1.474	1.4808
39	1.4732	1.476	1.4829
40	1.4752	1.478	1.48529
41	1.4771	1.48	1.4871
42	1.479	1.482	1.4893
43	1.481	1.484	1.4914
44	1.4829	1.486	1.4936
45	1.4849	1.488	1.4958
46	1.4868	1.49	1.4979
47	1.4888	1.492	1.5
48	1.4907	1.494	1.5023
49	1.4927	1.496	1.5044
50	1.4947	1.498	1.5065
51	1.4967	1.5	1.5086
52	1.4986	1.502	1.5107
53	1.5005	1.504	1.5128
54	1.5025	1.506	1.515
55	1.5044	1.508	1.5171
56	1.5064	1.51	1.5193
57	1.5083	1.512	1.5214
58	1.5103	1.514	1.5236
59	1.5122	1.516	1.5258
60	1.5142	1.518	1.5279
61	1.5161	1.52	1.53
62	1.518	1.522	1.5322
63	1.52	1.524	1.5343
64	1.522	1.526	1.5364
65	1.5239	1.528	1.5386
66	1.5259	1.53	1.5407
67	1.5278	1.532	1.5428
68	1.5298	1.534	1.545
69	1.5317	1.536	1.5472
70	1.5337	1.538	1.5492
71	1.5356	1.54	1.5514
72	1.5375	1.542	1.5535
73	1.5395	1.544	1.5557
74	1.5414	1.546	1.5578

No.	n_C	n_D	n_F
75	1.5433	1.548	1.56
76	1.5453	1.55	1.5621
77	1.5472	1.552	1.5642
78	1.5492	1.554	1.5664
79	1.5512	1.556	1.5686
80	1.5531	1.558	1.5708
81	1.5551	1.56	1.5729
82	1.557	1.562	1.575
83	1.559	1.564	1.5772
84	1.5609	1.566	1.5792
85	1.5628	1.568	1.5814
86	1.5648	1.57	1.5835
87	1.5668	1.572	1.5856
88	1.5687	1.574	1.5878
89	1.5706	1.576	1.5901
90	1.5725	1.578	1.5922
91	1.5745	1.58	1.5946
92	1.5763	1.582	1.5967
93	1.5782	1.584	1.599
94	1.5801	1.586	1.6013
95	1.5821	1.588	1.6035
96	1.584	1.59	1.6058
97	1.5859	1.592	1.6081
98	1.5878	1.594	1.6102
99	1.5898	1.596	1.6125
100	1.5917	1.598	1.6148
101	1.5937	1.6	1.617
102	1.5995	1.602	1.6191
103	1.5975	1.604	1.6214
104	1.5994	1.606	1.6236
105	1.6013	1.608	1.6259
106	1.6032	1.61	1.6281
107	1.6051	1.612	1.6304
108	1.607	1.614	1.6326
109	1.609	1.616	1.6349
110	1.6109	1.618	1.637
111	1.6128	1.62	1.6393
112	1.6147	1.622	1.6415

No.	n_C	n_D	n_F
113	1.6166	1.624	1.6439
114	1.6185	1.626	1.6459
115	1.6204	1.628	1.6484
116	1.6224	1.63	1.6506
117	1.6243	1.632	1.6528
118	1.6262	1.634	1.655
119	1.6281	1.636	1.6573
120	1.63	1.638	1.6594
121	1.632	1.64	1.6617
122	1.6338	1.642	1.6638
123	1.6357	1.644	1.6662
124	1.6377	1.646	1.6684
125	1.6396	1.648	1.6706
126	1.6415	1.65	1.6728
127	1.6435	1.652	1.6751
128	1.6454	1.654	1.6773
129	1.6473	1.656	1.6795
130	1.6492	1.658	1.6818
131	1.6512	1.66	1.684
132	1.6531	1.662	1.686
133	1.655	1.664	1.6883
134	1.657	1.666	1.6904
135	1.6589	1.668	1.6926
136	1.6609	1.67	1.6948
137	1.6628	1.672	1.6969
138	1.6648	1.674	1.699
139	1.6667	1.676	1.7013
140	1.6686	1.678	1.7032
141	1.6706	1.68	1.7056
142	1.6725	1.682	1.7077
143	1.6744	1.684	1.7099
144	1.6764	1.686	1.712
145	1.6783	1.688	1.7142
146	1.6803	1.69	1.7164
147	1.6822	1.692	1.7185
148	1.6842	1.694	1.7207
149	1.6861	1.696	1.7229
150	1.688	1.698	1.725

No.	n_C	n_D	n_F
151	1.69	1.7	1.7273

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