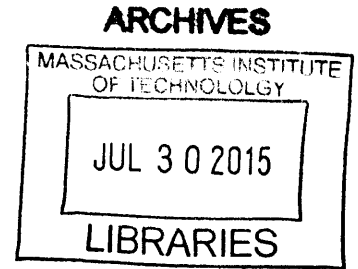


Optical Characterization of Emulsions and Applications in the Dairy Industry

by

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B.Tech. Production and Industrial Engineering
Indian Institute of Technology Delhi, 2012



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Requirements for the Degree of Master of Science in
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ABSTRACT

Milk and milk products are an integral part of diet for a major segment of human population. Safety and quality of the industry's products is therefore essential as it directly affects human health and wellbeing. The present thesis discusses work towards developing analytical technologies and methods for the dairy industry in India, to improve product safety and quality and eventually consumer health. A user-centric product design and development approach has been followed in the project. The needs and opportunities have been identified through repeated stakeholder interactions. Proposed concepts have been shortlisted based on value and impact, commercial potential, and technological feasibility. The thesis introduces a novel digital imaging based method of online spectrophotometric measurements on raw milk without any sample preparation. Multiple LED's of different emission spectra are used as discrete light sources and a digital CMOS camera is used as an image sensor. The absorption and scattering characteristics of samples are derived from captured images. Despite of the presence of multiple scattering, the extinction of incident radiation can be unequivocally quantified using the proposed method. The dependence of multiple scattering on power of incident radiation is exploited to quantify scattering. These can be related to the fat concentrations and globule sizes of samples. The method has been validated by conducting experiments for the spectrophotometric response of milk with varying fat concentrations and fat globule sizes.

Thesis Supervisor: Sanjay E. Sarma
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Chapter 1

INTRODUCTION

Milk and milk products are an integral part of diet for a major segment of human population. This is especially true in India. The dairy industry in India plays an invaluable role by providing food and nutrition to over 1.25 billion people in the country. Safety and quality of the industry's products is therefore essential as it directly affects the health and wellbeing of almost the entire population.

The present research endeavors to develop analytical technologies and methods that may be used by the dairy industry to improve product quality, prevent wastage and spoilage, and eventually benefit consumer health as well as industry economics. This thesis captures the project at a point where key technologies and systems have been proposed and initial concepts have been successfully proven in practice.

A user-centric product design and development approach has been applied in the present endeavor. A significant thrust has been the repeated interaction with various stakeholders in the industry, which has been instrumental in correctly identifying needs and potential opportunities, and proposing appropriate systems and solutions. It has also helped elicit an interest among key decision makers in the industry that will eventually be helpful in commercializing the products of this research.

A major component of the work has been the development of analytical technologies that form the core of the proposed solutions. A new method for online spectrophotometric characterization of constituents in milk, and in any emulsion in general, has been proposed and developed. Absorption and scattering characteristics of samples is measured using LED's as discrete light sources and a digital CMOS camera as an image sensor. Hypotheses outlining the method have been developed and experimentally validated.

1.1 Motivation

The project started with an initial goal to develop sensors and instruments for applications specific to healthcare in India and other developing countries. The interest in food quality and safety stemmed from the direct impact it has on health and wellbeing of a country's population. Milk and milk products, specifically, reach a wide consumer base in India due to the prevalent dietary habits. In light of several recent media reports and public litigations that have been pointing towards rampant adulteration of liquid milk, it was evident that the industry has unmet needs in ensuring product quality and safety to its customers. The efforts in the project were hence directed towards developing analytical technologies and methods that may be used by the dairy industry in India to improve product quality, prevent wastage and spoilage, and eventually benefit consumer health as well as industry economics.

India is the world's largest milk producer and consumer. In 2013, milk production in India was estimated to be over 135 Million Metric Tons (MMTs), comprising 15% of the estimated world production of 750 MMTs (FAO, 2013). While providing food and nutrition to the country's population, the dairy industry in India also provides a sustainable source of livelihood to over 75 million smallholder dairy farms in the country (Hemme, 2010). The interest of the present project is to propose solutions that have a concrete commercial application and significant value addition for stakeholders in the industry spanning from rural dairy farmers to urban consumers.

In the process of proposing solutions and systems in various embodiments at different nodes in the dairy supply chain, it was found that available technologies are severely limited in their potential for application within the imposed constraints. While cost and infrastructure are obvious constraints in low resource settings, other constraints and requirements, like in-line and rapid instrumental analysis, are more generic to the global dairy industry. In-line and rapid instrumentation is essential for analysis of small and continuous batches of milk, a requirement in village-level milk collection centers as well as large-scale mechanized dairy farms. Technology developed for applications specific to India can hence also be translated for applications in other parts of the world.

1.2 Method

A user-centric product design and development approach has been applied in the present research. In the present project, significant thrust has been the repeated interaction with various stakeholders in the industry. This has been instrumental in correctly identifying needs and potential opportunities, and proposing appropriate systems and solutions. It has also helped elicit an interest among key decision makers in the industry that will eventually be helpful in commercializing the products of this research.

Interacting with stakeholders has been a challenging and a rewarding part of the exercise. Over three trips to India, spanning a total of twelve weeks, key stakeholders were identified and involved in the process to understand the industry structure, existing concerns and potential opportunities of intervention. Discussions were had with buyers and consumers, farmers and producers, procurement officials and executives, industry specialists and experts, equipment manufacturers and suppliers, and government officials in different parts of the country during the process. The challenges have been in connecting with the right stakeholder at the right time, in communicating effectively during the exploration phase without concrete ideas, and in combining a diverse set of perspectives into a single solution. Despite of all its challenges, stakeholder interaction has been realized as an essential ingredient in the present research.

Working towards solutions for low-resource settings is an intriguing challenge that is frequently discussed and debated. A common thread that runs through most projects targeted to such settings is the cost-effectiveness of solutions. As an entrepreneur, it is obvious that implementing solutions in a market that cannot pay sufficiently is difficult, if not impossible. A preferred strategy is to focus on solutions that have identified customers who can and are willing to pay. In the present project, this strategy has been central in deciding to develop “Point of Procurement” solutions instead of “Point of Consumption” (Section 4.1) solutions among proposed concepts.

In the course of the present research, several limitations in available technology were identified. New scientific concepts were proposed that push the current technological paradigm forward. The developed technology is applicable not only to the chosen

application in low resource settings, but also for applications in the dairy industry around the globe. This project could hence be an example of reverse innovation.

1.3 Thesis Scope and Organization

The present thesis details both the technology and the systems dimensions of the project. Chapters 2 and 3 provide the background of the project. Chapter 3 gives key insights into the dairy industry in India and the world, in general. It describes the technology, operations and current practices in the industry. Practices related to quality assurance are provided in detail with a discussion on existing and future concerns. The discussion is drawn from findings in literature, stakeholder interaction and personal observations. Chapter 3 describes the composition and analysis of milk, with a focus on the technology dimension of the project. The major and minor constituents of milk are described with details relevant to quality and safety. Physical and physiochemical properties of raw and processed milk are also described. The chapter further gives an overview of the state of the art in chemical and instrumental analysis of milk.

Chapter 4 and 5 provide details of the proposed product and technology and the development of the same. Chapter 4 describes the potential opportunities of intervention in the dairy supply chain, as identified through the stakeholder interaction process. Various product embodiments and potential technologies are discussed. The chapter goes on to describe the chosen concept in detail and the prior art for the same. Chapter 5 is a detailed description of the experiments performed to validate the proposed concept. The experimental setup and the image and data analysis methods are described in extensive detail. Results from experiments conducted on raw and processed cow's milk are also presented. Appendix A contains a record of all obtained results.

Chapter 6 concludes the thesis with a discussion on the proof of concept, the proposed minimum viable product and future direction of work.

Chapter 2

BACKGROUND: THE DAIRY INDUSTRY

The dairy industry in India is the largest in the world in terms of annual production. The industry has grown continuously at a rapid pace since 1970, following major policy revisions and investments in infrastructure. Consumer demand for milk and milk products has also grown with increasing production. Other than serving the food and nutrition needs of a large population base, the industry also serves as a major source of livelihood for millions of farmers throughout the country.

The dairy industry in India is unique compared to its counterparts in other major milk producing countries due to the fact that a majority of milk is sourced from smallholder farmers. Smallholder farmer families usually have two to ten milk giving animals each, and milk beyond the household requirement is sold as produce to traders and processing plants. The industry follows a unique supply chain that collects milk from smallholding farmers at the village level and provides processed products to consumers throughout the country, connecting the producer and the consumer.

Product quality and safety in the industry are prime concerns both for consumers and producers. Several international and national standards govern the best practices to ensure regulatory compliance. The quality and safety front has improved over years as the country's economy has evolved, however, unaddressed or unsolved concerns still exist in the industry.

Section 2.1 gives an overview of the dairy industry and details of the supply chain in India. Section 2.2 discusses the quality control standards and concerns. The discussion is drawn from findings in literature, stakeholder interaction and personal observations.

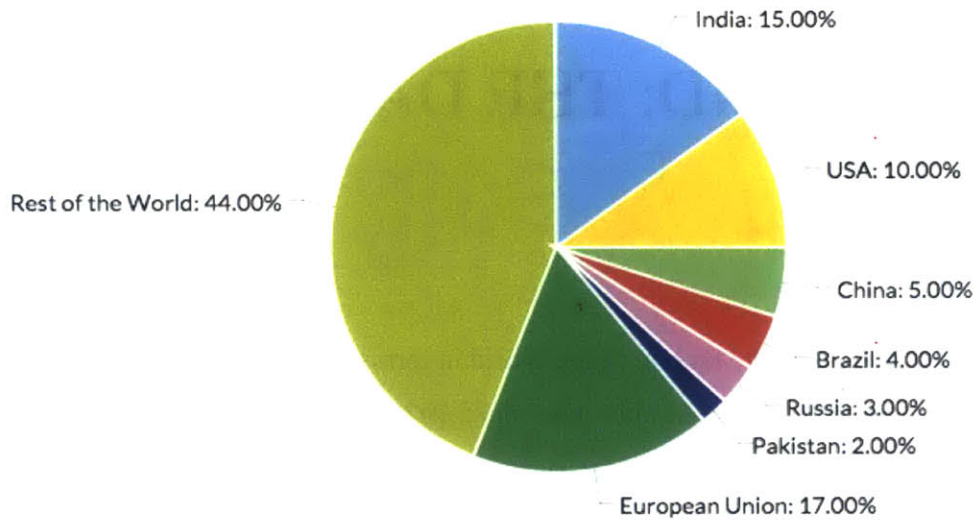


Figure 2.1 Global milk production with country % shares (2013) Data retrieved from FAO Statistics Division (2015)

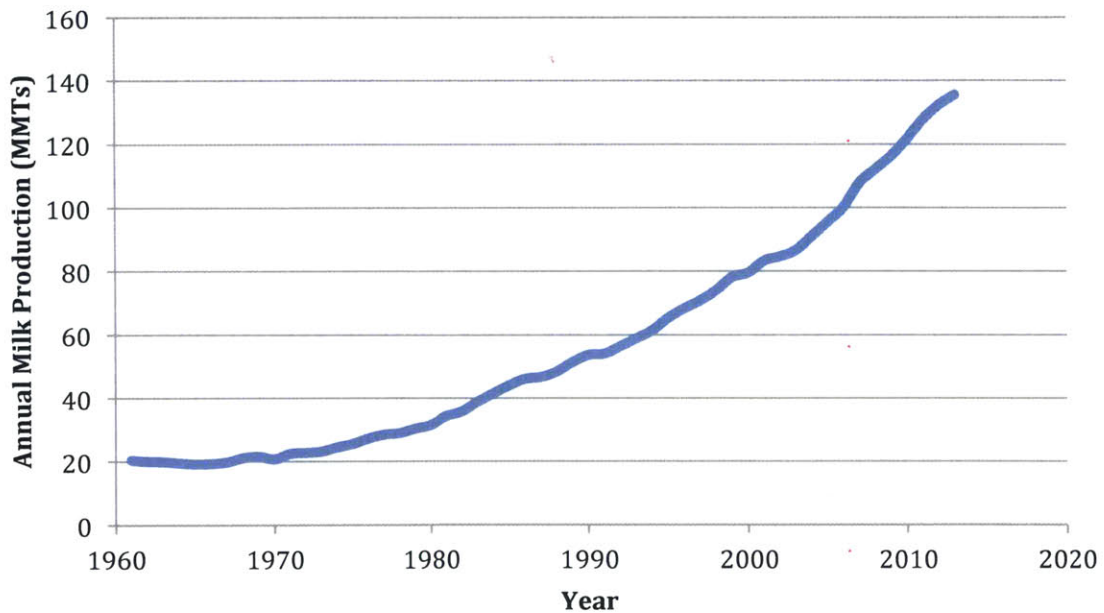


Figure 2.2 Annual milk production in India (1961 - 2013) Data retrieved from FAO Statistics Division (2015)

2.1 Industry Profile

India is the world's leading milk producer as well as consumer. In 2013, milk production in India was estimated to be over 135 Million Metric Tons (MMTs), an estimated 15% of world production (FAO, 2013). Figure 2.1 compares total milk production in 2013 among the top producers and the rest of the world. Annual milk production in India has been increasing constantly and rapidly from approximately 21 MMTs in 1970 to the recent 135 MMTs. Figure 2.2 shows the rise in production over the years. The industry generated revenues of over 5.2 Billion USD in 2012 (MarketLine, 2013). These are predicted to grow to 7.4 Billion USD by 2017, corresponding with a production of over 205 MMTs. The industry plays a vital role in India's economy and provides a source of livelihood to millions of farmers throughout the country (Jha, 2004).

According to an FAO estimate, India has over 75 million dairy farms, a majority of which are smallholder farms (Hemme, 2010). Smallholder farmer families usually have two to ten milk giving animals each, and milk beyond the household requirement is sold as produce to traders and processing plants. A variety of milch animals, including buffalos, cows, sheep, goat and camel are used for milk production in India. Buffalos and cows give approximately 50% and 45% of the total milk produced (Armentano, 2006).

Milk is a major source of vital nutrients as well as various micronutrients. The per capita availability of milk in India was 118 kg per person per year in 2011. It is higher than the world average of 105 kg per person, but lower than that of many other developed and developing countries (IFCN, 2013). The per capita availability is not a representative of milk consumed by all individuals, which may vary significantly among individuals belonging to different economic strata of the society.

Over 90% of the produce is currently sold as liquid milk in both packed and loose forms. Milk processing and distribution in India is dominated by the informal or traditional sector, which handles approximately 75% of the marketed produce (Jha, 2004). This sector primarily distributes loose unprocessed liquid milk to households and small businesses.

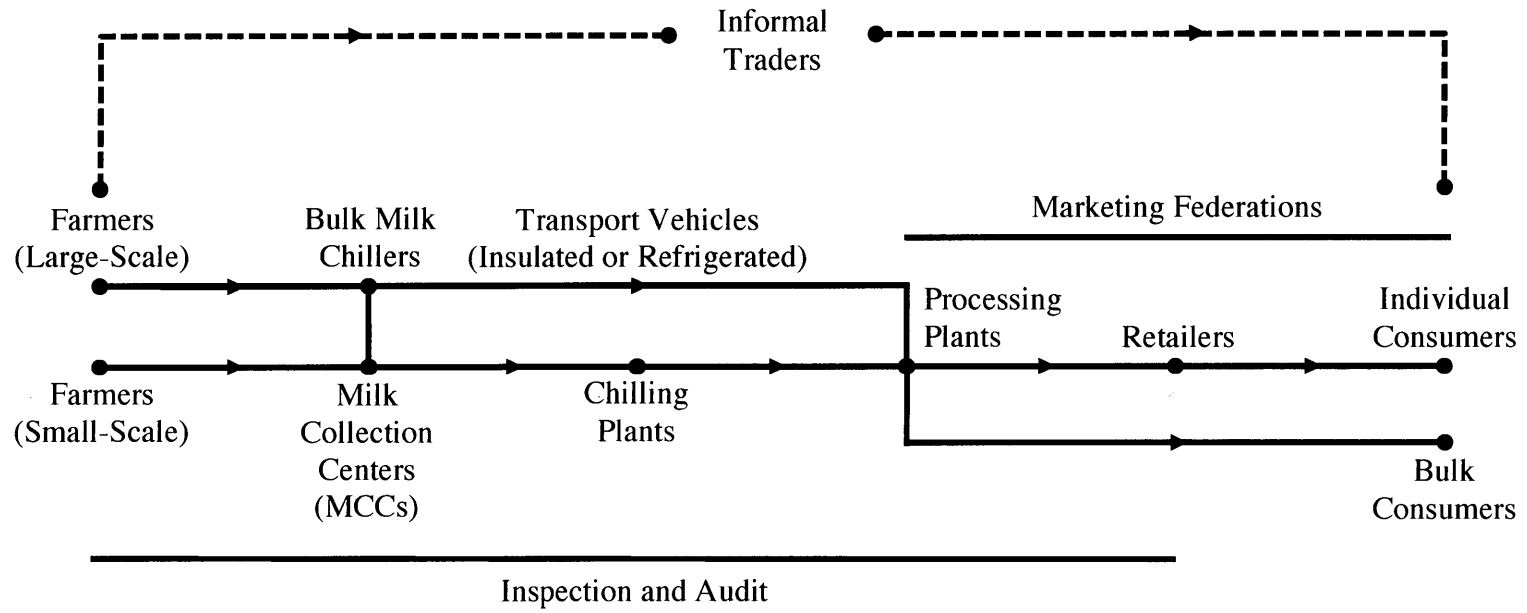


Figure 2.3 Formal (solid line) and informal (dashed line) supply chains in India's dairy industry

In the formal sector, cooperatives play a leading role. In 2013, cooperatives handled 12 MMTs of raw milk (9% of marketed produce), an increase of about 14% from the previous year (NDDB, 2013). The cooperatives underwent a national expansion in 1970 following major policy revisions and investments in infrastructure. Since then, they have grown at an increasing rate (Candler, 1998). Private dairies handle the rest of the marketed produce.

The cooperatives in India follow a unique three tier model to procure, process and market milk and milk products (Kurien, 2007), acting as a link between producers and consumers. The model is commonly known as the Anand pattern. The first tier is the Village-level Dairy Cooperative Society that owns and operates a Milk Collection Center (MCC). MCCs procure milk from member dairy farmers. The second tier is District-level Milk Producers' Union that receives milk from member MCCs and processes and packages it as liquid milk or other dairy products. The third tier is State-level Milk Marketing Federation that markets the produce of member unions. The supply chain is elaborated in the next subsection.

2.2 Supply Chain and Operations

The dairy industry in India is characterized by a unique supply chain that collects milk from smallholding farmers at the village level and provides processed products to consumers throughout the country. An informal sector operates in parallel with the formal sector. This is illustrated in Figure 2.3. The formal sector connects the urban demand and the rural supply in a way that is beneficial for both producers and consumers. The chain consists of various nodes starting from farmers and ending at consumers. Milk moves from farmers to processing plants through MCCs and Bulk Milk Chillers or regional Chilling Plants. Processed milk and value added products are distributed nationally through retailers or directly to bulk customers. Jha (2004) and Mu et al. (2013) discuss the supply chain and producer networks in more detail.

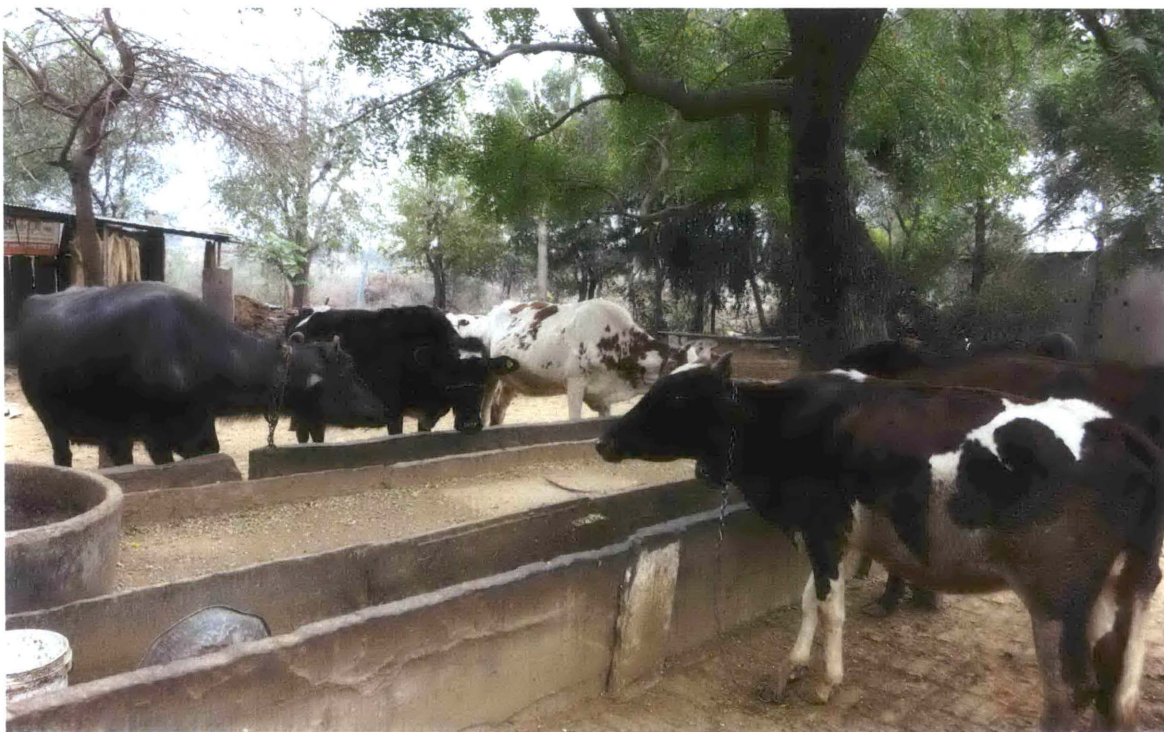


Figure 2.4 Photograph of a smallholder dairy farm near Jaipur, Rajasthan



Figure 2.5 Photograph of a milk collection center near Anand, Gujarat

A smallholder farmer may typically have two to ten milk giving animals at an instance. Figure 2.4 is a photograph of a smallholder farm in a village near Jaipur, Rajasthan. Milk Collection Centers (MCCs) are village level stations that purchase and procure milk from local dairy farmers. An MCC may be operated by a Village-level Dairy Cooperative Society in a cooperative model or by private individuals. Figure 2.5 is a photograph of an MCC operated by a society in a village near Anand, Gujarat. MCCs are hence analogous to individual large-scale farms. MCCs usually purchase milk from farmers based on the net fat content. Fat content is of interest specifically in the Indian dairy market due to high value of *ghee*. Fat is tested using chemical or analytical methods on site. Turbidimeters (REIL, 2015) and Ultrasonic Spectrometers (Mikotronics, 2015) are commonly used in MCCs for fat analysis. A turbidimeter is visible in Figure 2.5. Draaiyer & Mounsey (2009) describe the various analytical methods and other best practices for field-level institutions like MCCs. MCCs may have an onsite bulk milk chiller, depending on facility size and available infrastructure. Milk is rapidly chilled to below 4°C to prevent bacterial proliferation and spoilage. In absence of bulk milk chillers, milk is regularly collected and transferred to a regional chilling plant. Milk is then transported to processing plants in insulated tanks. Processing plants are usually district level facilities and are equipped with state-of-the art instruments and processes. Milk is tested for major constituent concentrations and inspected for abnormalities before intake into the plant.

Processed and packaged liquid milk and other value-added products are distributed to consumers through national and state-level networks of retailers. Marketing federations, in the cooperative model, play an important role in creating an appropriate market demand for the industry's products. Gujarat Cooperative Milk Marketing Federation (GCMMF), popularly known as Amul, is a good case in point.

Government regulatory bodies interact with all nodes in the supply chain. Samples are collected in random inspections and sent to central laboratories for testing and inspection. The newly created Food Safety and Standards Authority of India (FSSAI) plays an important role in creating and enforcing food safety and quality standards throughout the country.

2.3 Quality Control

Quality control is an important concern in all operations when handling food and related products due to its direct impact on consumer's health and wellbeing. Concerns over quality in the dairy industry's products are even stronger due to milk's vulnerability to spoilage, contamination and adulteration. Several international and national standards and regulations govern the best practices to ensure regulatory compliance of milk and other dairy products. AOAC International, IDF and ISO are among the most prominent standard-setting bodies. IDF (2000) has published an inventory of the relevant standards established by the three bodies for sampling and analysis of milk. Food Safety and Standards Authority of India (FSSAI) establishes standards applicable to food products sold within India.

Raw milk quality is ensured by employing analytical methods at source and before raw milk is fed into a processing plant. Currently available instrumentation limits testing at source to basic constituents and subjective organoleptic properties. Although milk is examined for multiple parameters at the processing plant, the incoming milk is pooled from several thousands of farmers, and individual inconsistencies are diluted.

In milk processing, Hazards Analysis and Critical Control Point (HACCP) systems are employed to ensure product quality and safety. HACCP is a preventive approach to managing food safety by identifying, analyzing and controlling potential chemical, biological and physical hazards in all stages of supply chain. In the dairy industry, it helps identify and target potential hazards in a systematic and controlled manner (Frye, 2008). Internationally, HACCP is implemented and enforced by Food and Agriculture Organization (FAO) and World Health Organization (WHO).

2.4 Existing and Future Concerns

The quality and safety front of India's dairy industry has improved over years as the country's economy has evolved and available technology has improved. However, unaddressed or unsolved concerns still exist in the industry. Several concerns in the dairy

industry were identified in the early stages of the project. The identified concerns are based on findings in literature, stakeholder interaction and personal observations.

Adulteration: Adulteration of milk and milk products is a major concern for India's dairy industry. Adulteration is the fraudulent addition of foreign substances, like water, vegetable oils, urea, detergents, sugar and neutralizers to augment volume, improve perceived product quality and counteract natural souring. It is a major hazard to economic value of the product and to the health of consumers.

A national survey (FSSAI, 2012) reported rampant adulteration in retailed milk in both packed and loose forms. The prominence of adulteration varied between regions of the country and also between urban and rural settings. 69% of samples from urban areas were found non-conforming to standards. For a few states in India, over 80% of collected samples were non-conforming. The results of the survey are however disputed by academic and industry experts for spurious results.

The most common non-conformity reported is dilution with water. Water is readily miscible in milk and is fraudulently added to augment quantity. Other adulterants include vegetable oils, detergents, urea and foreign sugars. Vegetable oils increase the measured fat content of watered-down milk. Detergents act as emulsifiers to mix foreign fats in milk. Urea and sugars increase the measured solid non-fat. Neutralizers like soda ash are added to reverse natural souring of milk.

Despite strong concerns over adulteration among consumers, interaction with stakeholders and personal observations gave an impression that adulteration, although present, is not a widespread problem. Further, most instances of adulteration are reported in milk handled by the informal sector. Tracking adulteration at every node in the supply chain is hence challenging in terms of logistics, technology and commercialization.

Spoilage: Milk is a rich biological fluid and is susceptible to microbiological growth. Milk is received at 37°C from animals' udders. If it is not chilled immediately, microbial proliferation happens exponentially and dramatically reduces the shelf life of raw milk. Spoilage of milk is a major concern in parts of India where ambient temperature may go

as high as 46°C. Chilling is usually delayed due to unavailability of refrigeration systems and electricity at the village household level. Several milk collection centers throughout the country have installed bulk milk chillers and diesel generators for mitigating spoilage concerns.

Hygiene: Hygiene of milk collection and handling processes is exceptionally important to prevent introduction of foreign, and potentially harmful, microbial species into milk. Hygiene and sterilization are at the core of HACCP systems. However, HACCP is not implemented in village level collection centers. Milk is still handled manually at the initial nodes in the dairy supply chain and that jeopardizes the safety of raw milk.

Although processed milk is heat-treated in pasteurization, bactofugation and UHT processes, the poor hygiene of incoming raw milk affects the processes and the products negatively.

Cattle Health: Cattle health directly affects the quality of raw milk. It is a concern in the dairy industry around the globe because poor cattle health may make milk unfit for consumption, prevent milking during and after medical treatment, and introduce antibiotics in the milk stream post care.

Mastitis, an udder infection disease, is common among milch animals and directly affects milk quality. The somatic cell count in milk increases dramatically during mastitis as a result of the animal's immune response. Absence of robust methods to regularly measure somatic cell count in raw milk currently prevents preclinical diagnosis.

Chapter 3

BACKGROUND: MILK COMPOSITION AND ANALYSIS

Milk is a complex biological fluid with multiple constituents dispersed or dissolved in a continuous aqueous medium. Analysis of these constituents and other physical properties, in the field or in laboratories, is essential for quality control in the dairy industry. The importance of the various available analytical methods at different stages in supply chain was described and emphasized in Section 2.3. Although the present research has a focus on quantitative in-field analysis of raw milk, it is important to appreciate all other available methods in order to develop an instrument that fits in the existing technology ecosystem.

This chapter provides a background on the composition of milk and the existing technologies and methods for its analysis. Section 3.1 details the composition and properties of milk in order to identify the various analytes of interest. Relevant major and minor constituents are described with details on their concentrations and physical forms. Physical and physiochemical properties of milk are also described. Section 3.2 reviews existing technologies, methods, and instruments for analysis of milk. The state of the art is described with reference to its present importance in the dairy industry.

3.1 Composition and Properties of Milk

Major constituents of milk include fats, proteins, lactose and minerals. Fat globules and casein micelles are dispersed in an aqueous medium with dissolved lactose, whey proteins and minerals. Due to the different particle sizes of fat globules and casein micelles, milk is characterized as a polydisperse emulsion. The aqueous medium takes an 80 - 90% w/w share of whole milk's mass while the rest is quantified as total solids.

Composition varies significantly among milk sourced from different animals. Table 3.1 gives the typical concentrations of major constituents and the total solids in milk obtained from common milch animals. The composition further varies between milk from animals of the same species, and between milk from the same animal depending on the season, feed and lactation stage.

Table 3.1 Typical composition of milk from common milch animals (Jost, 2012)

(% w/w)	Cow	Buffalo	Goat	Sheep
Total Solids	12.8	17.8	13.2	17.1
Fat	3.8	8.0	3.9	6.3
Protein	3.3	4.0	3.7	5.3
Lactose	4.8	5.1	4.8	4.7
Minerals	0.7	0.8	0.8	0.9

Milk also contains residues and contaminants like somatic cells, pesticides, veterinary drugs and heavy metals. It is rich in microbiology and acts as a good medium for microorganism growth.

This discussion is drawn from multiple references, especially McSweeney and Fox (2006, 2009 and 2013), Jost (2012) and Wong (1988). Details necessary in the present thesis are included. The reader is referred to the references for further information on milk composition and properties.

3.1.1 Major Constituents

Fat and Lipids: Fat in milk is largely composed of neutral lipids, dominated by triacylglycerols that dictate its physical and chemical properties. Diacylglycerols, phospholipids and sterols are present in relatively minor quantities (McSweeney, 2006). Milk from milch animals has a high proportion of saturated fatty acids, unlike human milk, which is richer in unsaturated fatty acids (Jost, 2012).

Fat in milk is in the form of dispersed spherical globules. Depending on several intrinsic and extrinsic factors, it may or may not be crystalline within the globule. The diameter of the globules in raw milk varies with species and seasons. It is usually between 3 - 5 μm and up to 10 μm , and follows a lognormal distribution. Fat globules in raw milk are prone to physical instability, and tend to separate by gravitational separation (creaming) and globule aggregation (coalescence and flocculation). In processed milk, the size is decreased to 0.7 - 2 μm by homogenization to increase globule surface area and hence promote emulsion stability. In homogenization, fat globules are subjected to pressure for breaking them down into smaller globules. The difference in globule sizes in raw and processed milk is visible under an optical microscope, as shown in Figure 3.1. Globule aggregation is visible in Figure 3.1 (a).

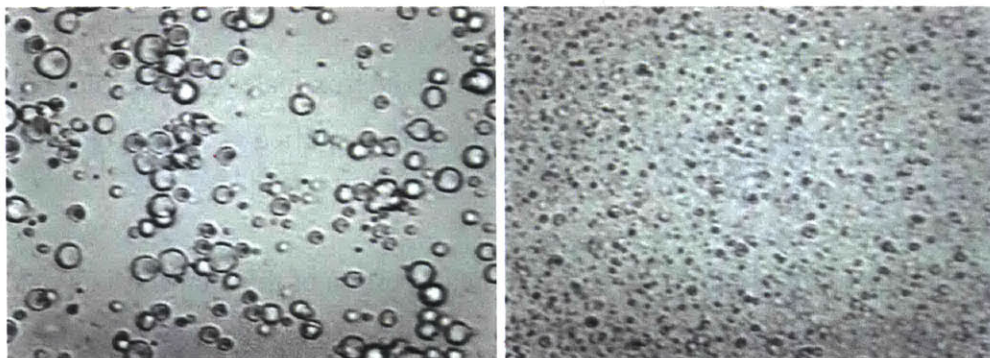


Figure 3.1 (a - left) Raw milk and (b - right) processed milk viewed under an optical microscope with 40X magnification (The two images are at the same scale.)

A layer of Milk Fat Globule Membrane (MFGM) emulsifies the fat globules in raw milk. MFGM is a complex biological layer mainly composed of proteins and phospholipids. It is structurally similar to an animal cell membrane and makes up 2 - 6% of the total globule mass.

Proteins: Proteins account for approximately 95% of total nitrogen content in milk. Casein protein is present as large colloidal aggregates, known as micelles, in the dispersed phase. Whey or serum protein is present as monomers or small quaternary structures in the dissolved phase. Casein amounts to 75 - 80% of the total milk protein (Jost, 2012). In homogenized milk, both casein and whey proteins are adsorbed to smaller

fat globules as emulsifiers. They form a layer usually thicker than the original MFGM layer.

Caseins may be classified into α , β , κ and γ -caseins, of which α -casein is dominant in milk. Micelles are stable spherical particles with mean diameter between 100 - 150 nm, and range between 50 - 500 nm (McSweeney, 2013). Milk is hence a polydisperse emulsion with dispersed particles (fat globules and casein micelles) having widely different particle size distributions.

Whey proteins are composed of α and β -lactoglobulin, of which the latter is dominant. Trace amounts of blood serum albumin and immunoglobulins may also be present, which are more noticeable in colostrum. While β -lactoglobulin denatures with heat treatment and forms colloidal complexes with caseins, α -lactoglobulin is resistant to heat.

Milk has non-protein nitrogen (NPN), amounting to approximately 5% of total milk nitrogen. Urea is a major source of NPN in milk. It is quantified as Milk Urea Nitrogen (MUN) for quality analysis.

Lactose: Carbohydrates in milk constitute a major portion of the total solids. Lactose is the dominant dissolved carbohydrate in milk while glucose is present in relatively minor quantities (McSweeney, 2009). Lactose concentration varies significantly with the animal's lactation stage. In processed milk, it typically remains unchanged after homogenization.

Minerals: Other dissolved substances in the aqueous phase include vitamins and minerals. Phosphates, chlorides and citrates of potassium, sodium, magnesium and calcium are present as dissolved ions or in complexes with caseins (Gaucheron, 2005).

3.1.2 Minor Constituents and Microbiology

Somatic Cells: Somatic Cells are leukocytes naturally released into milk from the animal's udders. They are indicative of animal health, and especially of the incidence of mastitis. In milk obtained from healthy cows, cell density is in the order of 10^5 cells/ml (Belloque, 2009). Milk with higher density is usually considered unfit for consumption.

Contaminants and Residues: Several contaminants and residues may be found in milk in trace quantities, and are a concern to consumer health. Antibiotics and other veterinary drugs may be present in milk obtained from animals under medication. Applicable regulations and guidelines hence usually prohibit milking of animals recently under medical treatment to prevent drug resistance in consumers. Other common residues include hormones, pesticides and heavy metals. Animal feed and health, and milking equipment have a direct bearing on residual compounds.

Microbiology: Microbial activity in milk is minimal as it comes from the udders. However, warm temperature leads to exponential proliferation of microbes. Unhygienic equipment is also responsible for bacterial proliferation. To prevent spoilage and potential health hazards, milk should be rapidly chilled and hygiene should be maintained meticulously.

Untreated water may be infected with disease causing bacteria, otherwise absent in milk. Adulteration of milk with water may cause rapid increases in microbial activity, depending on the source of water.

3.1.3 Physical and Physiochemical Properties

Milk has several characteristic physical properties that depend on constituent form and concentration. These are occasionally exploited in analysis of milk for identifying constituents and adulterants.

Acid-Base Equilibria: Milk pH is usually between 6.5 and 6.7, but may vary to 6 or 7.5 for early-lactation milk and mastitis-infected cattle, respectively. The pH shows a strong dependence on temperature because of insolubilization of Calcium Phosphate with raising temperature.

Thermal Properties: Thermal properties of milk are strongly dependent on the fat content and total non-fat solids. They are hence selectively sensitive to added water. The freezing point of milk, usually between -0.515 and -0.550°C , is observed to increase with added water. Milk cryoscopes measure the freezing point and are commercially used to detect adulteration of milk with water.

Specific heat of milk shows non-linear dependence on composition, homogenization, and external temperature.

Electrical Conductivity: Electrical conductivity of milk usually varies between 4 - 5mS. It is principally determined by the presence of charged species in the emulsion. Pasteurization, homogenization and other processes may modify the ion content in the aqueous medium, and hence affect conductivity. A change in pH, from the usual 6.5 to 6.7, also affects conductivity due to dissolving of colloidal Calcium Phosphate. Ion sensitive electrodes may be used to identify concentration of individual charged species.

Optical Properties: Milk has interesting optical properties that are often exploited in its analysis. Absorption of specific wavelengths due to atomic or molecular excitation is characteristically observed in mid-infrared and near-infrared region of EM spectrum. In the visible region of the spectrum, absorption continuums are observed instead. This is because of the presence of both macroscopic and microscopic dispersed phase particles, with sizes comparable to the wavelength band of visible radiation.

Scattering is a dominant form of interaction with visible radiation. Both fat globules and casein micelles scatter light. The polydisperse nature of milk and dense particle concentration leads to different scattering regimes and multiple scattering events. This is the basis of the present work, and will be discussed in more detail in Section 4.2.2.

The refractive index of milk is usually between 1.34 and 1.39, which is greater than that of water (1.33). The refractive index varies linearly with total solids, and the contribution of different constituents is additive. It also varies with milk from different milch animals, principally because of different total solid concentrations (Wong, 1988).

Rheological Properties: Milk behaves as a Newtonian fluid when fat content is below 40% w/w and temperature is above 40°C. Viscosity of milk is affected by its composition, concentration, temperature and processing history. The concentration and particle size of dispersed phase have a stronger effect on rheological properties than the dissolved lactose and whey protein.

Acoustic Properties: The response of milk to low-intensity ultrasonic excitation is characteristic of the fat and solid non-fat concentration. Measurements of ultrasonic velocity and attenuation coefficients can be correlated with these analytes. The response is however sensitive to temperature, particle size and presence of air bubbles.

3.2 Analysis of Milk

Several methods and techniques are used for qualitative or quantitative analysis of constituents in milk. Analysis of milk may be done at different nodes in the dairy supply chain with different objectives. Major constituents, including fats, proteins and lactose, (Section 3.1.1) are frequently quantified in the dairy supply chain (Section 2.2) for ensuring product quality and for process control. Minor constituents and microbiology (Section 3.1.2) is quantified for ensuring product safety and compliance with applicable regulations. Methods of analysis may also be employed for detecting adulterants in milk or verifying authenticity.

A method may be chosen based on the following major criteria:

- Analytes of interest
- Selectivity to specific analytes
- Accuracy and precision
- Response and recovery time
- Destructive or non-destructive testing
- Calibration and maintenance constraints
- Equipment and operating expenses
- Operator skill and training requirement
- Available resources and infrastructure

Different methods find application at different nodes in the supply chain. Chemical methods of analysis use reagents that react selectively with the analyte of interest. In most cases, the reactions may either yield a binary color indication of the presence of an analyte, or may be titrated to determine the exact concentration. Chemical methods have

been used traditionally both in laboratory and in field. They, however, have now been replaced by instrumental methods in several applications. Instrumental methods indirectly quantify concentration of select analytes based on their physical and physiochemical properties. Spectroscopic techniques, which measure the interaction of an analyte with electromagnetic radiation, are the most common instrumental methods. In laboratories, instrumental methods may be coupled with separation processes like chromatography for more selective analysis.

This section discusses some important available methods for analysis of milk. The discussion is focused on analysis and quantification of major constituents in milk and only details necessary in the present thesis are included. The reader is referred to the references for further reading where appropriate. Standards published by AOAC International (2012) for individual methods are mentioned where applicable.

3.2.1 Chemical Methods

Chemical methods have been traditionally used for quantification of constituents in milk. A large variety of methods exist for both major and minor constituents. Although instrumental methods have displaced chemical methods in several applications, chemical methods are still preferred in select applications, both in laboratory and in field. Chemical methods compete with instrumental methods on selectivity, accuracy and precision, and calibration and maintenance requirements. They, however, usually have a longer response time, are dependent on operator skill, have significant recurring costs and are destructive in nature.

The Gerber method (AOAC 2000.18-2004), Babcock method (AOAC 989.04-2000), Roese-Gottlieb method (AOAC 905.02-1973) and Modified Mojonnier Ether Extract (AOAC 989.05-1992) are available chemical methods for analysis of fat in milk. Babcock method is most commonly used in field due to easy to operate and low-cost equipment.

The Kjeldahl method (AOAC 991.22-1994) and The Die Binding methods (AOAC 967.12-1970 and AOAC 975.17-1975) are available chemical methods for analysis of

proteins in milk. The former gives the total nitrogen content of milk. Total nitrogen content of milk included Non-Protein Nitrogen, mostly in the form of urea. (Bintsis, 2008)

Adulterants in milk are frequently detected using chemical methods. Chemical methods for detecting common adulterants, including urea, ammoniates, nitrates, starch and neutralizers, are available and extensively used (NDDDB, 2015).

3.2.2 Spectroscopic Methods

Spectroscopic methods, in general, measure the interaction of electromagnetic radiation with a sample of milk in a controlled environment and relate the present chemical species and physical forms. Spectroscopic methods are a family of technologies, roughly classified based on the wavelength band of electromagnetic radiation measured.

Depending on the spectroscopic method, the interaction may be measured in the form of transmittance, absorbance, reflectance, elastic scattering or inelastic scattering. Acoustic or material waves may also be measured in some methods.

Spectroscopic methods are routinely used in quantification of both major and minor milk constituents in milk (Belloque, 2009). Spectroscopic methods are also used for detecting adulterants or verifying authenticity of milk and milk products (Reid, 2006; Ellis, 2012; Karoui, 2007).

Methods commonly used for analysis of milk include FTIR (Fourier Transform Infrared) Spectroscopy in the Near Infrared (NIR) or Mid-Infrared (MIR) ranges, Fluorescence and Ultraviolet-Visible (UV-VIS) Spectrophotometry, Nuclear Magnetic Resonance (NMR) Spectroscopy, Mass Spectroscopy and Raman Spectroscopy.

FTIR Spectroscopy: In FTIR spectroscopy, the absorbance of radiation in the NIR (780 - 2500 nm) band or MIR (2500 nm - 25000 nm) band is measured. Both major and minor chemical species in milk have prominent and characteristic signatures in MIR absorbance. Although signatures can also be identified in NIR absorbance, significant overtones are present at multiple wavelengths, affecting selectivity and sensitivity. MIR spectroscopy is hence widely used in laboratories for quantification of fat, protein, lactose

and total solids concentrations in milk (AOAC 972.16-1972) (McSweeney, 2006; McSweeney, 2013). NIR offers a simpler and lower cost alternative to MIR spectroscopy and used in certain applications.

UV-VIS Spectrophotometry: In UV-Vis spectrophotometry, silicon photodetectors are used to measure absorbance of radiation in the 300 nm - 1100nm band. In Turbidimetry and Nephelometry, the scattering response is measured instead of absorbance.

Turbidimetry is widely used in fat determination in field-level applications (AOAC 969.16-1969). Radiation in UV-VIS spectrum has a strong interaction with the physical form of fat globules and casein micelles in milk samples. Scattering is hence a dominant effect.

3.2.3 Separation-based Methods

Separation methods are usually coupled with spectrophotometric methods for increasing precision, accuracy, and selectivity of measurements. Chromatographic methods, including High Pressure Liquid Chromatography (HPLC) and Gas Chromatography (GC) are commonly used in laboratory environments. Capillary Electrophoresis (CE) is another commonly used separation process.

3.2.4 Other Methods

Ultrasonic Spectroscopy: Ultrasonic spectroscopy uses measurement of attenuation and phase change of ultrasound waves through samples of milk to quantify multiple parameters. Although, commercially available devices claim a wide choice of analytes (McSweeney, 2006), most applications use ultrasonic spectroscopy for quantification of fat and solid non-fat concentrations. Ultrasonic spectroscopy is sensitive to the sample's temperature, and hence requires samples to be heated or cooled to calibration temperature.

Biosensors: Biosensors, in general, use enzymes and other biological reagents to sense and quantify minor analytes in milk. Dipsticks and Lateral Flow Immunoassays are commercially used in field-level applications for detecting urea in milk.

Organoleptic Sensing: The taste and smell of milk are important parameters for consumer perception of quality (Drake, 2007). However, subjective evaluations of taste and smell are not reliable on a commercial scale. Recent developments in electronic noses (Ampuero, 2003) are attempting to solve this problem.

Chapter 4

OPPORTUNITIES AND SELECTED CONCEPT

The motivation (Section 1.1) of the present research has been to develop analytical technologies and methods that may be used by the dairy industry, specifically in India, to improve product quality, prevent wastage and spoilage, and eventually benefit consumer health as well as industry economics. For identifying specific challenges and opportunities, the author utilized multiple opportunities to interact with key stakeholders in the industry and observe current practices. In the course of the product design and development process (Section 1.2), several intriguing points of intervention were identified and potential product and technology ideas were generated. Out of the multiple generated concepts, one was selected and developed in detail.

Section 4.1 describes the various identified points of intervention in the supply chain and the generated concepts. The concepts rely on different branches of chemical and physical analysis that are briefly explained. The chosen concept is described in detail in Section 4.2, with a focus on the underlying technology and relevant prior art. Experiments and results from the chosen concept are discussed later in Chapter 5.

4.1 Opportunities

The value chain and operations in the dairy industry in India were discussed in Section 2.2. Quality control requirements and concerns were discussed in Section 2.3. Existing methods and instruments, applicable to various nodes in the supply chain, were discussed in Section 3.2. It is found by observation that despite the several available technologies and instruments, there are many voids in the supply chain that can benefit from appropriate instrumentation. Different forms of interventions at different points can strongly and positively affect the industry economics and eventually consumer health.

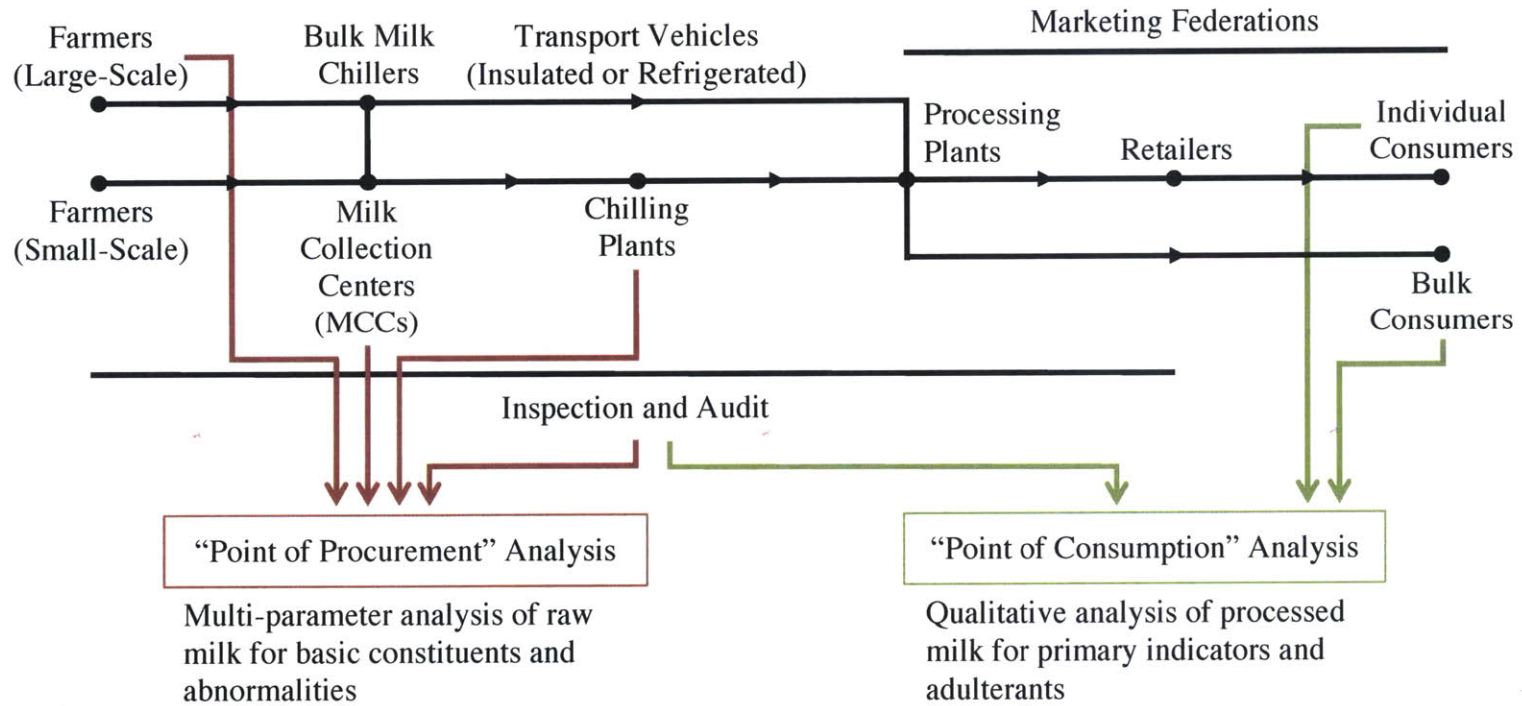


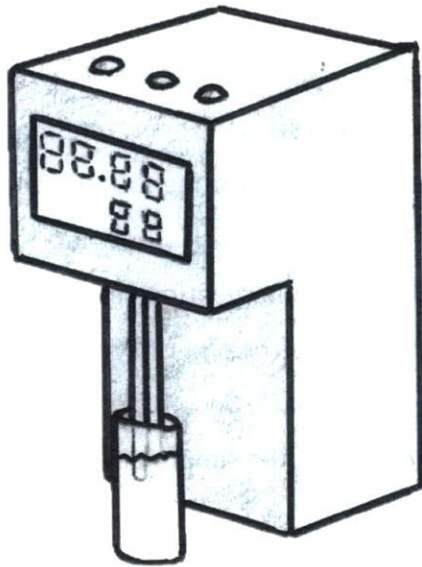
Figure 4.1 “Point of Procurement” and “Point of Consumption” interventions on the dairy industry supply chain

4.1.1 Points of Intervention

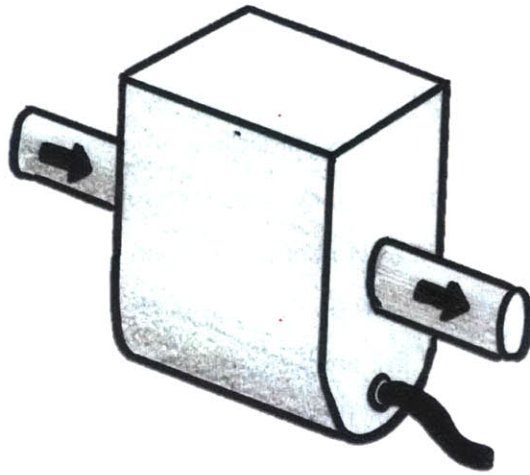
In the dairy supply chain, most current interventions are centralized and found in the middle of the supply chain, near or at the processing plant. Chemical and instrumental methods are readily used for quality control and process analysis. However, intervention at a single node is inadequate to ensure product quality and safety throughout the chain. This is true for dairy supply chains in countries other than India as well (Frye and Kilara, 2008). Other than centralized intervention, two key classes of interventions can be identified based on the applicable part of the supply chain. First, “Point of Procurement”, is for raw milk analysis near the producer end of the supply chain. Second, “Point of Consumption”, is for processed milk near the consumer end of the supply chain. These are illustrated on the supply chain flowchart in Figure 4.1.

Point of Procurement: “Point of Procurement” analysis is a class of interventions near the producer end of the supply chain to ensure quality of raw milk prior to pooling and processing. Farmers, MCCs and Chilling Plants can potentially be equipped with instruments that quantify or qualify primary quality markers, including composition and concentrations, adulteration, and spoilage. Other than preventing pooling with poor raw milk, such analysis may also help understand cattle feed implications, conduct pre-clinical diagnosis of cattle diseases and disorders, and allow precision dairy practices. Further, analytics on quality statistics from production stages can improve process control and aid decision-making. Inspection and Audit can also be facilitated with such analyses, to prevent malpractices in real time with reduced testing costs and lead times.

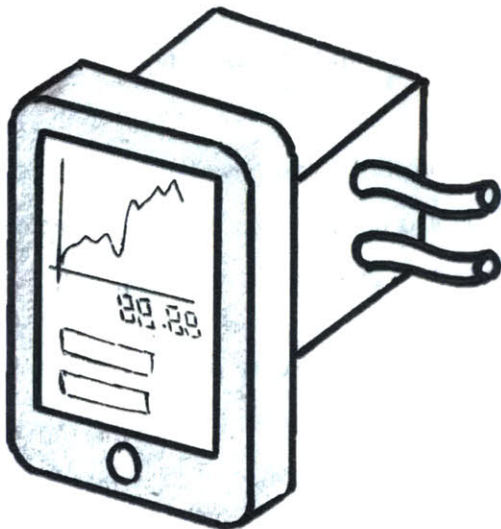
Instruments for “Point of Procurement” analysis have a shared set of design constraints. They all need to function on raw milk, which is challenging because of the large variability in raw milk’s physical and chemical properties. They also need to be field deployable, and hence power consumption, calibration, maintenance, and operator skill are important design parameters. Other important parameters include modularity, cost and portability.



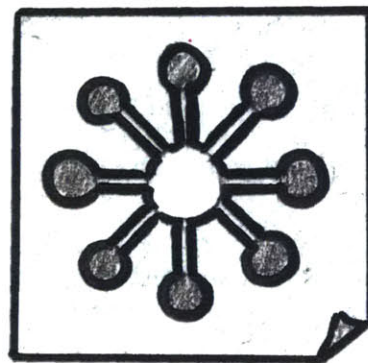
(a)



(b)



(c)



(d)

Figure 4.2 Illustrations and sketches for (a) Standalone Instrument, (b) Inline Instrument (c) Portable Instrument, and (d) Single Use Test

Point of Consumption: “Point of Consumption” analysis is a class of interventions near the consumer end of the supply chain to ensure product quality and safety prior to consumption. Adulterated or spoiled products directly affect consumers, both individuals and businesses. Instruments, devices or systems may be developed that empower consumers to make better decision when buying products, and to reject and report unsafe ones. This ensures consumer health and directly affects the industry, both economically and legally, and incentivizes it to improve current practices in order to root out existing malpractices. Inspection and Audit can also be facilitated with such analyses, to readily sample products, primarily liquid milk, and perform on-site qualitative analysis as a primary test.

Instruments for “Point of Consumption” analysis also have a shared set of design constraints. They need to function on both raw and processed milk, which is further challenging due to significant differences between the two. They need to inspect multiple parameters and multiple adulterants (if adulteration is being tested) for ensuring product quality and safety. Little or no operator skill should be required for widespread adoption of such a product or system.

4.1.2 Embodiments and Systems

There are four embodiments of the proposed instrument, as illustrated in Figure 4.2 (a) - (d) and explained below.

Standalone Instrument: A standalone instrument is installed at sites where loose milk is received or vended. It may function without any additional hardware, or may be coupled with systems that acquire data from the instrument for storage, transmission or printing. It may be used to perform multiparameter analysis of milk that addresses the existing quality and safety concerns.

Examples of existing standalone instruments are turbidimeters and ultrasonic milk testers (See Section 3.2.3) that are used for fat and SNF quantification. Turbidimeters require sample dilution and homogenization, and can only quantify fat. Ultrasonic testers do not destroy the samples, but are slower and hence not used frequently, especially at peak

operation hours. They are limited in the number of analytes, and hence are unable to address important needs and concerns.

Large-scale farmers, MCCs and bulk consumers can use standalone instruments. It is hence applicable to both the points of intervention.

Inline Instrument: An inline instrument can perform the same function as a standalone instrument, but in an automated setup. It is useful in applications like automatic milking parlors and chilling units, where loose milk is not handled manually or trained operators are unavailable or expensive. Although automation is not presently witnessed at MCCs, benefits of automation and its decreasing capital and operating costs may lead to changes in near future.

Such an instrument has advantages compared to standalone instruments. Bypassing any manual handling of milk improves hygiene and reduces scope for manhandling. Online instruments may also be controlled remotely, allowing automated receiving and vending of milk. Cleaning and maintenance can optionally be automated reducing sensor degradation due to human errors or negligence. A challenge with inline instruments is routine calibration, repair and down time. When deployed in remote locations, inline instruments cannot be transported to service centers.

Portable Instrument: A portable instrument may perform the same functions as a standalone instrument, depending on the form factor and size of components. It is useful in all applications at all nodes in the supply chain, and is especially attractive for inspection and audit personal.

Different kinds of portable instruments are possible. Illustrated in Figure 4.2 (c) is a smartphone-mounted instrument. Another possibility is a dedicated device. Smartphone-mounted instruments are especially attractive because of the available high computing power on even low-cost smartphones. Further, smartphone are seamlessly connected to the Internet or other telecommunications networks, allowing real-time data collection and integration.

Single Use Test: Single use tests are distinguished from the previously described instrument embodiments by relatively low capital expenditure and higher per-test cost. An example of a single analyte qualitative test, applicable at Point of Consumption, may be a strip that changes color when Na^+ content in milk is higher than a threshold, indicative of fraudulent neutralizer addition. An example of a test useful at Point of Procurement is a quantitative test for somatic cells that allows farmers monitor their cattle's health accurately.

Different kinds of single use tests are possible. They may be quantitative or qualitative, and may test for single or multiple analytes. Illustrated in Figure 4.2 (d) is a conceptual paper microfluidic strip for testing major adulterants like urea, detergents and vegetable oil.

4.1.3 Potential Methods and Technologies

The embodiments of an instrument described above cannot be developed using available methods and technologies for analysis of milk. Several constraints, including required analytes, sensor characteristics, form factors and cost, restrict applicable methods and technologies. Three shortlisted approaches are listed here with applicable concepts. UV-Vis spectrophotometry, discussed first, is further described and developed in the present thesis.

UV-Vis Spectroscopy: UV-Vis Spectroscopy, introduced in Section 3.2.3, has several potential applications that fit the described embodiments and systems. UV/Vis spectrum is especially attractive for field-level instrumentation because of the low cost of silicon photodetectors sensitive to wavelengths between 300 - 1100 nm. Use of low power LED's as discrete light sources reduces costs further. It can potentially be used for rapid non-destructive analysis of milk for qualifying or quantifying multiple parameters.

Available instruments for spectrophotometry and turbidimetry typically require sample dilution to minimize the effects of scattering, especially that of multiple scattering. Several opportunities of using multiple scattering for analysis are yet unexplored.

Electrochemical Sensing: Electrochemical sensing has interesting and novel applications in milk. Ion Sensitive Electrodes (ISEs) may be used to quantify the relative concentrations of different charged species in raw or processed milk. The ions are indicative of several indirect quality parameters. Although the technology cannot be used to quantify major constituents, variation in ion concentrations may be indicative of abnormalities and adulteration. An existing application of non-selective electrochemical sensing is to qualitatively inspect somatic cells in raw milk for pre-clinical diagnosis of mastitis.

Biosensors and Chemical Sensors: Biosensors and chemical sensors fall in a family of technologies that rely on selective enzymatic or chemical interactions among analytes and introduced reagents. These interactions may be quantified by appropriate transduction methods, as is done in blood sugar monitors, or visually inspected based on color changes, as is done in pregnancy detection kits. Several existing applications of lateral flow immunoassays are found in the dairy industry. An example is of urea test strips for Milk Urea Nitrogen (MUN) quantification.

Paper microfluidics have especially interesting applications in detecting multiple analytes on a low-cost single use test. Single use tests, as described earlier, may be used in different use cases and applications.

4.2 Proposed Instrument and Technology

Section 4.1 described the diversity in opportunities for developing instruments to improve product quality in the dairy industry. The identified opportunities are evaluated on the parameters of impact potential, commercial viability and technical feasibility. An opportunity that clearly stands out is of leveraging UV-Vis Spectroscopy for developing Point of Procurement instruments for analysis of raw milk. Point of Procurement instruments economically benefit the industry directly by decreasing wastages and isolating hazards. Such instruments are more compelling than Point of Consumption instruments due to obvious technological and economic advantages of the former.

Recent works (see Section 4.2.2) on developing new methods for analysis of milk have shown an increasing interest in optical measurements using UV/Vis spectroscopy for estimating fat, protein and lactose concentrations. However, effects of multiple scattering, which is dominant in UV-Vis spectroscopy of milk, is usually suppressed and ignored. In the present research, multiple scattering in UV-Vis spectroscopy is studied to understand its potential in quantifying quality parameters of milk.

The objective is to use UV-Vis Spectroscopy in diverse product embodiments (standalone, inline or portable instruments) for multi-parameter analysis of raw milk. For the purpose of product development and deployment, a coarse list of functional requirements for a minimum viable product is populated. This is presented in Table 4.1. The following subsections describe the prior art and the proposed technology in details.

4.2.1 Working Principle

In spectrophotometry, interaction of UV/Vis radiation with analytes present in a sample is measured and related to the physiochemical characteristics of the analytes, in order to quantify their respective concentrations and physical forms. Radiation in the UV/Vis spectrum incident on a sample of milk attenuates as it interacts with the dispersed phase, the aqueous medium and the solutes dissolved in the aqueous medium. This attenuation or extinction is a combined effect of the absorption and scattering characteristics of the multiple interacting substances. With traditional spectrophotometric instruments, only pure absorption of incident radiation is measured, while scattering is intentionally suppressed or purged from observations. Samples are usually diluted and homogenized to be able to ignore multiple scattering events. Further, scatter correction methods like standard normal variate transformation are employed to process data before chemometric regression methods are used for calibration and analysis. Such instruments usually require sample pretreatment that is not practical in an inline instrument, especially when continuous monitoring is desired, or sample wastage is unacceptable.

Results from absorption based UV/Vis spectrophotometry have been found to be less precise compared to those from NIR spectroscopy (Aernouts, 2011). However, the results have been shown to improve when scatter correction methods are not employed in

Table 4.1 Functional requirements for a Minimum Viable Product, and good-to-have requirements (marked with *)

Requirement	Description	Design Parameter
Multi-constituent Analysis	Fat and Solid Non Fat (SNF)	Fat (0% - 15%) [†] SNF (3% - 15%) [†]
	Fat, Casein Protein, Whey Protein, Lactose*	Proteins (2% - 8%) ^{†*} Lactose (0% - 6%) ^{†*}
	Adulterants, Microbial Activity, Residues*	Others (Traces)* [†] w/w
Milk Types and Calibration	Both Raw and Processed Milks	Fat Globule Sizes (0.5µm to 10µm)
	Milk from different milch animals	See Table 3.1
Non-Destructive Testing	No dilution, mixing or homogenization Automatic sampling, inline applications*	
Field Ready	Rapid measurement and analysis	30 seconds (maximum)
	Wide operating temperature and humidity range	0 - 50°C; Rel. Humidity 30 - 80%
	Clean-in-place, Low maintenance	
Cost	Low/moderate fixed and per test cost	USD 1,000 (max) fixed cost
		USD 0.05 (max) per test cost
Suitable for Low Resource Settings	Minimal operator training	
	Low/intermittent power operation*	

analysis (Bogomolov, 2012). Reflectance measurements, indicative largely of the scattering characteristic of the sample, have also been shown to give more precise results (Aernouts, 2011) than pure absorption measurements.

Particle size variations in raw milk samples, and the polydisperse nature of milk as an emulsion reinforce the importance of scattering measurements for unequivocal quantification of constituents, especially fat and casein protein. Scattering measurements are traditionally done using turbidimeters and nephelometers to estimate the fat content in milk. Turbidimetry, in other applications, is used as a method for particle size analysis in colloids and suspensions (Kourti, 2006). Typically, samples of milk are diluted and homogenized, and mixed with a chelating agent like solutions of Ethylene Diamine Tetra Acetate (EDTA) to isolate fat globules in the dispersed phase (AOAC 969.16-1969, AOAC International, 2012). Radiant power along or at an angle to the incident beam of light is measured as it exits the sample. Depending on the average globule size relative to the wavelength, Rayleigh or Mie scattering theories are used to relate observations with the globule size distribution and volume fraction, assuming spherical isotropic globules and absent multiple scattering events. For an inline instrument, it is essential to develop methods to measure scattering without modification or pretreatment of samples. Scattering measurements may be combined with absorption measurements to characterize samples of raw milk on both physical and chemical parameters.

4.2.2 Prior Art

Spectrophotometry for quantitative constituent analysis of milk, especially raw milk, has been proposed and studied in the past. Different methods of data collection and analysis have been reported. Use of LED's as light sources has been proposed in a few instances, however results have been reported to have significantly higher errors.

Tsenkova et al. (1999) studied the use of Near Infrared (NIR) wavelengths between 700 nm and 2400 nm for spectroscopic analysis of raw milk. Results obtained from absorbance in the 700 nm - 1100 nm range, Short-Wave NIR (SW-NIR) to which silicon photodetectors are sensitive, was compared with those obtained from the 1100 nm - 2500 nm range. While absorbance values in both bands were reported to be comparably

accurate and precise for fat and lactose concentrations, the former were less precise for protein concentration. Absorbance in both bands was reported to be more precise for fat concentration than for lactose and protein concentrations.

Šašić and Ozaki (2001) specifically studied the use of absorbance in the SW-NIR band for quantitative constituent analysis of homogenized milk. Compared to results for raw milk presented by Tsenkova et al. (1999), similar precision and accuracy were observed for fat and lactose concentrations. However, precision and accuracy for protein analysis was reported as significantly improved, and comparable to that for fat analysis. Kalinin et al. (2013) also discussed the approach for fat and protein analysis with similar results.

Crofcheck et al. (2000) studied the use of absorbance in the 400-1000 nm range, VIS-NIR spectrum, for quantification of fat content in skim milk. First-order scattering of light was observed as a dominant effect, especially with increasing path length. Correlation of absorbance at specific wavelengths with fat concentration was discussed. The results were limited to fat concentration below 0.2% w/w due to increasing multiple scattering at larger concentrations.

Muñiz et al. (2009) studied the use of VIS-NIR spectrum for quantification of fat, protein and lactose in raw milk. Two methods of data analysis, Principal Component Regression (PCR) and Partial Least Square (PLS), were compared and found to give similar results. The precision and accuracy of predicted concentrations were not reported. Pinsky et al. (2013) presented an online instrument with an approach similar to that proposed by Muñiz et al. (2009). The instrument used discrete LED's as light sources in VIS-NIR spectrum and photodiodes for transmittance and reflectance measurement in raw milk. Kaniyamattam and De Vries (2014) compared the results from the instrument presented by Pinsky et al. (2013) with those from a calibrated Mid Infrared (MIR) spectrometer. Correlations were found to be moderate for all analytes.

Aernouts et al. (2011) studied the use of reflectance and transmittance values in VIS-NIR spectrum for quantification of fat, protein and lactose in raw milk. PLS regression and scatter correction methods were employed in analysis. Correlation of both reflectance and transmittance with fat and protein concentrations was found to be comparable with results

presented earlier by Tsenkova et al. (1999) and Šašić and Ozaki (2001). For lactose concentration, transmittance was found to have a better correlation than reflectance. Villar et al. (2012) presented an instrument with an approach similar to that proposed by Aernouts et al. (2011). Fat and fatty acid content in raw milk were quantitatively analyzed using VIS-NIR spectroscopy.

Bogomolov et al. (2012, 2013) studied the use of absorbance values in VIS-NIR spectrum for quantification of fat and protein content in raw milk. Unlike previous approaches, no scatter correction methods were employed. Results were reported to have improved over prior art. They were also stated to be comparably precise and accurate with results obtained from NIR spectroscopy.

Kucheryavskiy et al. (2014) presented a method to measure and analyze spectroscopic data for milk using LED's as discrete light sources and a digital camera as an image sensor. Images were processed to extract intensity histograms that were correlated with fat and protein concentration using regression methods. Compared to prior art, the root mean errors were significantly higher for both fat and protein content.

No promising results have been reported in literature with use of LED's as discrete light sources and a digital camera as an image sensor. However, promising results have been reported using conventional spectrophotometry measurements (Aernouts, 2011) and without the use of scatter correction (Bogomolov, 2013). Hypotheses described in Section 4.2.4 develop on the prior art to extend capabilities of LED's and digital cameras in the proposed instrument to give results better than those obtained from conventional spectrophotometry.

4.2.3 Spectrophotometry of Milk

Absorption and Scattering in Milk: The presence of multiple chemical constituents, in different physical forms, and varying concentrations significantly complicates interaction of electromagnetic radiation with milk. Most fundamental signatures due to atomic or molecular absorption from lipids, proteins and lactose lie in the mid-infrared region (2500 - 25000 nm) of the electromagnetic spectrum. The near-infrared region (780 - 2500

nm) also exhibits absorption signatures from milk constituents, however with overtones and band overlapping. Both mid-infrared and near-infrared spectrums are hence used in laboratory instruments for constituent analysis of milk. Electromagnetic radiation is also scattered by milk due to the presence of particles of different sizes. In laboratory measurements, samples are diluted and homogenized to prevent the effects of scattering on observations.

In the UV/Vis region (300 - 1100 nm), instead of specific bands and peaks of absorbance, a continuum is observed. Interaction is dominated by the scattering of light from fat globules and casein micelles that is strongly dependent on the ratio of particle diameter and incident wavelength. Turbidimeters used for estimation of fat concentration measure the extinction of incident light due to scattering by fat globules. To isolate the scattering effect of fat globules, casein protein is dissolved by addition of chelating reagents. Milk is also diluted to prevent multiple scattering that is not accounted for in analysis.

Effect of Multiple Scattering: The radiative transfer equation explains the absorption, scattering and extinction of electromagnetic radiation passing through an interacting medium. If a sample of milk is present between two parallel transparent flats with a discrete light source at one end, the system can be modeled as a plane-parallel problem, as illustrated in Figure 4.3. The equation of transfer for the problem, adapted from Chandrasekhar (1960), is then given by:

$$\mu \frac{dL(\tau, \vartheta, \varphi)}{d\tau} = L(\tau, \vartheta, \varphi) - S(\tau, \vartheta, \varphi)$$

L is the radiance and S is the source function along a vector at any given point. ϑ and φ are the polar and azimuth angles respectively according to the chosen coordinate system. τ is the optical thickness of the sample, and a product on the extinction coefficient ϵ , analyte concentration c and path length l . μ is a symbol used in place of cosine of ϑ . The formal solution to the problem described in Figure 4.3 is as follows:

$$L(0, \vartheta, \varphi) = L(\tau_1, \vartheta, \varphi)e^{-\tau_1/\mu} + \int_0^{\tau_1} S(t, \vartheta, \varphi)e^{-t/\mu} \frac{dt}{\mu}$$

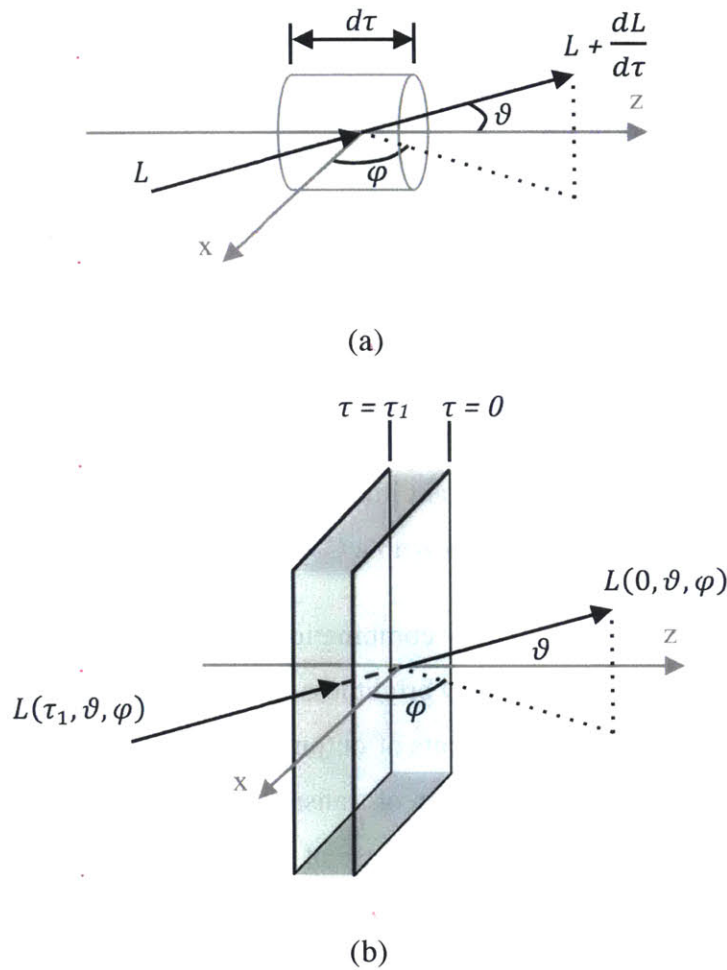


Figure 4.3 (a) Extinction of a beam passing through an infinitesimal mass of optical thickness $d\tau$; (b) Extinction of a beam passing through a finite plane-parallel system of optical thickness τ_1

The scope function \mathbf{S} depends on the radiance \mathbf{L} and a phase function \mathbf{p} . The phase function \mathbf{p} is the angular distribution of scattered radiation about a point scattering source, and is defined by the scattering regime applicable (Rayleigh or Mie). The Mie scattering regime is applicable for particles with diameters comparable with or greater than incident wavelength. This is true for milk fat globules and casein micelles subjected to UV/Vis radiation. Forward scatter is dominant in the Mie scattering phase function.

From the equations, it is evident that absorption follows the Beer-Lambert Law stating an exponential relation between extinction, and analyte concentration and path length.

Further, it is apparent that scattering or spread depends also on the power of incident radiation because of the radiance-dependent source function S .

4.2.4 Method and Hypotheses

The proposed method for online spectrophotometric measurements on raw milk and for extraction of both scattering and absorption characteristics of the samples from captured data is introduced here. Samples of milk are channeled into a flow-through sampling cell of a known uniform path length. UV/Vis radiation is directed on one transparent window of the sampling cell from an optical fiber. The radiation is sourced from discrete LED's with different emission spectra. Resultant images on the opposite window are captured using a coaxially placed digital camera with a CMOS sensor.

Multiple images are captured for every combination of milk sample and peak wavelength, at different values of LED brightness. LED's of different peak wavelengths are powered separately and in increments of output power. The captured images are processed to derive the radial distribution of transmitted radiation, which is further used to derive the extinction and scattering response of the samples.

The method is divided into the following series of hypotheses for sequential experimentation and development:

1. A CMOS camera (image sensor combined with a focusing lens) may be used to observe and quantify transmittance of light passing through a sample, similar to that presently done using photodiodes.
2. A CMOS camera may additionally be used to observe and quantify the scattering of light passing through a sample, without changing the setup used to quantify transmittance.
3. LED's may be used as light sources to quantify transmittance and scattering, as hypothesized in 1 and 2 above, at discrete wavelengths.

4. Transmittance and scattering measurements may be performed, as hypothesized in 1-3 above, for samples of milk without dilution or homogenization.
5. Fat content in samples of milk with the same fat globule size distribution may be quantified using transmittance and scattering measurements on milk, as hypothesized in 1-4 above.
6. Samples of milk with different fat globule size distributions may be distinguished using transmittance and scattering measurements on milk, as hypothesized in 1-4 above.
7. Solid Non-Fat (SNF) content in samples of milk may be quantified using transmittance and scattering measurements on milk, as hypothesized in 1-4 above.
8. Other analytes in milk, specifically lactose, casein protein, whey protein, somatic cells etc., may be selectively quantified using the proposed method with additional wavelengths or multiple sensors.

The proposed method has been systematically analyzed with experiments on raw and processed milk for response with different fat concentrations and fat globule sizes.

Hypotheses 1-6 are examined in the present research.

Chapter 5

EXPERIMENTS AND RESULTS

An online instrument for “Point of Procurement” analysis of raw milk is proposed in the present research. UV-Vis spectroscopic analysis with both absorbance and scattering measurements is hypothesized as a potential technology for the instrument. The exact hypotheses are described in Section 4.2.4.

Multiple experiments are performed on milk samples to test the validity of said hypotheses. The experimental setup was fabricated to embody the method outlined in Section 4.2. Samples of raw and processed milk were collected for every set of experiments. Multiple samples were derived from the original samples to get a suitable variety. The experimental setup was used to acquire images of spectral response from the original and derived samples. The acquired images were processed to quantify absorbance and scattering characteristics of the samples. These were compared with the analyte concentrations in samples, known from standard reference tests, to verify the said hypotheses.

This chapter describes the procedure, preparation and results of the experiments performed. Section 5.1 describes the hardware design and fabrication for the experimental setup. Section 5.2 gives details of image acquisition and data processing. Section 5.3 describes the design of experiments with details of the method followed for each experiment, sample collection and preparation, and reference tests. Section 5.4 discusses the results obtained with inferences.

5.1 Experimental Setup and Equipment

Figure 5.1 gives a schematic illustrating the major components of an experimental setup for the proposed instrument. The components are detailed in the following subsections.

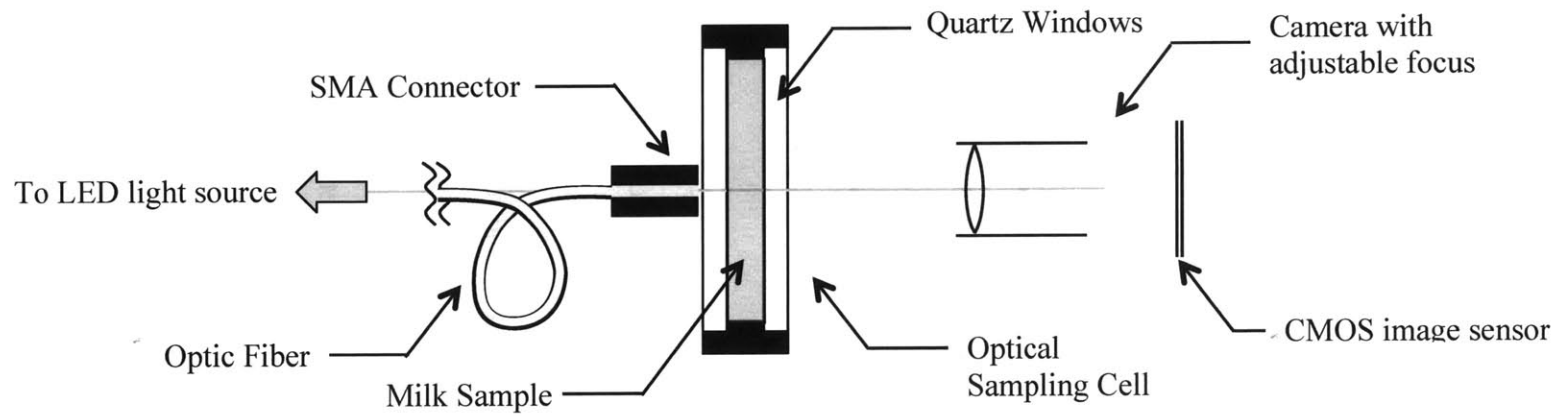


Figure 5.1 Major components of the experimental setup (Illustration not to scale with actual dimensions)

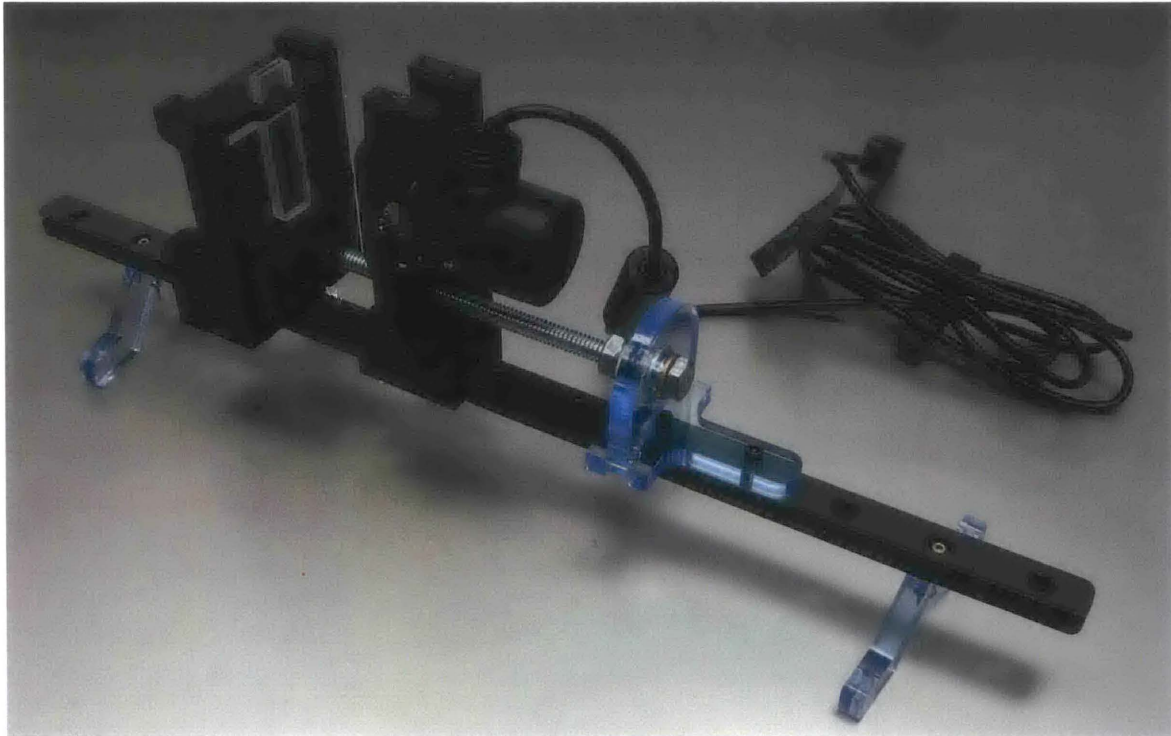


Figure 5.2 A photograph of the bench-level prototype

A sample of milk is introduced in the optical sampling cell either manually or by pumping it. The sampling cell has two transparent quartz windows separated by a fixed and known path length. Radiation in the UV-VIS spectrum, at desired wavelength and intensity, is incident on one window. The choice of wavelength and intensity is limited by the LED light source. A component of the incident radiation transmits through the sample while the rest is absorbed or backscattered. Transmitted radiation is observed in the form of an image captured using a digital CMOS camera with adjustable focus. The sample is removed from the cell manually, or by pumping. The cell is subsequently cleaned. The setup is placed inside an enclosure to isolate it from external radiations in the UV/Vis spectrum. All surfaces and inside walls of the enclosure are painted black to prevent stray reflections.

Two prototypes of the proposed instrument were fabricated and used to perform experiments. The first, a bench-level prototype (Figure 5.2) and the second, a field-level prototype (Figure 5.3) were developed in different stages of research. The bench-level prototype was used to perform experiments that suitably demonstrated the relevance of

the concept and its potential for further development. The field-level prototype was used to perform more detailed experiments and validate the hypotheses. The design and fabrication of only the latter is described here.

5.1.1 Sampling Cell

The sampling cell holds the fluid sample between two quartz windows with a fixed and known gap between them. The path length, equal to the gap between the inner surfaces of the quartz windows and the thickness of the fluid section, is chosen to be 1.5 mm. The sampling cell has an inlet and an outlet for sample introduction and removal. These can be connected with a pumping system to control the motion of the liquid. The parts of the sampling cell are illustrated in Figure 5.4. Figure 5.5 is photograph of the fabricated sampling cell with attached tubing.

Cleaning and Hygiene: A critical functional requirement of the sampling cell is that the transparency of quartz windows is maintained throughout operation. The various components in milk, especially caseins and minerals, tend to adhere to surfaces, and hence require frequent meticulous cleaning. A related requirement is prevention of microbial growth.

The hygiene and cleaning requirements are similar for all dairy equipment. Clean-in-Place (CIP) practices are usually followed when operating equipment that handles milk or other food products. The objectives of a CIP process are product recovery, rinsing, and disinfection. Tamime (2008) discusses the current good practices and also outlines equipment design principles for hygiene requirements. These are employed in determining suitable material choices and part geometries.

The CIP process for the present instrument should preferably be the same as that used for standard milk handling equipment. Typically the equipment is cyclically rinsed with alkaline and acidic solutions at specific temperatures. The same may be used for the present sampling cell. Material choice ensures corrosion-resistance to standard CIP rinsates. In preliminary examination, a 70% w/w iso-propyl alcohol was cycled through a soiled cell. Results are illustrated in Figure 5.6.

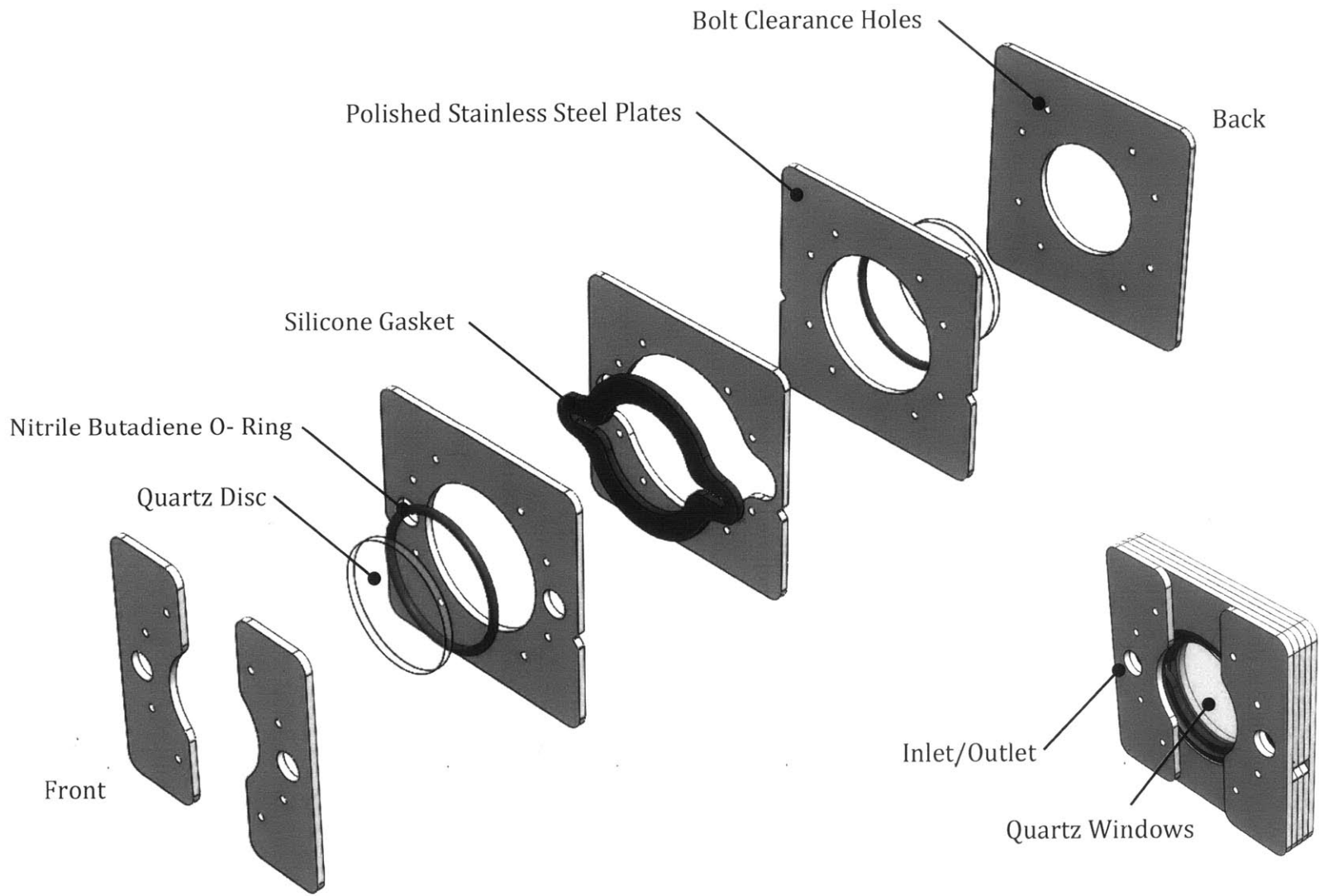


Figure 5.4 Parts of the sampling cell

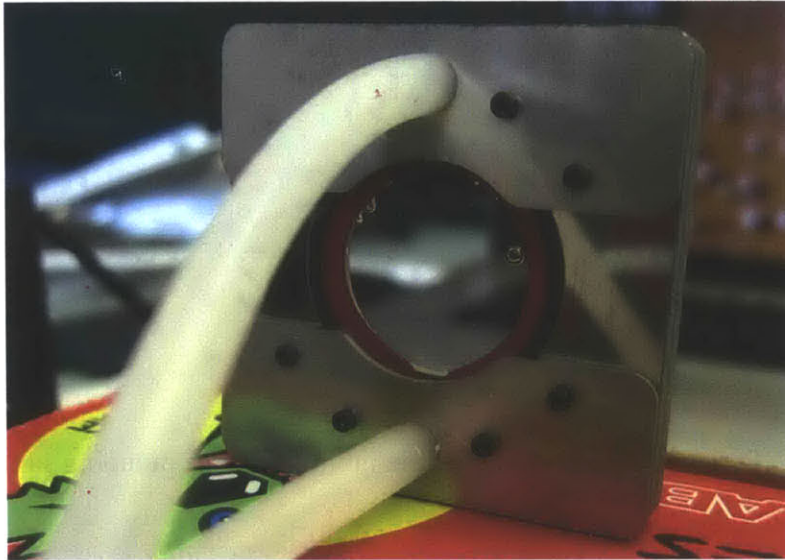


Figure 5.5 A fabricated sampling cell with attached tubing

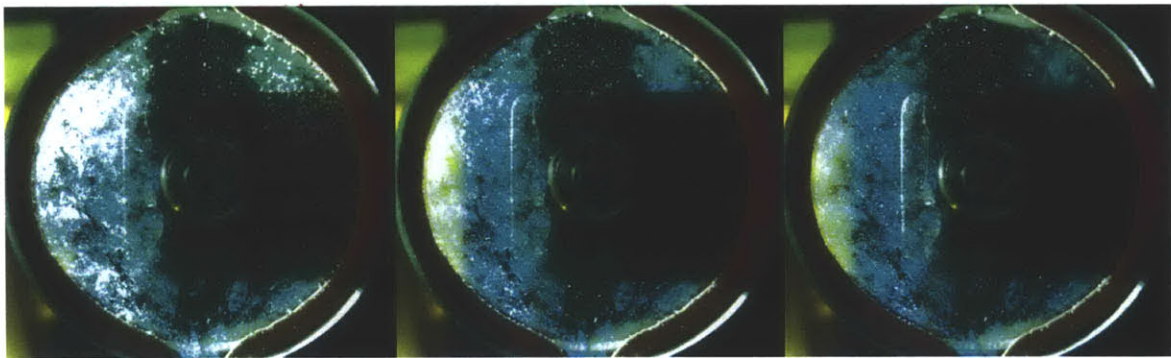


Figure 5.6 (Left to Right) A soiled sampling cell being cleaned using Iso-Propyl Alcohol. The window is illuminated with a white light from the back to highlight particles adhered to quartz surfaces after experimentation.

Materials: The materials of construction should be chosen to have the following characteristics:

- Resistant to chemical interaction with milk constituents
- Resistant to corrosion or degradation due to cleaning materials
- Non-Toxic. Should not contaminate the sample

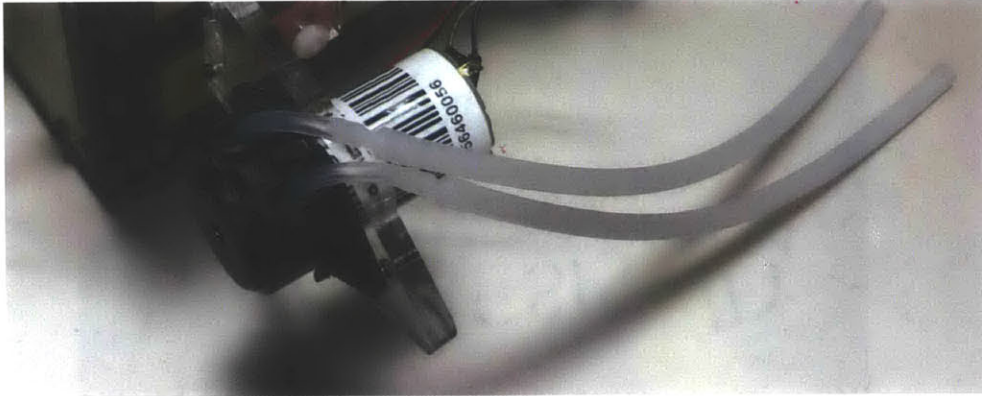


Figure 5.7 Peristaltic pump and silicone tubing used for pumping sample in and out of the sampling cell

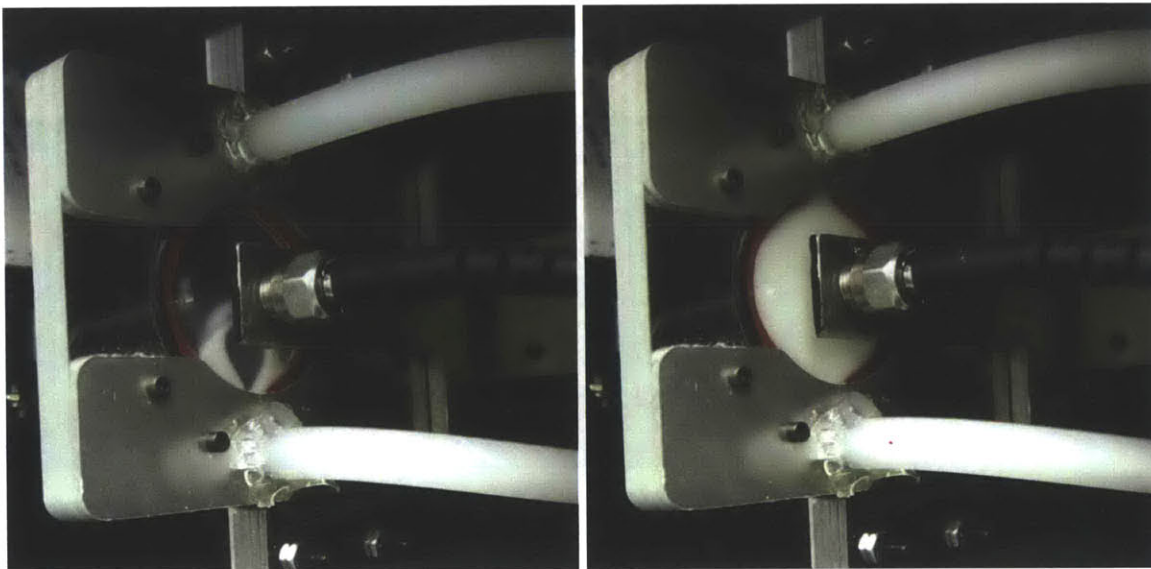


Figure 5.8 (Left to Right) Milk being pumped into a sampling cell

Stainless steel (Grade 304) is a common choice due to its corrosion resistance and weldability. Polished stainless steel was used in the present construction. Several easy-to-clean and chemical resistant polymers are also available. PTFE (Teflon®) is a common choice in industrial equipment. Silicone, Vitron®, Nitrile Rubber, N-Butyl Rubber are frequently used elastomers. Silicone rubber is used in the present construction for gaskets and tubing. The materials chosen and purchased were such that all surfaces that interact with milk are smooth, non-porous and free from crevices.

Geometries: The parts were designed to avoid sharp corners, stagnant pools and obstructions to flow. Although it is preferred that the sampling cell is self-emptying and draining, the present design does not give these features. It is acceptable for limited experiments, and may be changed in future designs.

Pumping: A peristaltic pump (Figure 5.7) was used to introduce and recover samples of milk to and from the sampling cell. Silicone tubing was used to connect the sampling cell and the pump. A diaphragm pump may also be used instead. Peristaltic and diaphragm pumps are easier to clean as only the carrying tube interacts with the pumped fluid.

Milk was successfully pumped in and out of the sampling cell using the peristaltic pump. No macroscopic bubbles were observed in introduced samples (Figure 5.8). The cleaning fluids were also pumped using the pump.

5.1.2 Light Source

In the proposed instrument, LED's are used as discrete light sources for the spectroscopic measurements. A multicolor LED with three embedded diodes with different wavelength bands (red, green and blue) was used in the present prototype. The spectral response of the three wavelength bands, measured using a calibrated spectrophotometer, is presented in Figure 5.9. For red, green and blue bands, peaks were observed at 602, 508 and 460 nm respectively.

For every sample, the LED's were controlled to sequentially emit radiation in specific wavelength bands and at specific relative intensities. The emitted radiation was channeled from the LED to the input quartz window using an optical patch cord. Since the wavelength bands were all in the Visible range of the spectrum, Plastic Optical Fiber (POF) was used. A quartz optical fiber patch cord may be used for LED's emitting radiation in the IR spectrum. The LED and the patch cord were coupled using a standard Sub-Miniature version A (SMA) connector. The coupling was sealed and painted black to prevent stray radiation from entering the POF patch cord. This is illustrated in Figure 5.10.

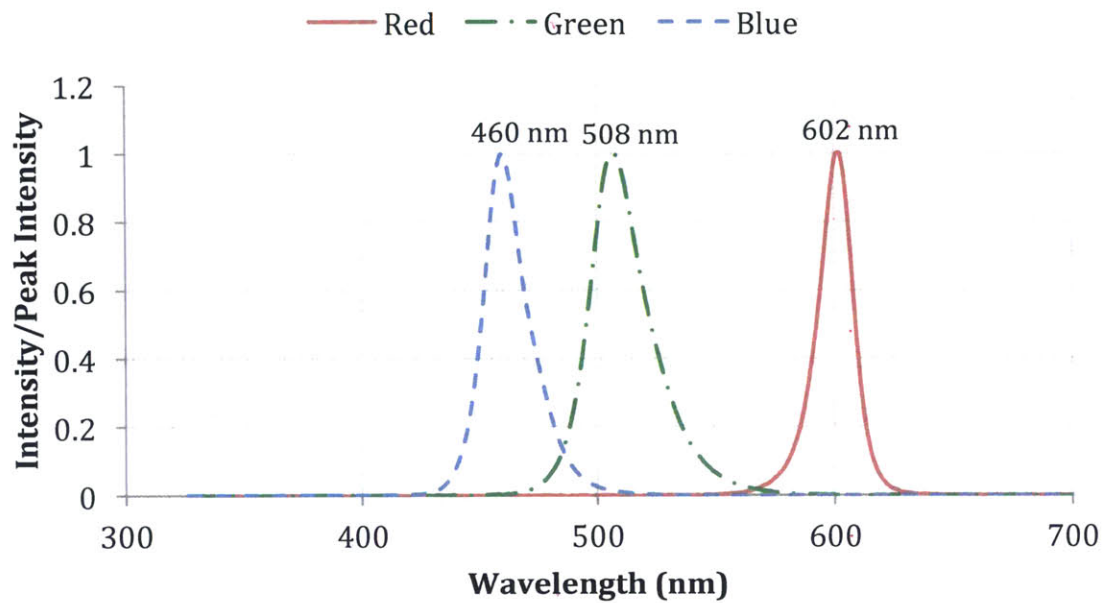


Figure 5.9 Measured emission spectra of the three wavelength bands. Intensities are shown relative to measured peak intensities.

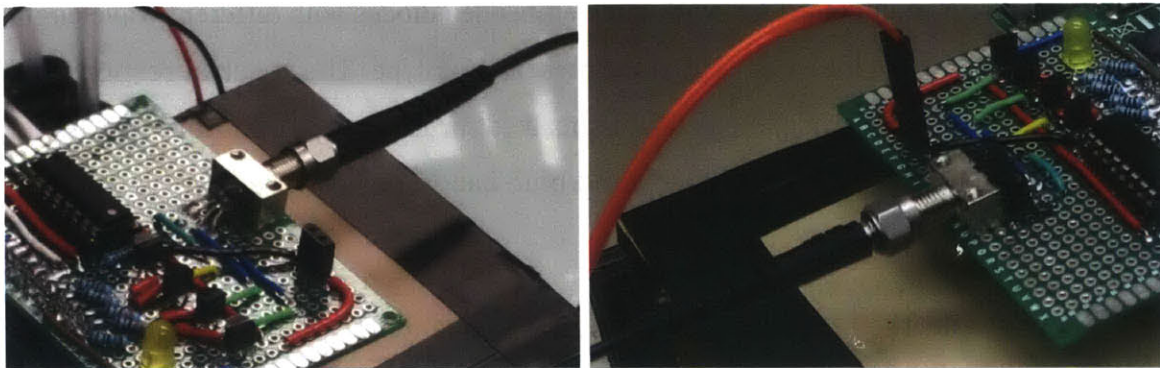


Figure 5.10 (Left) Coupling between LED and POF patch cord using a standard SMA connector; (Right) Coupling after sealing

The other end of the POF patch cord was affixed to the input window of the sampling cell, as is seen in Figure 5.8. A fixed fraction of the light emitted by the LED is hence transmitted to the sampling cell for spectroscopic measurements. The sampling cell is placed within an enclosure with black inside walls to prevent stray reflection from affecting quality of collected data.

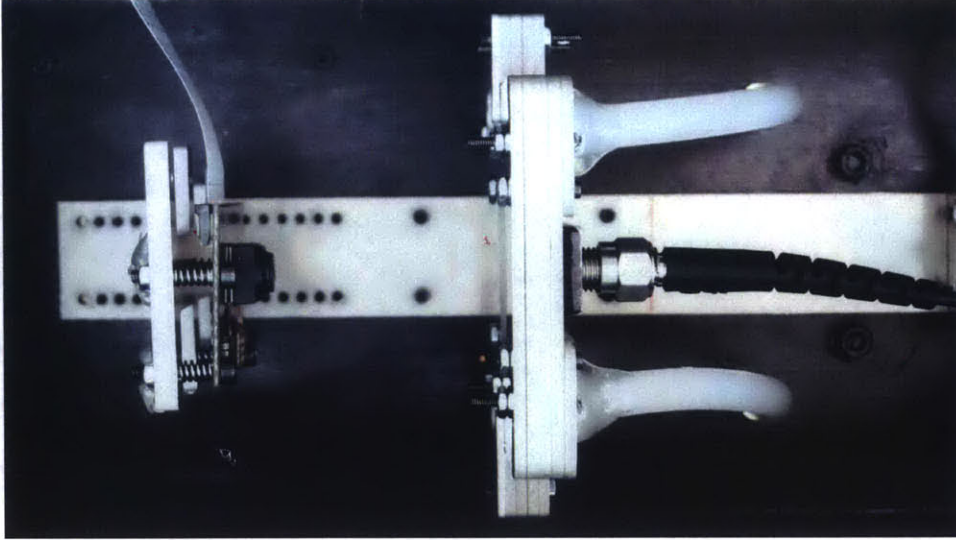


Figure 5.11 Coaxial assembly of camera, sampling cell and light source; Camera mounted on adjustable bolts

5.1.3. Camera and Imaging Optics

A CMOS camera was coaxially placed on the side of the sampling cell opposite to the one with the POF patch cord. The camera was used to capture multiple images for each sample at different wavelength band and relative intensities. In the present prototype, a digital camera for use with Raspberry Pi Modules was used. The camera had a 1/4" CMOS QSXGA (5 Megapixel, 2592×1944) image sensor manufactured by OmniVision Technologies, Inc. The camera also had a focusing lens with adjustable distance between the lens and the image sensor. The sensor was placed coaxially with the light source, as is seen in Figure 5.11. The camera was mounted using four spring loaded bolts to allow fine adjustments in the sensor orientation during hardware calibration. A camera calibration cell with dot-matrix pattern (Figure 5.12) was fabricated and used to adjust camera orientation and focus.

The camera was controlled using the Raspberry Pi B+ System-on-Chip board. The board also controlled the light source, and hence synchronized the capture of images with every combination of wavelength band and relative intensity. The image sensor (OmniVision OV5647) had onboard image processing capabilities that could be suitably controlled

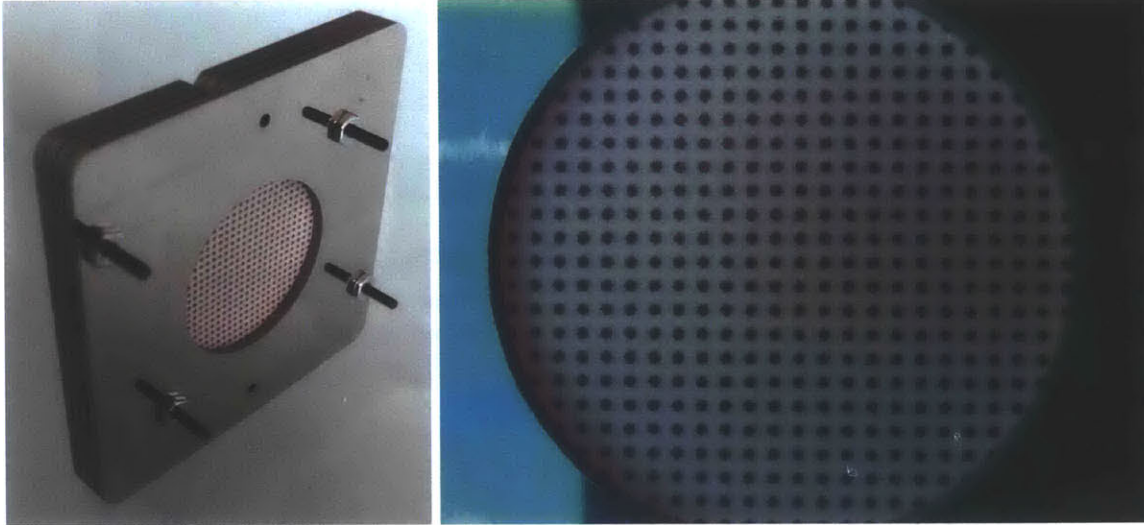


Figure 5.12 (Left) Camera calibration cell; (Right) Image captured by onboard camera during hardware calibration

using the Raspberry Pi. For consistent images, the onboard image processing parameters were fixed. Following are some of the fixed parameters:

Auto White Balance (AWB): AWB adjusts the relative gains in Red, Green and Blue (RGB) channels to interpret white surfaces correctly. The relative gains were leveled and fixed in the present prototype.

Exposure: Exposure is a multi-parametric property that strongly affects image output. It is principally impacted by analog and digital gains, shutter speed and metering mode. The parameters were individually set to constant values where possible. Values of analog and digital gains could be fixed but not be commanded by the controller. The values were allowed to stabilize to a specific value and fixed before images were captured.

Image Enhancement: Images are enhanced onboard by modifying image properties, including brightness, contrast, saturation and sharpness. Image enhancement feature on the image sensors was fixed by establishing constant values for all governing parameters.

Image De-noising: The onboard camera was preprogrammed to remove various sources of noise in captured images. It removed black noise, speckle or shot noise and vignetting effects. The de-noising features were retained during experiments.

5.2 Image and Data Analysis

For every sample introduced, images were captured for different individual wavelength bands (with peaks at 460, 508 and 602 nm) at different values of LED brightness varying from 5% to 95% of the peak brightness. The brightness was adjusted by supplying a PWM of corresponding duty cycle to the light source. The peak brightness values were different for different LED wavelengths due to difference in effective internal resistance.

The CMOS image sensor and the onboard signal processor were programmed to have a constant shutter speed and gains in order to get the same exposure in all images (Section 5.1.3). Images were captured in full resolution, denoised and demosaiced, and stored with lossless compression. The eight-bit readings (0 - 255) of the RGB channels for each pixel were assumed proportional to the power of incident EM radiation in the corresponding wavelength bands. While all channels were sensitive to all incident wavelengths, the R, G and B channels were individually most sensitive to the 602, 508 and 460 nm bands respectively, as expected. Readings from only the most sensitive channel were used in the analysis. Figure 5.13 illustrates the process with an example image captured using the experimental setup. The image is taken from multiple images captured with processed whole milk in the sample chamber, subjected to a 602nm wavelength band at 50% brightness. The presented image is cropped from a larger 2592×1944 pixel image to show detail. Image and data analysis done on images drawn from this sample are used for illustration in later examples as well.

For every wavelength band and LED brightness, one image was obtained by averaging three captured images to reduce temporal noise. The images visually appeared like blurred discs with bright centers, which were coincident across all images. A radial distribution of the chosen channel's pixel readings about the center was obtained for every combination and LED brightness. These distributions were used to extract absorbance and scattering data for each sample.

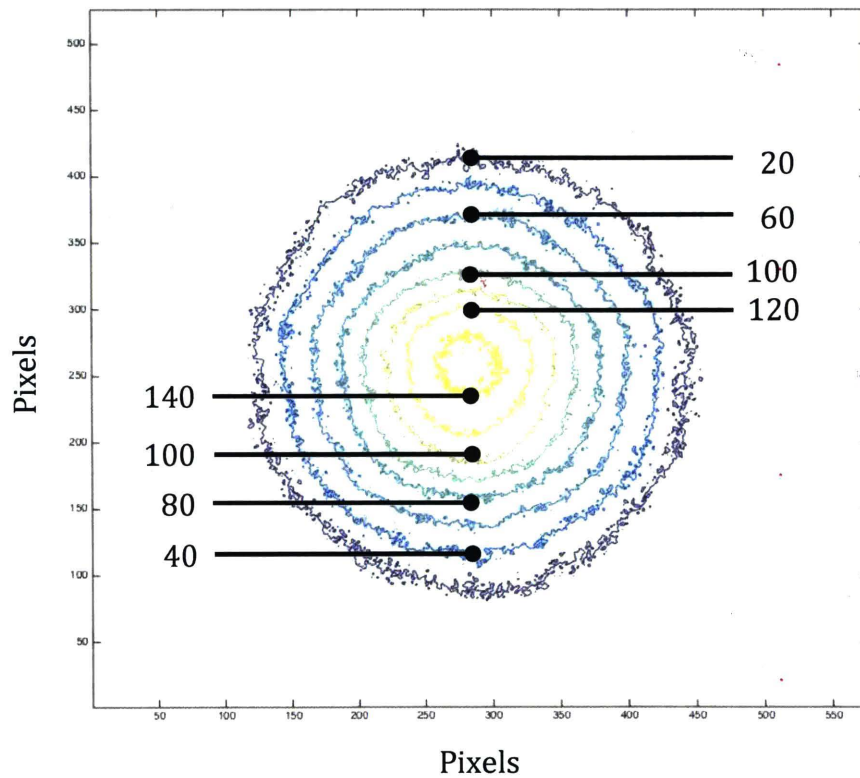


Figure 5.13 (Top) A typical captured image; (Bottom) Contour plot of the readings at individual pixels in the dominant channel

5.2.1 Image Preparation and De-noising

Results from every image acquisition process have noise that affects the interpretations and conclusions. It is therefore important to reduce noise in the images obtained from the present experimental setup before relevant parameters are extracted from it. The following is a description of the major noise components observed and how they were reduced to acceptable levels. Mizoguchi (2006) and Yoshida (2006) provide details of sources of image noise.

Temporal Noise: Temporal noise is defined as the component in the output signal for each pixel that fluctuates over time, without any change in incident radiation. There are multiple sources of temporal noise in images that may or may not be controlled deterministically. The noise is random in nature, and can be reduced by averaging of multiple images. Averaging n images reduces temporal noise on an average by \sqrt{n} . In the present experiments, three images for each wavelength-brightness combination are averaged for reducing temporal noise to a tolerable range.

Fixed Pattern Noise (FPN): FPN noise is defined as the non-uniformity of image data that does not change with time or incident radiation. FPN can be determined using dark images for a given image sensor. In the present experimental setup, FPN was determined separately for each channel. Over ten dark images were taken at different time instances and averaged. FPN was observed to be different for the three channels. The determined FPN is illustrated in Figure 5.14. Determined FPN for each color channel was subtracted from the obtained images to eliminate it accurately.

Gamma Correction: Gamma correction or tone curves are usually used in image sensors and digital cameras to enhance captured images to match human perception of brightness. Any applied gamma correction should be inverted for the analysis to give true measurements. The image sensor presently used has no inbuilt gamma correction.

Blooming: Blooming is the spread of pixel readings to surrounding pixels in case of saturation. Images with saturated pixel readings were hence rejected from analysis.

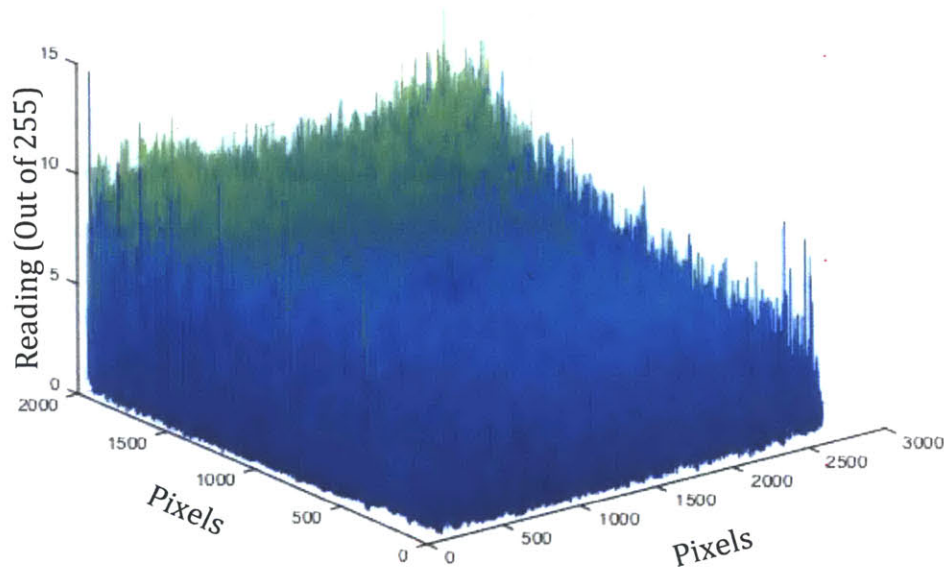
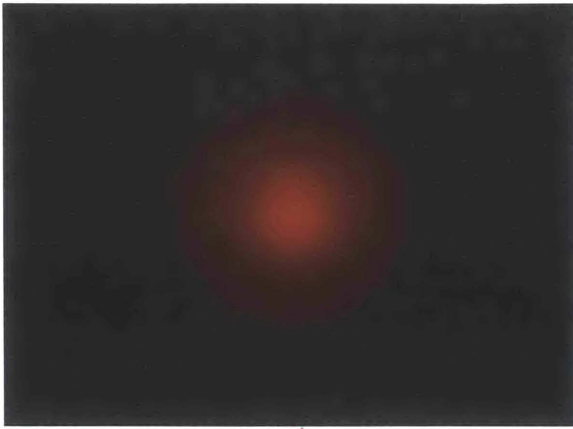


Figure 5.14 Fixed Pattern Noise (FPN) determined for the Green channel

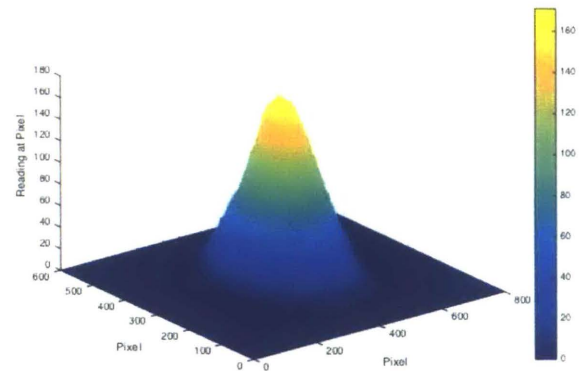
5.2.2 Radial distribution of Intensity

The prepared images for every wavelength-brightness combination were used to derive a radial distribution of intensity. The effective center of observed circular pattern (Figure 5.13) was found by fitting a 2-Dimensional Gaussian curve on the matrix of pixel readings. Since the experimental setup did not change between any two images, the determined centers were also found to be close, as expected. The location was determined by averaging the location found by Gaussian fit for fifteen randomly chosen images for different samples.

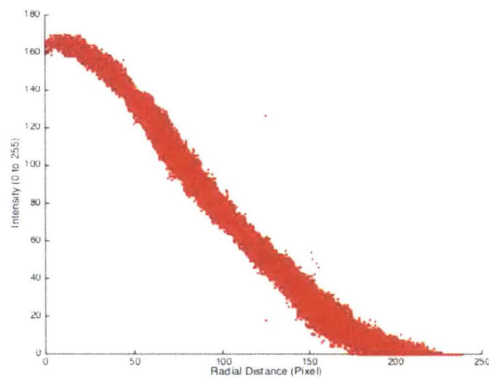
The radial distribution of intensity $I(\mathbf{r})$ (radiant flux per unit surface area with respect to radial distance) was found by computing the distance of each pixel to the determined center location. The readings at the pixels were separated into classes according to the computed radial distance \mathbf{r} . Each bin was 1 pixel wide. The averages of the readings obtained for each bin were used as the radial distribution of intensity $I(\mathbf{r})$. Figure 5.15 illustrates the process with an example from the same sample as in Figure 5.13.



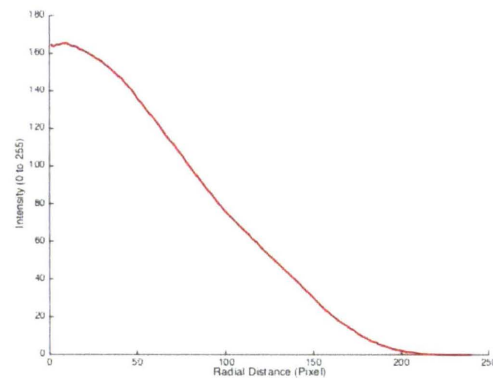
(a)



(b)



(c)



(d)

Figure 5.15 (a) An obtained image for 602nm-50% combination; (b) R-channel readings for all pixels; (c) Readings in bins according to radial distance; (d) Averages of binned readings, equivalent to radial distribution of intensity

Images obtained for a given sample subjected to a given wavelength band at different values of LED brightness can be compared by using the radial distributions of intensity for each image. Figure 5.16 illustrates this with the example of images obtained for a processed whole milk sample subjected to the 602 nm wavelength band, with LED brightness varying from 5% to 95% of peak brightness. Images for different samples at the same wavelength band and LED brightness can be similarly compared. Images obtained for different wavelength bands, however, cannot be compared similarly because absolute peak brightness of LED's and the sensitivity of the image sensor are different for different wavelength bands.

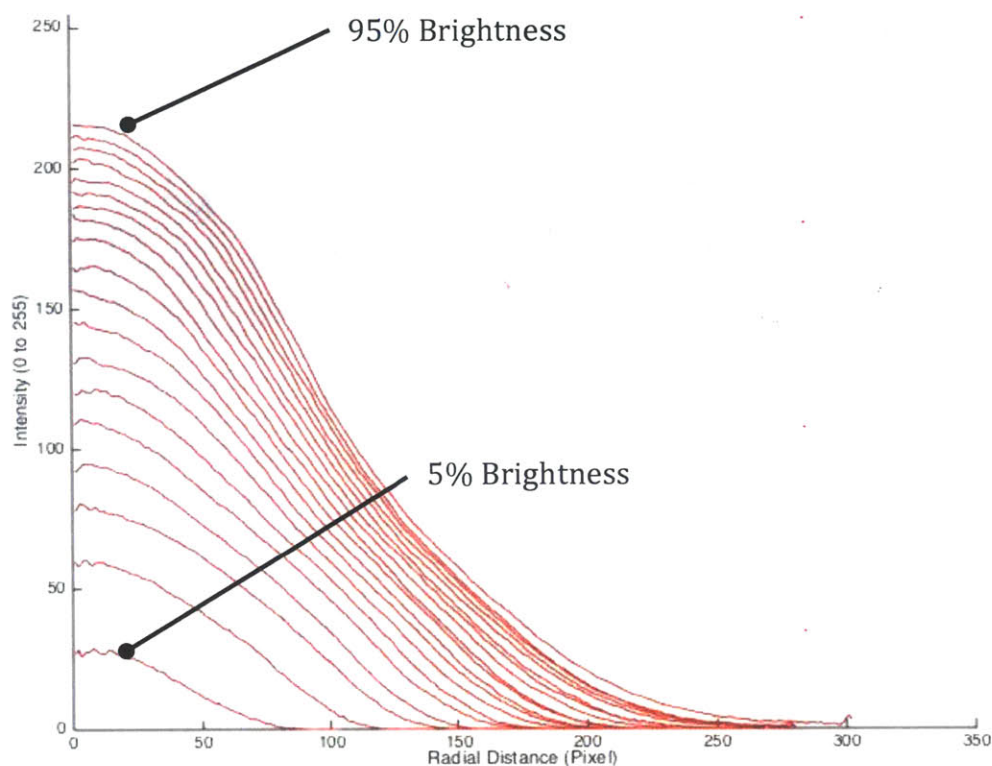


Figure 5.16 Comparison of radial distributions of intensity, obtained from images corresponding to same wavelength and sample and different relative brightness values

5.2.3 Transmittance and Absorbance

Transmittance T is defined as the ratio of transmitted and incident radiant intensity, I and I_0 respectively.

$$T = \frac{I}{I_0}$$

The Beer Lambert law, mentioned earlier with the radiative transfer equation in Section 4.2.3, is a guiding principal in most spectroscopic methods. It defines the relation between transmittance T , which is measured in a spectrometer, and the sample's bulk optical properties. A sample's bulk optical properties are in turn dependent on the extinction coefficient ϵ of the analyte of interest, and the concentration c of the analyte.

For a sample with a single analyte interacting with incident radiation, the relation is straightforward, and is defined as follows:

$$T = e^{-\epsilon cl}$$

Here, l is the path length of radiation transmitted through the sample. Measurement of I and I_0 is affected by the sensitivity of the image sensor or photodiode and the optical thickness of other components in the system. The effects are usually multiplicative in nature, and are hence eliminated in T .

Absorbance A is defined as the negative logarithm of transmittance T , for a linear relation with analyte concentrations.

$$A = -\ln(T) = \epsilon cl$$

For a sample with multiple (n) interacting analytes, the measured transmittance T is a product of individual analyte's transmittance values:

$$T = T_1 T_2 \cdots T_n$$

$$T = e^{-\epsilon_1 c_1 l} e^{-\epsilon_2 c_2 l} \cdots e^{-\epsilon_n c_n l}$$

Accordingly, measured absorbance A is a sum of individual analyte's absorbance value.

$$A = A_1 + A_2 + \cdots + A_n$$

$$A = \epsilon_1 c_1 l + \epsilon_2 c_2 l + \cdots + \epsilon_n c_n l$$

Measuring transmittance T and absorbance A is straightforward when a monochromatic laser beam is transmitted through a sample and measured using a photodetector. The present instrument however uses a diffuse source of light and an image sensor instead. Incident radiation is simultaneously absorbed and scattered. Transmittance and absorbance of a sample for a given source of radiation are independent of incident intensity. However, the measured intensity at individual pixels does not scale linearly with increasing incident intensity. This is evident in Figure 5.16.

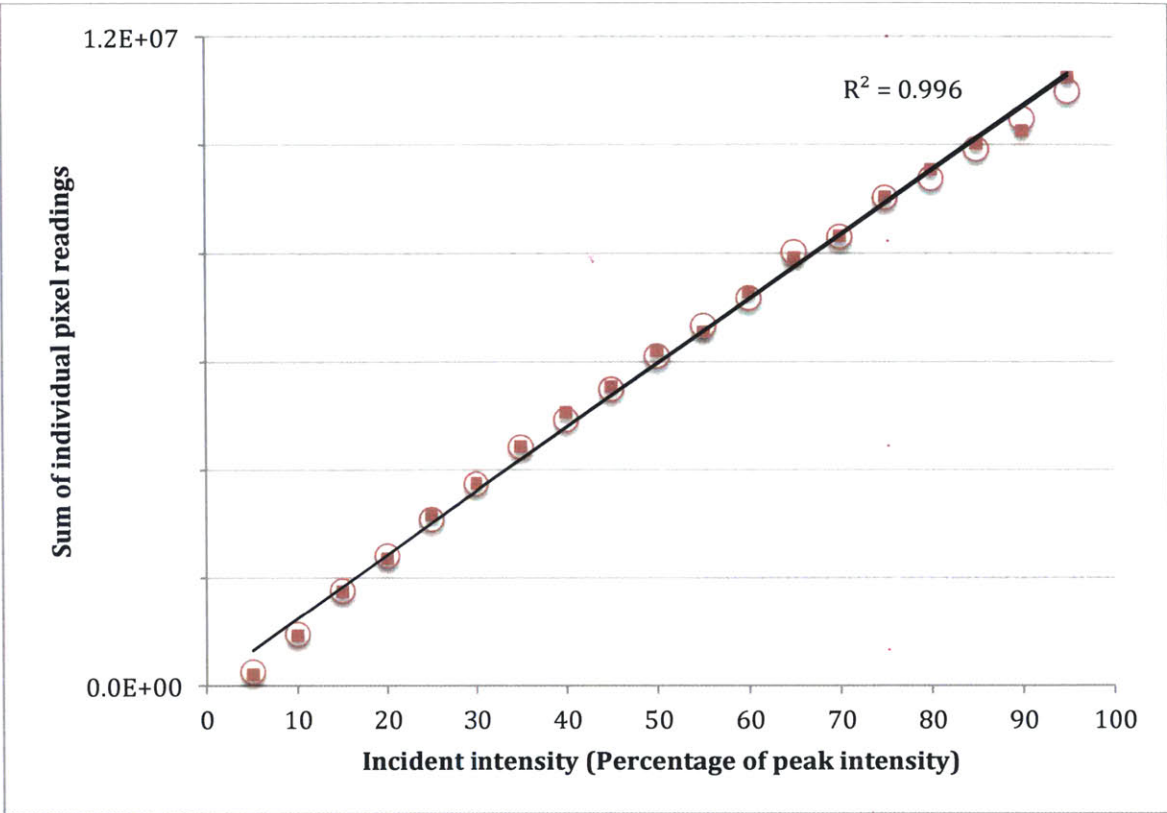


Figure 5.17 Sum of individual pixel readings for different relative incident intensities indicating a linear fit for two separate sets of measurements

In the course of the experiments, it was realized that instead of the individual pixel readings, the sum of individual pixel readings over the whole image scaled linearly with increasing incident intensity. This is illustrated in Figure 5.17 with an example, same as that used in Figures 5.15 and 5.16. Two separate sets of measurements are presented to validate repeatability of the observation. Similar trend was observed for different wavelength bands in all samples.

The sum of individual pixel readings is a measure of the transmitted radiant flux, or the total power of transmitted radiation. The slope of the linear relation is equivalent to the transmittance **T** of the sample.

$$T = \frac{\Delta I}{\Delta I_0}$$

Using the slope is a suitable metric of transmittance because it eliminates additive or subtractive sources of error in the measured intensity. Additive or subtractive sources of error are witnessed in the form of an X intercept of the best-fit straight line. These are otherwise not eliminated in conventional spectroscopy that independently measures incident and transmitted radiation.

Absorbance **A** is calculated from the measured slope by taking its negative natural logarithm. The relation of measured absorbance with fat concentration and particle size of different samples is discussed in Section 5.4.

5.2.4 Scattering Characteristic

Scattering is a result of the interaction of incident radiation with physical particles dispersed or suspended in a sample. Fat globules and casein micelles can be approximated as finite spherical particles that measurably scatter radiation in the UV-VIS spectrum. The response and the presence of multiple scattering was discussed in Section 4.2.3 using the radiative transfer equation.

The spread or diffusion of transmitted radiation is dependent on the incident intensity. This is evident in Figure 5.16. Pixel readings do not scale linearly with increasing incident intensity while the sum of all pixel readings does (Section 5.2.3). The spread of transmitted radiation, which is a qualitative indicator of the sample's scattering characteristic, hence compensates for the non-linear scaling of individual readings.

The spread of transmitted radiation, or the scattering characteristic of a sample, is traditionally quantified by measuring the angular distribution of transmitted intensity, also known as Bidirectional Scatter Distribution Function (**BSDF**). In the present experimental setup, BSDF for every wavelength band and relative LED brightness can be derived from the radial distribution of intensity, discussed in Section 5.2.2.

BSDF is defined for a point in space as the angular distribution of radiance in a polar coordinate system (Stover, 2012). This is illustrated in Figure 5.18.

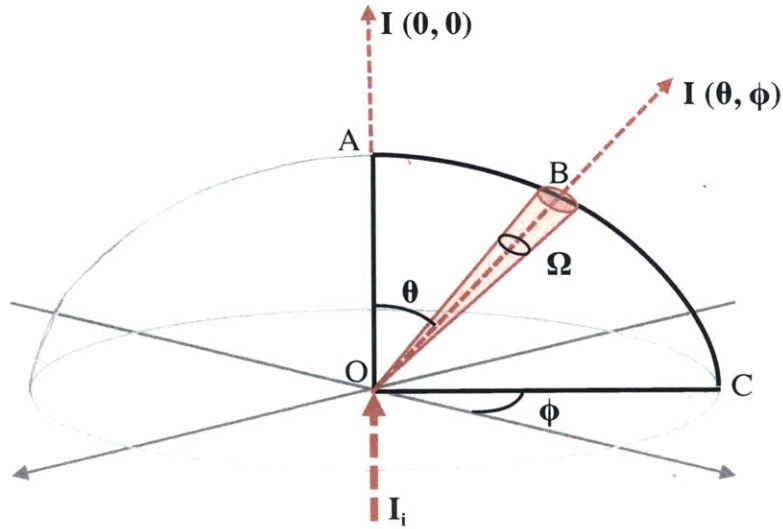


Figure 5.18 Bidirectional Scattering Distribution Function (BSDF) for a point, measured over a surrounding uniform sphere

$$\text{BSDF} = \frac{L(\theta, \phi)}{I_i} = \frac{I(\theta, \phi)}{\Omega I_i}$$

Here, \mathbf{I} is radiant intensity (radiant flux per unit subtended area) and \mathbf{L} is radiance (radiant flux per unit solid angle per unit subtended area). $\mathbf{I}(\theta, \phi)$ and $\mathbf{L}(\theta, \phi)$ are radiant intensity and radiance respectively at polar angle θ and azimuth angle ϕ . \mathbf{I}_i is the intensity of radiation incident at a point O in space. ABC is an arc on a uniform sphere of radius R , formed at a given azimuth angle ϕ with polar angle θ varying between 0 and 0.5π radians.

For the present experimental setup, intensity distribution has a rotation symmetry (is invariant with azimuth angle ϕ). Although absolute value of measured \mathbf{I} is unknown, it is fixed for individual wavelength bands and relative brightness values. BSDF is hence reduced to $\mathbf{L}(\theta)$ and is computed from the obtained radial distribution of intensity $\mathbf{I}(\mathbf{r})$ (Section 5.2.2). The radial distribution is measured over a plane equivalent to the plane of the image sensor. Figure 5.19 illustrates BSDF in such a configuration. Radiant intensity \mathbf{I} at a point B is measured as an eight-bit pixel reading. Since every pixel has a finite and equal area, the solid angle Ω is dependent on the position of the pixel B with respect to the center A .

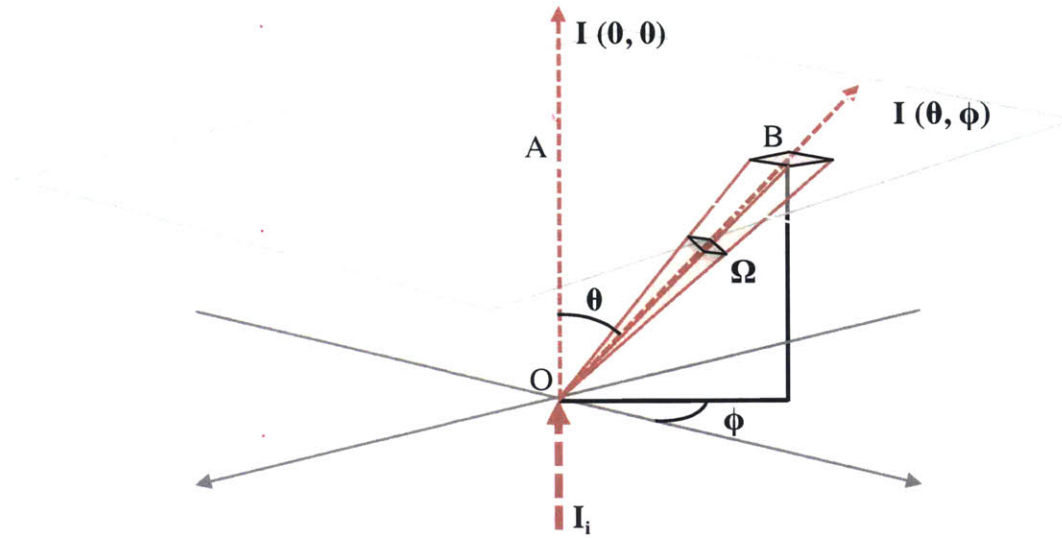


Figure 5.19 Bidirectional Scattering Distribution Function (BSDF) for a point, measured over a plane

For deriving **BSDF** from the radial distribution $I(\mathbf{r})$, it is assumed that the captured image corresponds to the radiation exiting the sample surface. OA is hence the path length l of the sample. AB is the distance r of a pixel from the observed center. θ can hence be expressed as follows:

$$\theta = \tan^{-1} \frac{r}{l}$$

For computing BSDF, the solid angle Ω is required, which is expressed as follows:

$$\Omega = \frac{\text{Pixel Area} * \cos^3(\theta)}{l^2}$$

BSDF is hence proportional to the ratio of intensity radial distribution and solid angle:

$$\text{BSDF} \propto \frac{I(\mathbf{r})}{\Omega} \propto \frac{I(\mathbf{r})}{\cos^3(\tan^{-1}(r/l))}$$

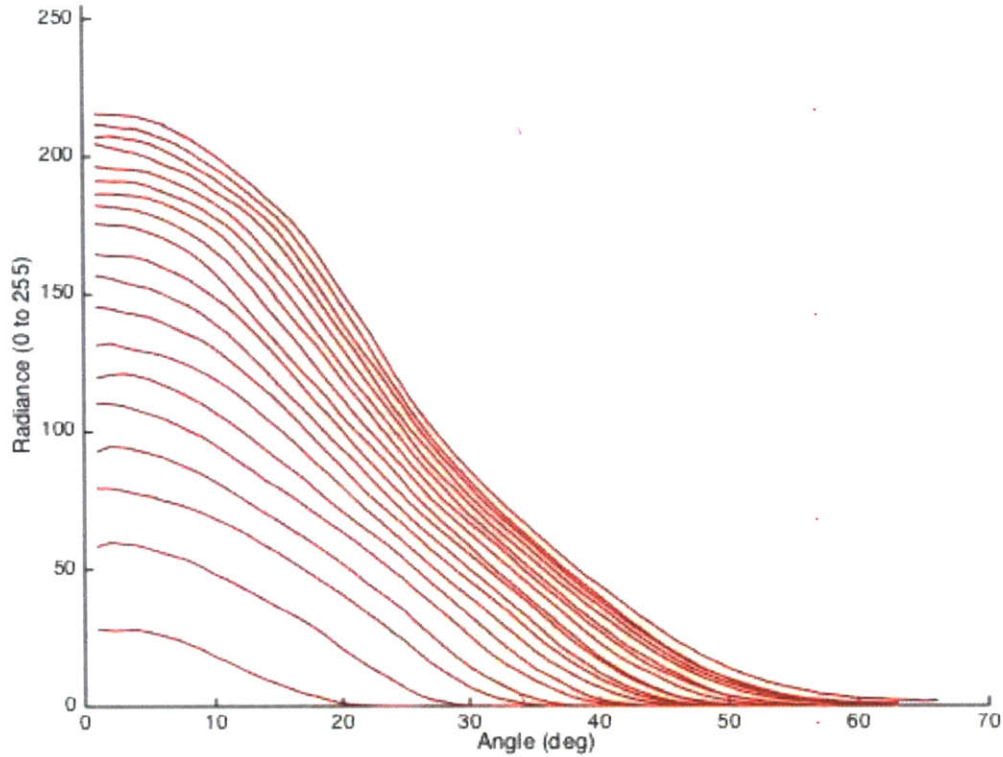


Figure 5.20 Comparison of BSDF, obtained from images corresponding to same wavelength and sample and different relative brightness values

The radial distribution of intensity was hence converted to a distribution proportional to BSDF. The proportionality factor depends on intensity and wavelength of incident radiation. Figure 5.20 illustrates this with the example of images obtained for a processed whole milk sample subjected to the 602 nm wavelength band, with LED brightness varying from 5% to 95% of peak brightness. The BSDF functions are not normalized to respective incident intensities for visual clarity.

In the course of the experiments, it was realized that the standard deviation of BSDF curves scaled logarithmically with increasing incident intensity. This is illustrated in Figure 5.21 with an example, the same as that used in Figures 5.20. A similar trend was observed for different wavelength bands in all samples. The various parameters of the logarithmic fit can therefore be used to quantify the scatter characteristic of a sample at different wavelengths.

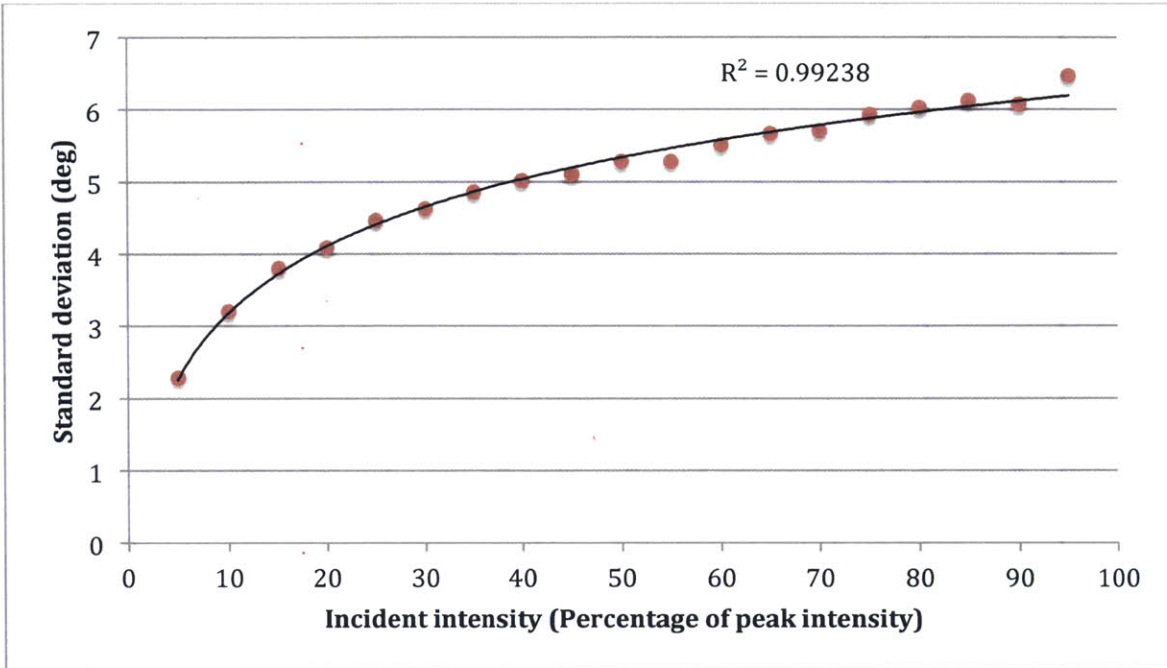


Figure 5.21 Standard deviation of derived BSDF for different relative incident intensities indicating a logarithmic fit

5.3 Design of Experiments

The purpose of the experiments conducted was to systematically analyze the proposed concept for validity of the hypotheses. Procured and prepared samples of raw and processed milk were used for the experiments. In multiple early experiments, the bench-level prototype was used to characterize the absorption and scattering response of different samples. The method followed and the results obtained evolved with each experiment. Results from experiments conducted using the bench-level prototype suitably demonstrated the relevance of the concept and its potential for further development. However, due to the nature of the setup, the results were however not robust enough to draw reliable inferences. The challenges were overcome by developing the field level prototype with robust construction and onboard automated milk sampling and data collection systems that increases precision and repeatability. The results and inferences obtained with later experiments were found to be significantly more repeatable and reliable. The design of only the latest experiments is described here for brevity.

Table 5.1 Primary and derived samples, with reference major constituent concentrations

Sample	Fraction of Primary Samples					Fat (w/w %)	Protein (w/w %)	Lactose (w/w %)	Nature of Sample
	A	C	F	K	G				
A	1.00					1.51	3.28	5.22	Raw
B	0.50	0.50				2.50	3.23	5.12	Raw
C		1.00				3.49	3.19	5.02	Raw
D		0.67	0.33			5.05	3.17	4.99	Raw
E		0.33	0.67			6.62	3.16	4.96	Raw
F			1.00			8.18	3.14	4.93	Raw
G					1.00	0.08	3.26	4.96	Processed
H				0.25	0.75	0.88	3.23	4.91	Processed
I				0.50	0.50	1.68	3.20	4.87	Processed
J				0.75	0.25	2.48	3.17	4.82	Processed
K				1.00		3.28	3.15	4.78	Processed
L		0.33		0.67		3.35	3.16	4.86	Combined
M		0.67		0.33		3.42	3.17	4.94	Combined

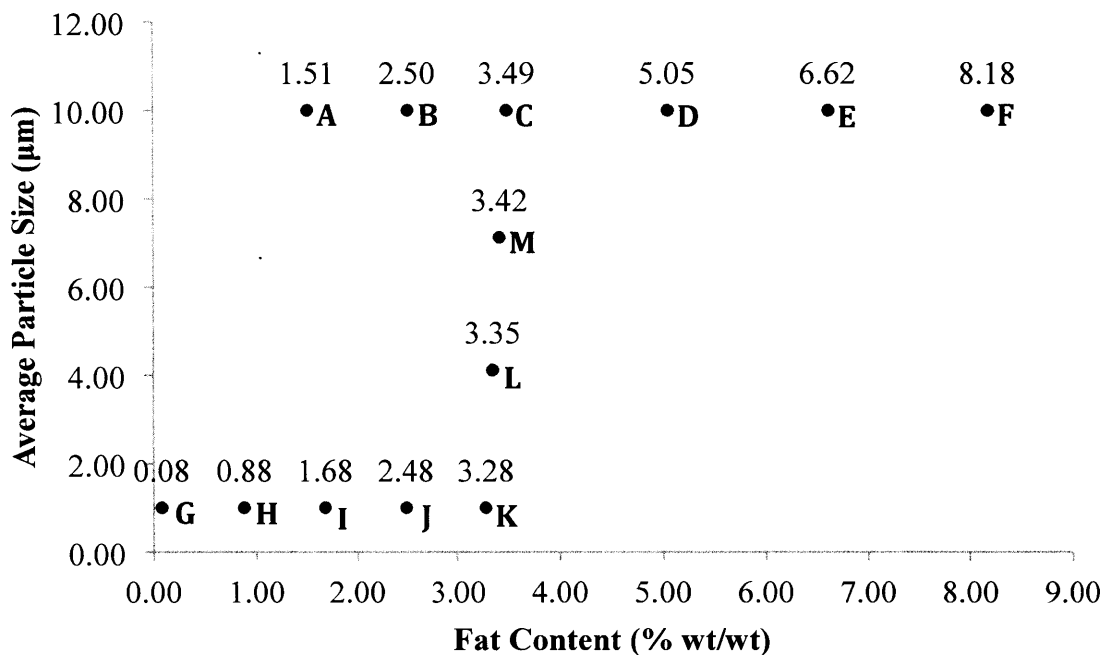
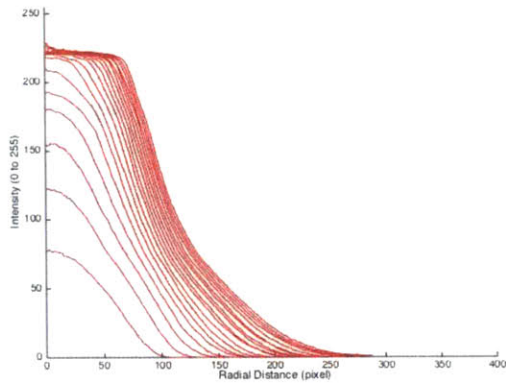


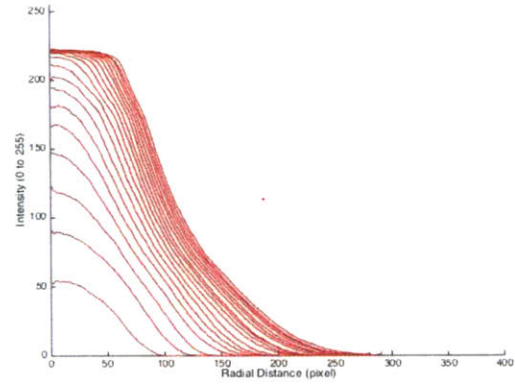
Figure 5.22 Fat concentration versus expected particle size for all samples

Multiple samples, listed in Table 5.1, of raw milk, processed milk, and combinations of raw and processed milk were examined. The samples were derived from five primary samples, A, C, F, K and G. A, C and F were samples of raw milk sourced from a regional dairy farm. A, C and F differ principally in fat content as they correspond to different layers in standing milk. K and G were samples of processed skim milk and processed whole milk, as sold at retail outlets. Different fractions of the primary samples were mixed to derive all other samples. The fractions are listed in Table 5.1

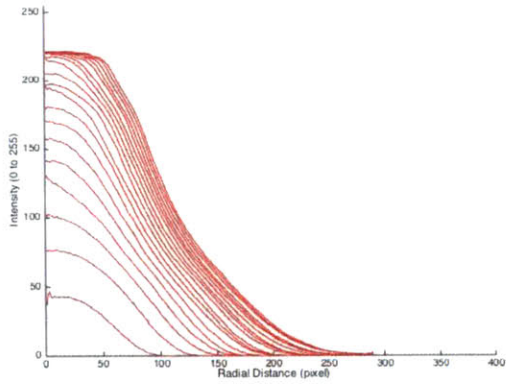
The primary samples were tested for concentration of major constituents using standard FTIR instrumentation at an accredited laboratory. For concentration of derived samples, concentration of primary samples were weighted according to fraction in sample and added. These are detailed in Table 5.1. The samples are indicated on a plot of fat concentration vs. expected average fat globule diameter (particle size.) The average particle size was assumed as 10 µm for all raw milk samples and 1 µm for all processed milk samples. The particle sizes are assigned strictly for convenience and have no bearing on current measurements, analysis and results. The three classes of samples, raw, processed and combined, can be clearly differentiated in Figure 5.22.



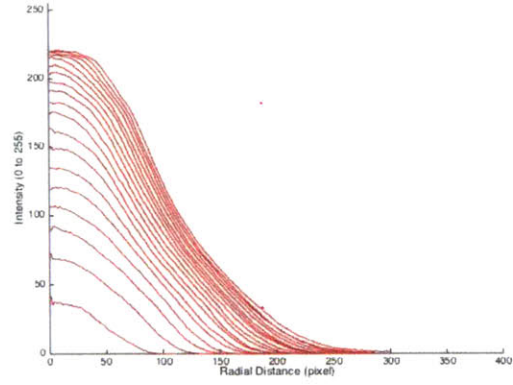
(a)



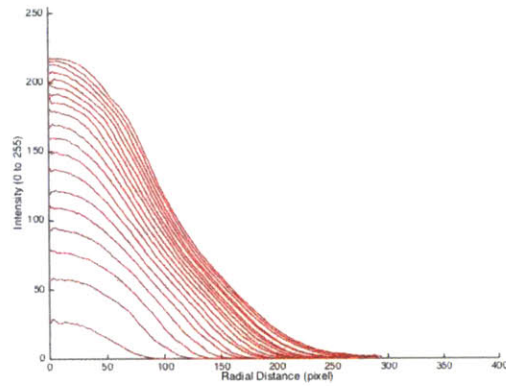
(b)



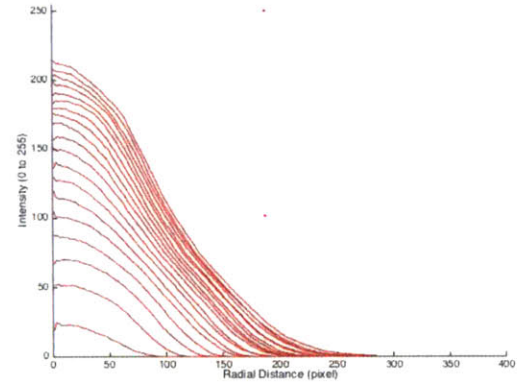
(c)



(d)



(e)



(f)

Figure 5.23 (a - f) Radial distribution of intensity for samples A - F (raw milk) in the 602 nm wavelength band at different LED brightness values

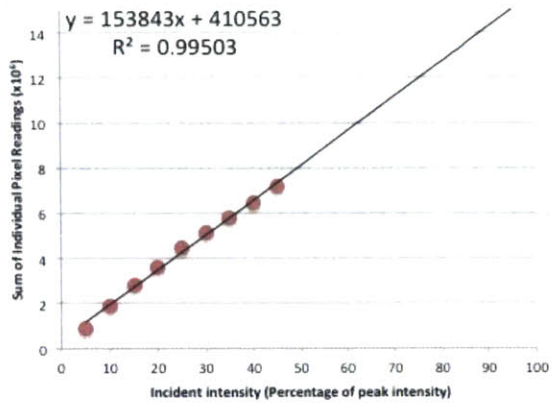
5.4 Results

The experimental procedure, described in Sections 5.1 and 5.2, was repeated for all primary and derived samples, described in Section 5.3. Multiple images were collected for the three wavelength bands (460, 508 and 602 nm) at LED brightness varying from 5% to 95% of peak brightness, in steps of 5%. These images were processed and analyzed to get radial distribution of intensity, and the transmittance and absorbance, and scattering characteristics of each sample. These were compared for different samples while knowing their relative constituent concentrations and physical form, given in Table 5.1. This section gives an overview of the results obtained from the process and the inferences drawn from them. The obtained radial distribution of intensity, transmittance and absorbance, and scattering characteristics are recorded for individual samples in Appendix A.

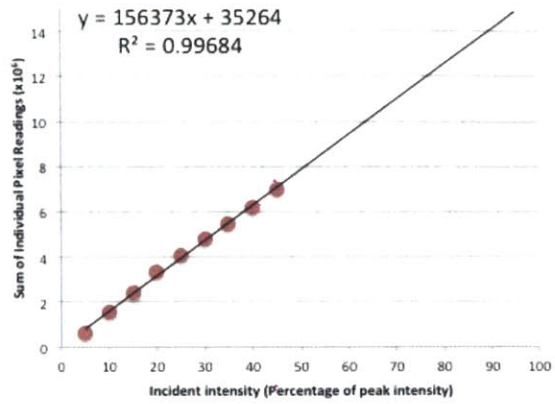
5.4.1 Radial Distribution of Intensity

The radial distribution of intensity was obtained from individual images as described in Section 5.2.2. Figure 5.23 compares the radial distribution of intensity obtained for raw milk samples (A - F) for the 602 nm wavelength band at LED brightness varying from 5% to 95% of peak brightness, in steps of 5% each. The radial distributions for all samples are reported in Appendix A. The samples had similar concentrations of constituents other than fat (Table 5.1). Their effects are hence similar in all. It is evident that absorbance and spread of transmitted radiation increases as fat concentration increases. Effects of multiple scattering are also evident from visual inspection. Images for skim milk (G) samples were always saturated (Fig. A.7), and hence were not used in drawing any transmittance and scattering characteristics of the sample.

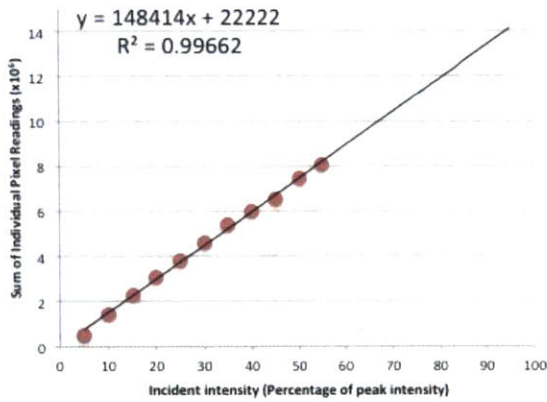
Similar plots were obtained for all samples. Appendix A gives the radial distributions for all samples at the three wavelength bands at different LED brightness values. Images with saturation were rejected and the rest were used for transmittance and scattering quantification.



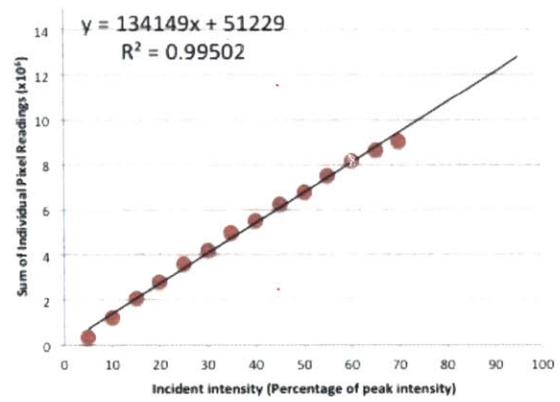
(a)



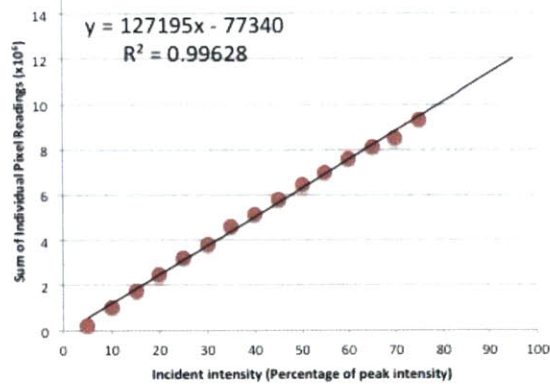
(b)



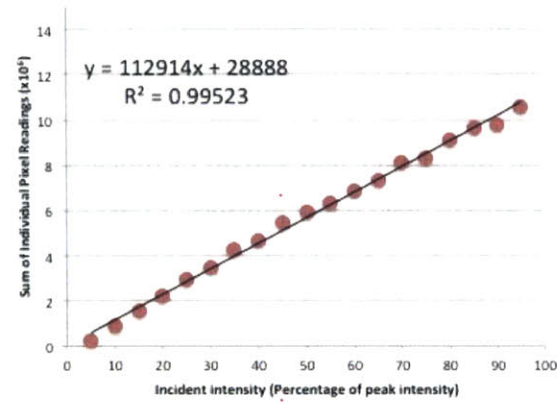
(c)



(d)



(e)



(f)

Figure 5.24 (a - f) Sum of all individual pixel readings for samples A - F (raw milk) in the 602 nm wavelength band at different LED brightness values

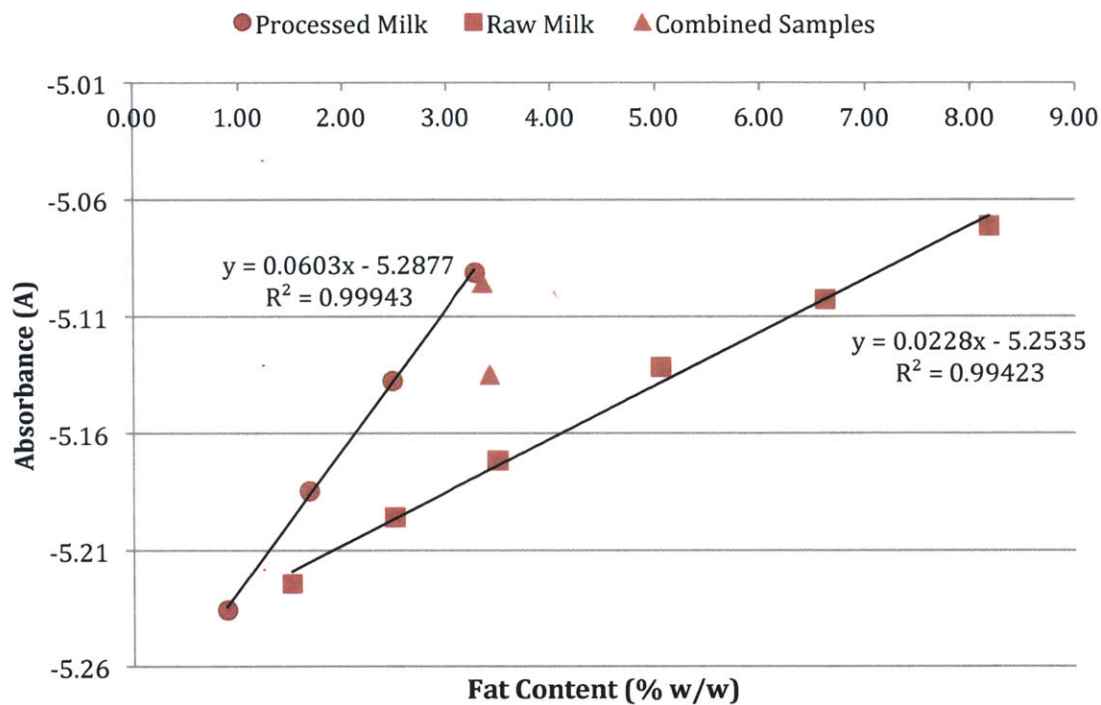
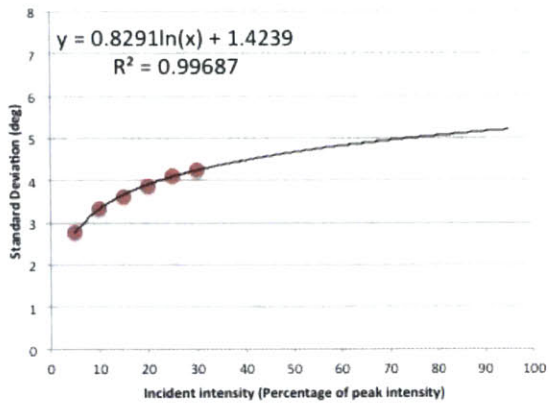


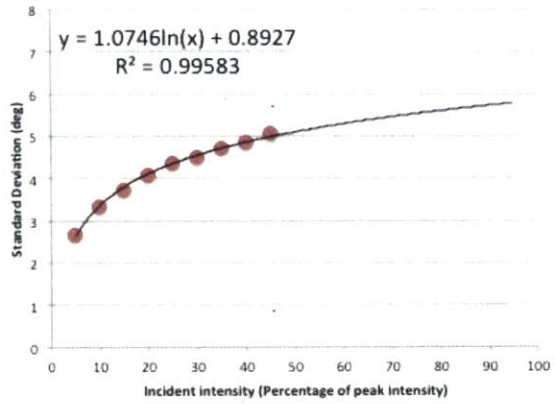
Figure 5.25 Measured Absorbance **A** in the 602 nm wavelength band versus reference fat concentration for all samples

5.4.2 Transmittance and Absorbance

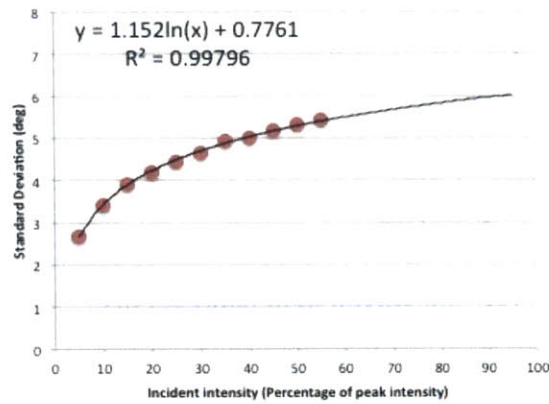
The transmittance and absorbance characteristics of the samples were obtained from individual images as described in Section 5.2.3. Figure 5.24 compares the sum of all pixel readings obtained for raw milk samples (A - F) subjected to the 602 nm wavelength band at different values of LED brightness. The brightness is increased in steps of 5% of peak brightness until the camera saturates. The sums of pixel readings for all samples subjected to the three wavelength bands are reported in Appendix A. The linear relation between the sum and the LED brightness (equivalent to incident intensity) is confirmed by $R^2 > 0.994$ for all samples at all wavelengths. The slope of the best linear fit is equivalent to the sample's transmittance, and the negative logarithm of the same is equivalent to the sample's absorbance. Absorbance for all samples was hence derived from the images. These are compared with reference fat concentration of all samples in Figure 5.25.



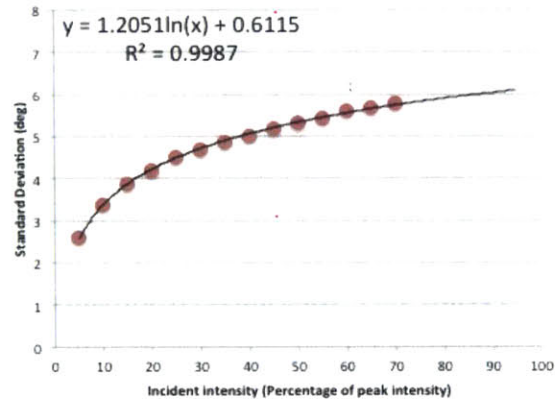
(a)



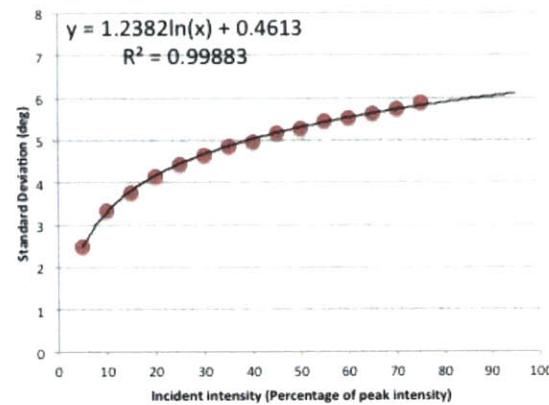
(b)



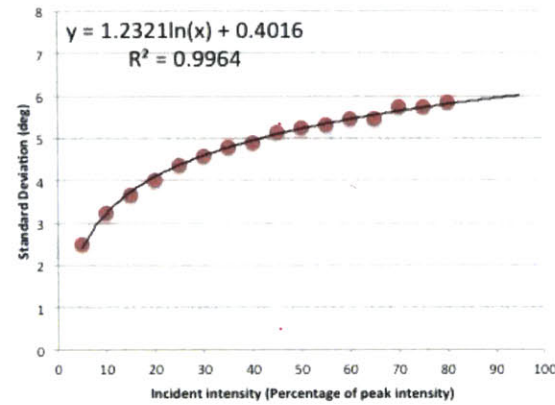
(c)



(d)



(e)



(f)

Figure 5.26 (a - f) Standard deviation of derived BSDF for samples A - F (raw milk) in the 602 nm wavelength band at different LED brightness values

Following inferences are drawn:

- With the proposed instrument and experimental setup, transmittance T and absorbance A of a given sample subjected to a wavelength band can be unequivocally quantified.
- A strong linear relationship between absorbance A and fat concentration c is established with extinction coefficient ϵ as the slope.
- The extinction coefficient ϵ of milk samples is observed to decrease with increasing particle size.
- The extinction coefficient ϵ is dependent on the wavelength of incident radiation, especially for processed milk due to comparable particle sizes.
- The effect of non-fat solids is recorded as the y-intercepts for the two linear fits in Figure 5.25. The similar fit validates the observation.
- From preliminary qualitative examination, it is inferred that both the particle size and non-fat solids can be estimated by using multiple wavelengths.

5.4.3 Scattering

The scattering characteristics of the samples were obtained from individual images as described in Section 5.2.4. Figure 5.26 compares the standard deviation of derived BSDF obtained for raw milk samples (A - F) subjected to the 602 nm wavelength band at different values of LED brightness. The brightness is increased in steps of 5% of peak brightness until the camera saturates. The standard deviations of derived BSDFs for all samples subjected to the three wavelength bands are reported in Appendix A.

The logarithmic relation between standard deviation and percentage of incident intensity is defined by the coefficient of $\log(\text{percentage of incident intensity})$ and intercept on the y (standard deviation). For the purpose of preliminary examination, the coefficient is assumed as a representative of the scattering response of the sample. Figure 5.27 gives the scattering characteristic (coefficient) of different samples in the three wavelength bands.

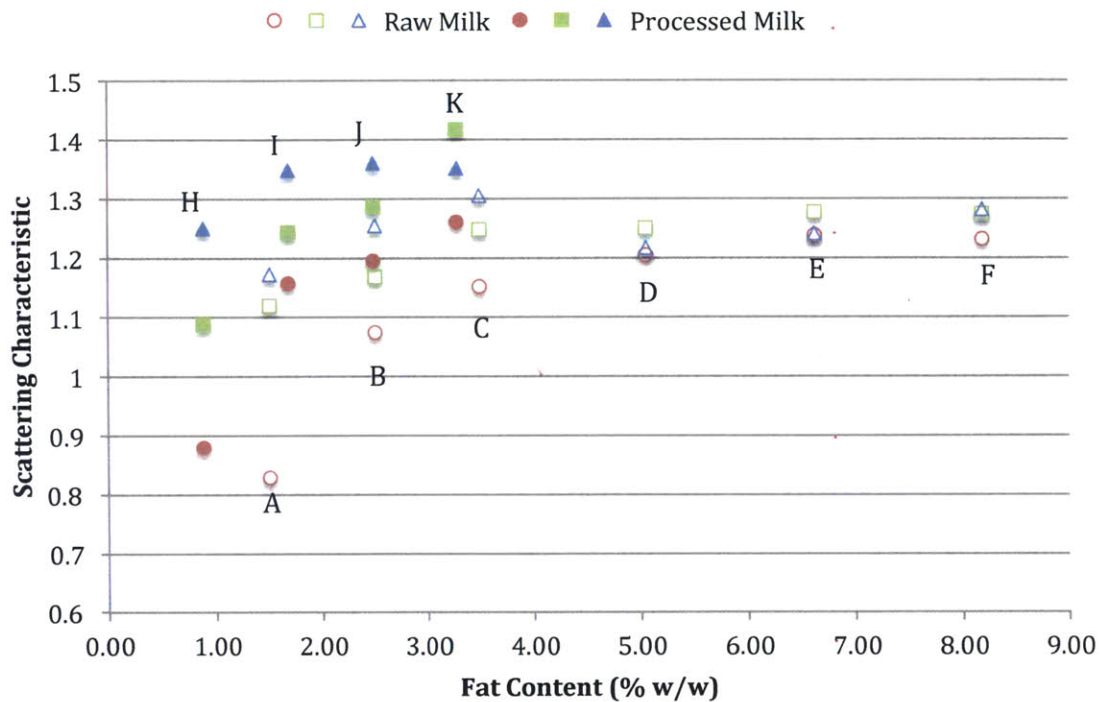


Figure 5.27 Scattering characteristic in the three wavelength bands versus reference fat concentration for raw and processed milk samples

Following inferences are drawn:

- With the proposed instrument and experimental setup, multiple scattering of radiation through a given sample can be suitably quantified. The measurement and analysis process can be improved further for unequivocal quantification.
- The measured scattering characteristic is lower for raw milk samples compared to processed milk samples with similar fat concentrations. This effect can be explained by the larger number of particles in processed milk with same fat concentration.
- The measured scattering characteristic is different for different wavelength bands. It is more pronounced in processed milk samples. This effect can be explained by the smaller globule and casein particle sizes in homogenized milk. The sizes are closer to the 460 nm wavelength band.
- The measured scattering characteristic could potentially be used for quantifying particle size in unknown milk samples.

Table 5.2 Samples, with reference fat concentration, used for spectrophotometric measurements

Sample	Fat (% w/w)	Nature of Sample
A	1.51	Raw
C	3.49	Raw
C + F	5.84	Raw
F	8.18	Raw
G	0.08	Processed
I	1.68	Processed
K	3.28	Processed
K + C	3.39	Combined

5.4.4 Comparison with Conventional Spectrophotometry

The experimental results were validated by comparison with results obtained using a conventional spectrophotometer. A smaller set of samples was examined on a spectrophotometer. Table 5.2 lists the samples used, preserving the existing nomenclature. Samples were diluted with 99 parts of deionized water for every part of the sample. The spectrophotometer measured a sample's absorbance (effectively extinction coefficient) at wavelengths varying between 300 nm and 1100 nm. The path length for all samples was chosen as 10 mm.

Figure 5.28 gives the measured absorbance versus wavelength for samples of raw milk (A - F). Figure 5.29 gives the measured absorbance versus wavelength for samples of processed milk (G - K). Figure 5.30 gives the measured absorbance versus wavelength for the samples K, K+C and C, representing the combined set.

The absorbance in visible region is more level in raw milk than in processed milk. Processed milk has higher absorbance at smaller wavelengths. This is potentially an artifact of the scattering of light by particles of comparable size in processed milk.

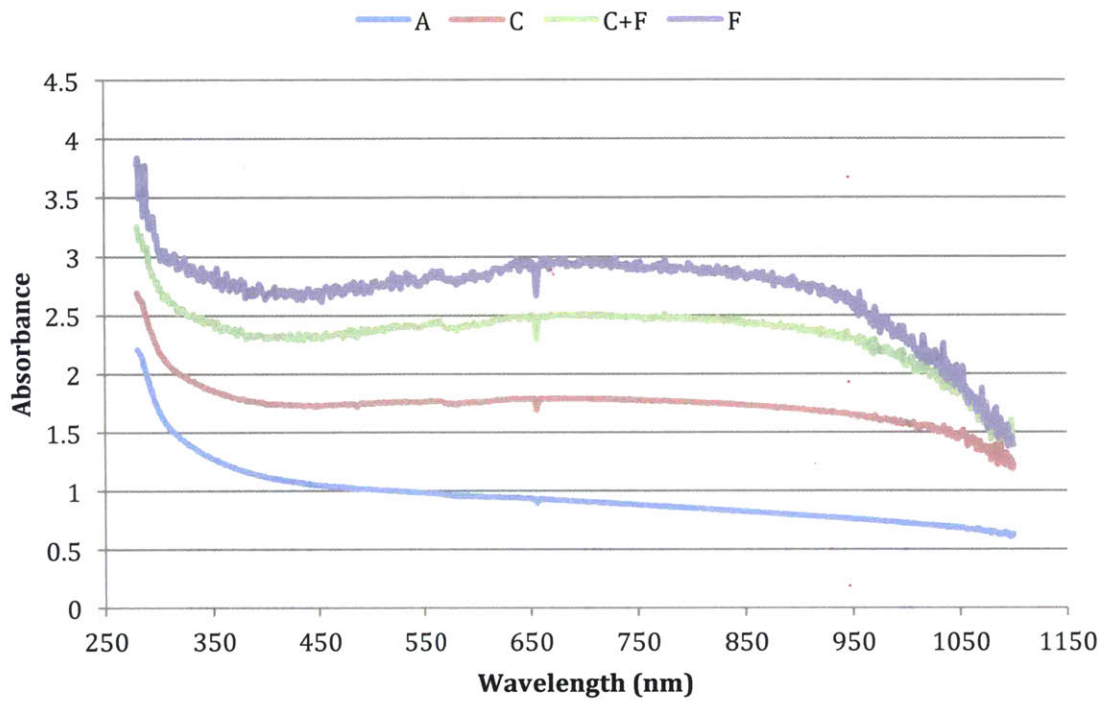


Figure 5.28 Absorbance for raw milk samples

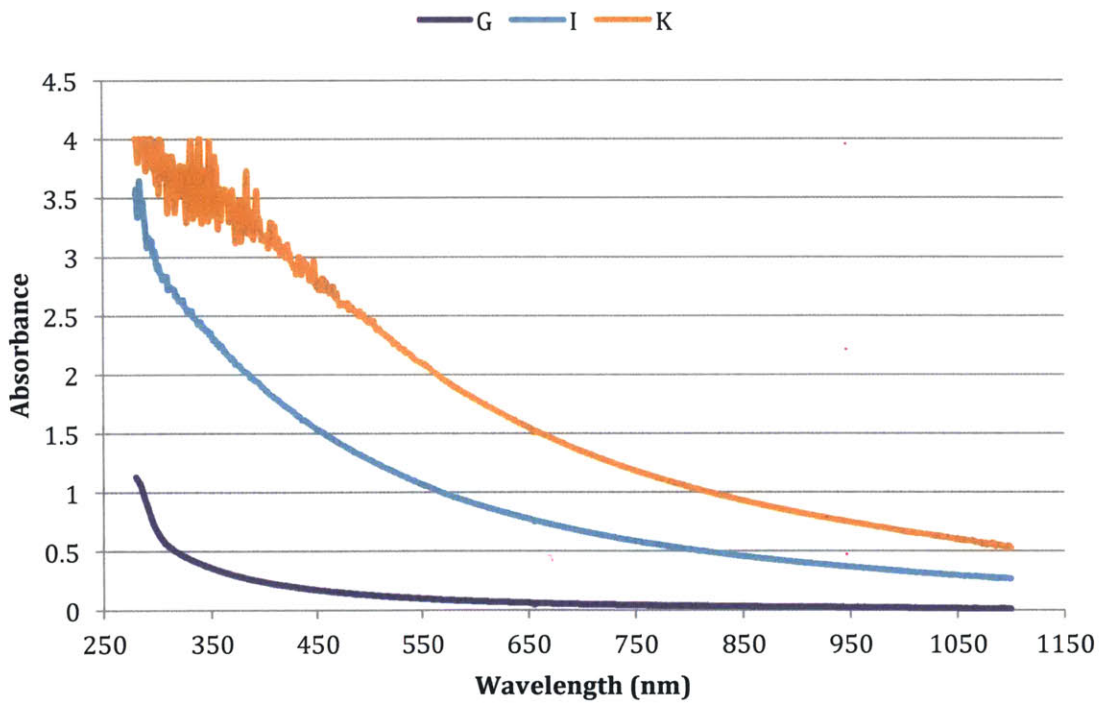


Figure 5.29 Absorbance for processed milk samples

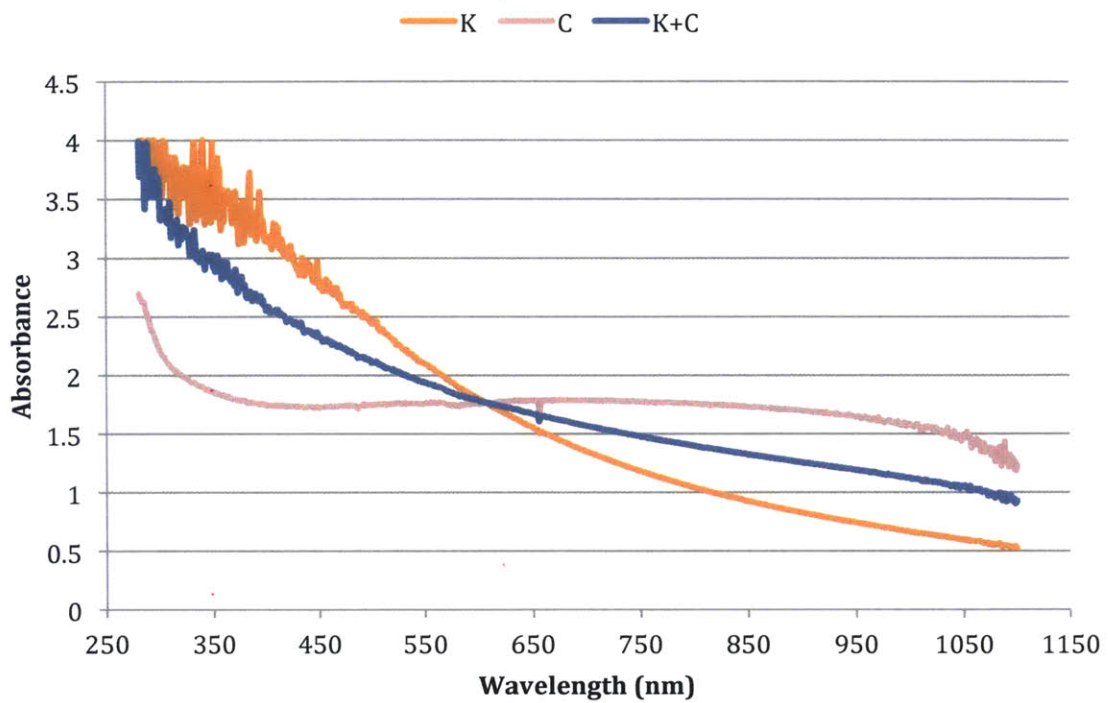


Figure 5.30 Absorbance for combined milk samples

The present results cannot be directly related to results from spectrophotometry due to unknown peak brightness values for different wavelength bands. However, preliminary qualitative examination indicates a strong relation between conventional spectrophotometry and the measurements made using the proposed instrument and the field-level prototype.

Chapter 6

CONCLUSIONS

The presented research is a continuing effort towards development of analytical technologies and methods for the dairy industry, specifically in India, to improve product quality, prevent wastage and spoilage, and eventually benefit consumer health as well as industry economics. This thesis captured the project at a point where key technologies and systems have been proposed and initial concepts have been successfully proven in practice.

Following a user-centric product design and development approach, significant thrust has been given to repeated interaction with various stakeholders in the industry. Interactions with a diverse set of stakeholders, personal observations and findings through literature have been leveraged in identifying needs and opportunities in the industry. Specific points of intervention at the various nodes in the dairy supply chain have been identified and classified. “Point of Procurement” applications have been chosen as primary target application of any product and system developed due to strong potential for impact, commercial viability and technical feasibility.

Various embodiments of analytical instruments ranging from in-line instruments for process monitoring and control to handheld instruments for rapid analysis have been proposed. A major component of the work has been the development of the technology that forms the core of the proposed solutions. A novel method of UV-Vis spectroscopic analysis with both absorbance and scattering measurements has been hypothesized as a potential technology for the instruments.

The proposed method, which uses LEDs as discrete light sources and a digital camera as an image sensor, has been experimentally tested and validated. The results obtained are encouraging for further development and eventual commercialization.

6.1 Proven Hypotheses

The proposed analytical method was divided into a set of hypotheses for sequential experimentation and development. These were listed in Section 4.2.4. Experiments conducted, as described in Chapter 5, were directed towards testing these hypotheses. Based on the results obtained, it is concluded that the following hypotheses are valid:

1. A CMOS camera (image sensor combined with a focusing lens) may be used to observe and quantify transmittance of light passing through a sample, similar to that presently done using photodiodes.
2. A CMOS camera may additionally be used to observe and quantify the scattering of light passing through a sample without changing the setup used to quantify transmittance.
3. LEDs may be used as light sources to quantify transmittance and scattering, as hypothesized in 1 and 2 above, at discrete wavelengths.
4. Transmittance and scattering measurements may be performed, as hypothesized in 1-3 above, for samples of milk without dilution, sonication or homogenization.
5. Fat content in samples of milk with the same fat globule size distribution may be quantified using transmittance and scattering measurements on milk, as hypothesized in 1-4 above.

Further experiments and testing are needed for drawing conclusions on the validity of the following hypotheses:

6. Samples of milk with different fat globule size distributions may be distinguished using transmittance and scattering measurements on milk, as hypothesized in 1-4 above.
7. Solid Non-Fat (SNF) content in samples of milk may be quantified using transmittance and scattering measurements on milk, as hypothesized in 1-4 above.

8. Other analytes in milk, specifically lactose, casein protein, whey protein, somatic cells etc., may be selectively quantified using the proposed method with additional wavelengths or multiple sensors.

6.2 Minimum Viable Product

In the course of the project three major classes of embodiments of an instrument have been proposed, namely standalone, inline and portable (Section 4.1.2). Although the developed spectrophotometric method for characterization of milk may be translated into any of these embodiments, the most tractable embodiment is that of the standalone instrument. The embodiment presents the least technological challenges in development. Compared to other embodiments, it should be easier to operate, maintain and calibrate. At present, standalone instruments are extensively used in the dairy industry in India, and adoption of a similar form factor may be faster compared to that of other embodiments.

Functional requirements of a minimum viable product were described in Table 4.1. In summary, an instrument that can distinguish and quantify fat and solid non-fat in a rapid and repeatable manner offers significant value to the dairy industry, to be able to justify a case for switching to the proposed technology. The developed technology is exciting as it may eventually be used to quantify several other parameters that have an economic value for the industry.

6.3 Future Work

This thesis captured the project near the conclusion of a critical technology development phase. Of the proposed hypotheses, the most critical ones have been validated. Results are encouraging for further experimentation and theoretical validation of the proposed technology. Thorough experimentation is required for unequivocal characterization of any developed instrument prior to commercialization. Next steps in the project are development of a minimum viable product followed by field trials and user validation. The ongoing efforts are focused on eventually creating a strong and positive impact.

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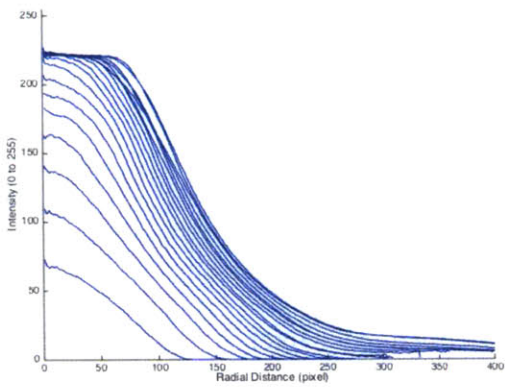
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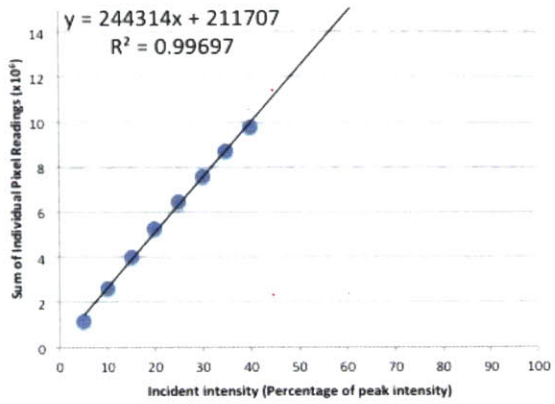
Appendix A

DETAILED RESULTS

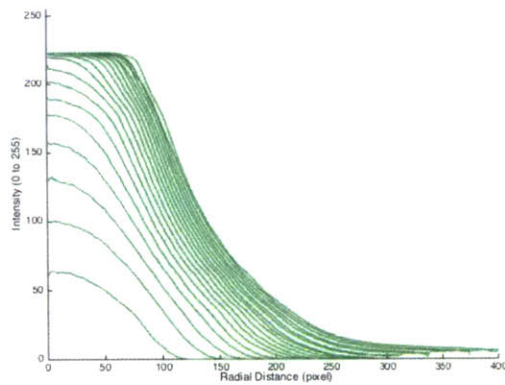
A record of the detailed results of experiments, described in Chapter 5, is provided here. Section 5.4 provides an overview of these results and the inferences drawn from them. Figures A.1 to A.13 give the radial distributions of intensity, transmittance, and scattering characteristics derived from individual images for each sample (A - M respectively).



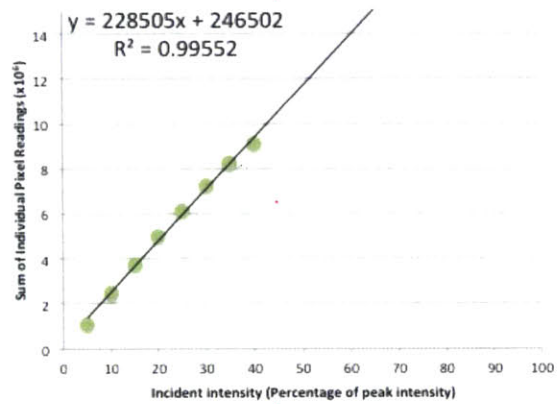
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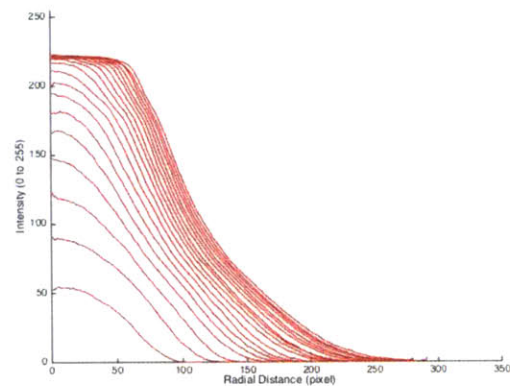
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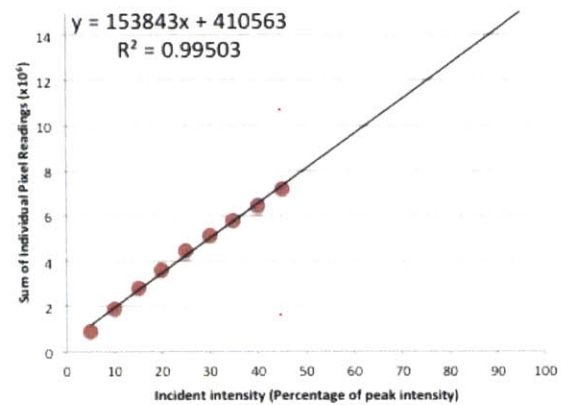
(b)



(e)

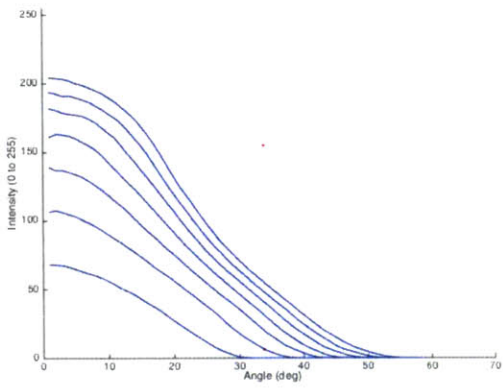


(c)

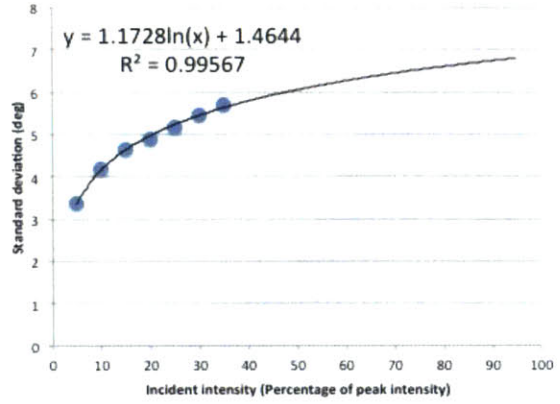


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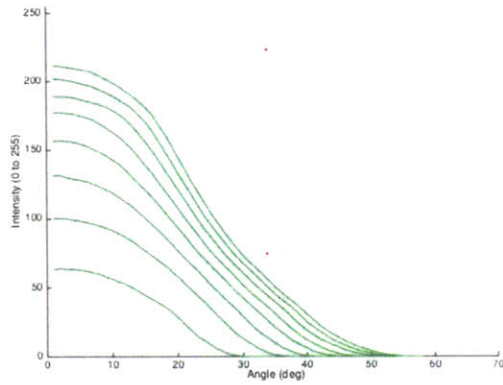
Figure A.1 Detailed results for Sample A for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (a - c) Radial Distribution of Intensity; (d - f) Sum of individual pixel readings, slope indicating transmittance



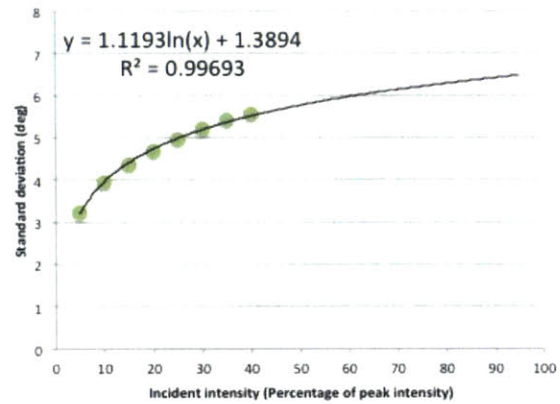
(g)



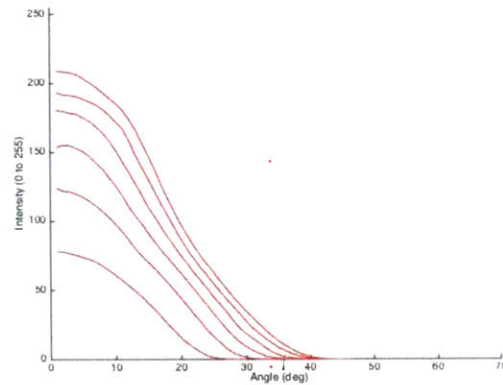
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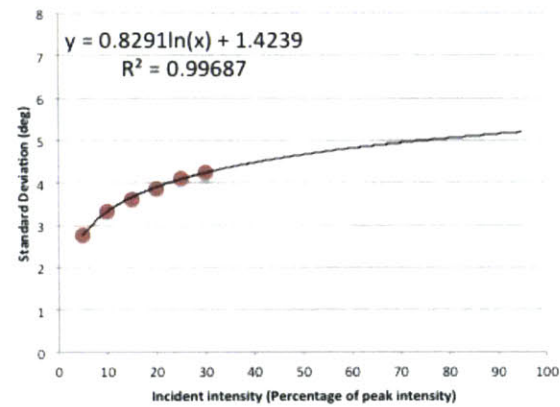
(h)



(k)

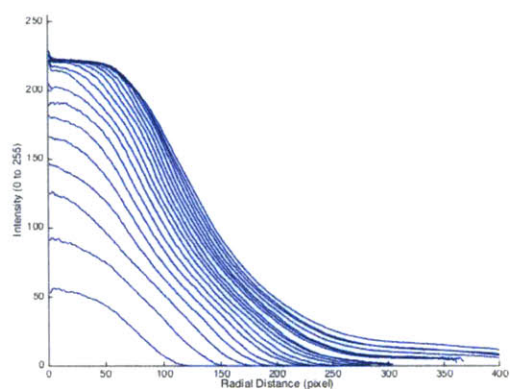


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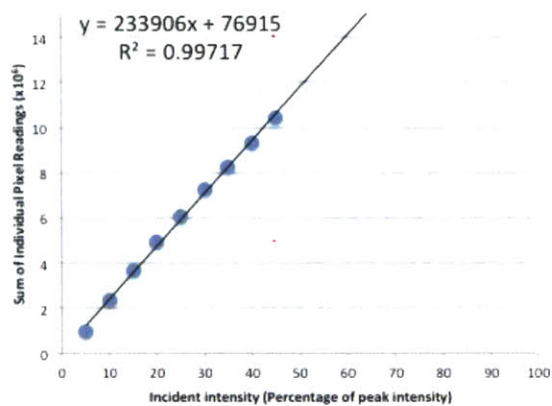


(l)

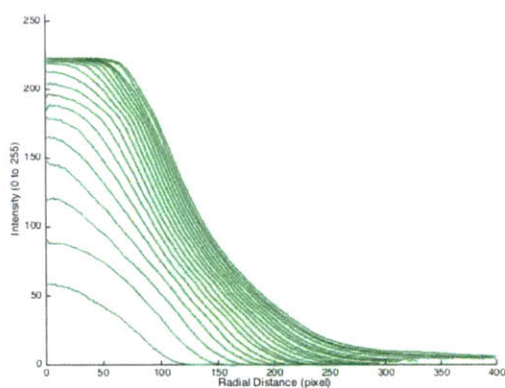
Figure A.1 Detailed results for Sample A for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (g - i) derived BSDF; (j - l) standard deviation of derived BSDF



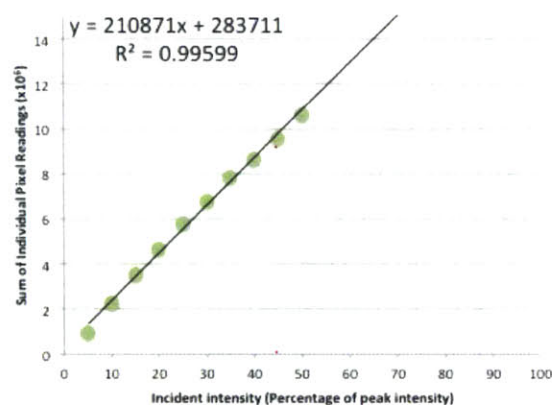
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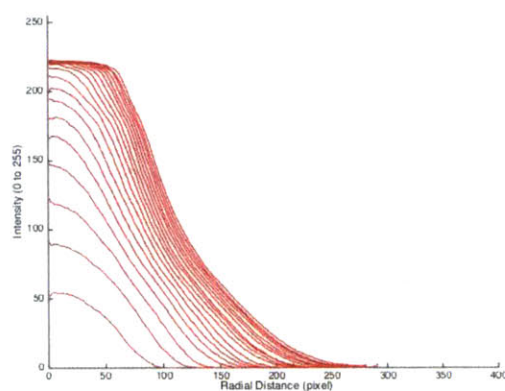
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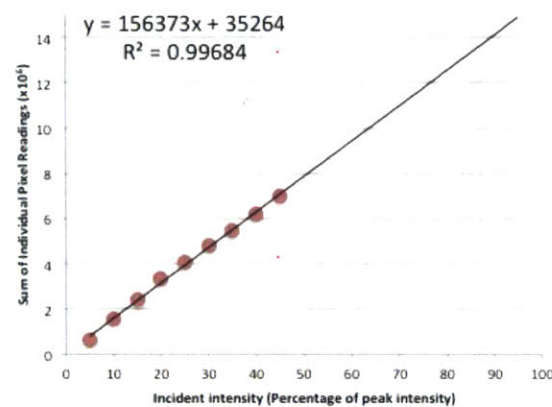
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(e)

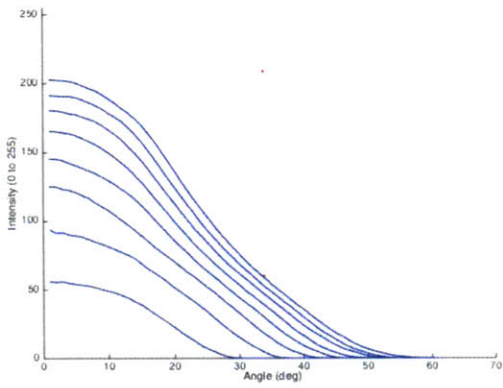


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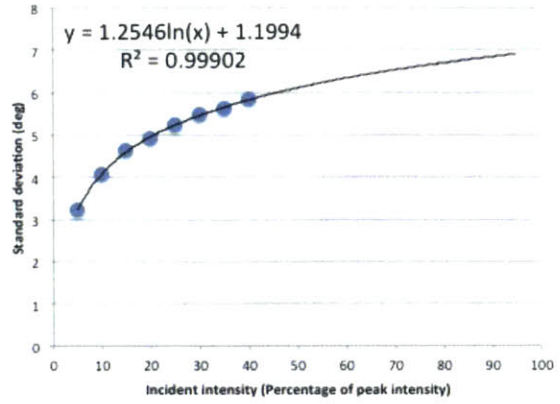


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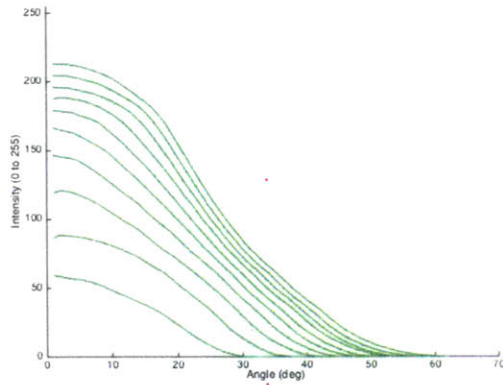
Figure A.2 Detailed results for Sample B for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (a - c) Radial Distribution of Intensity; (d - f) Sum of individual pixel readings, slope indicating transmittance



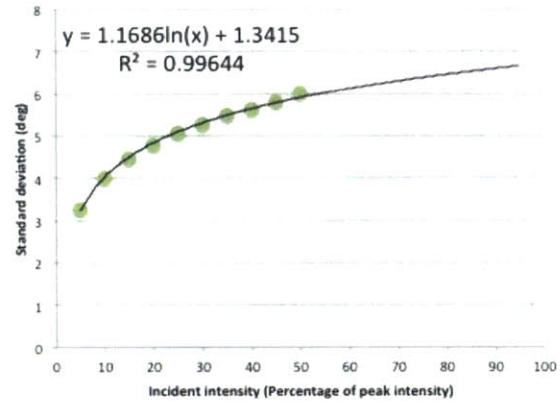
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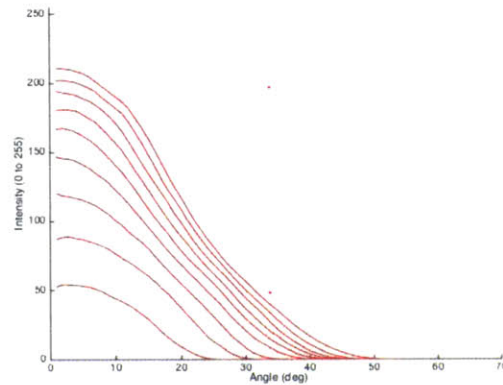
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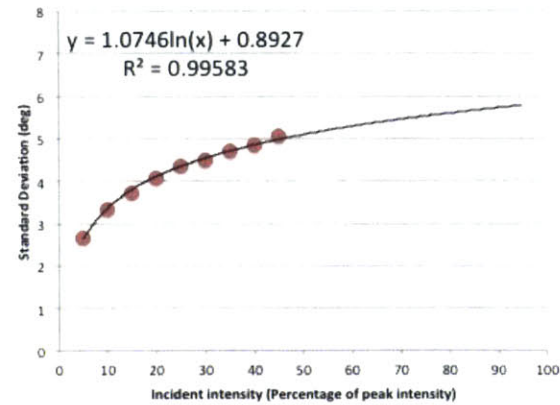
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(k)

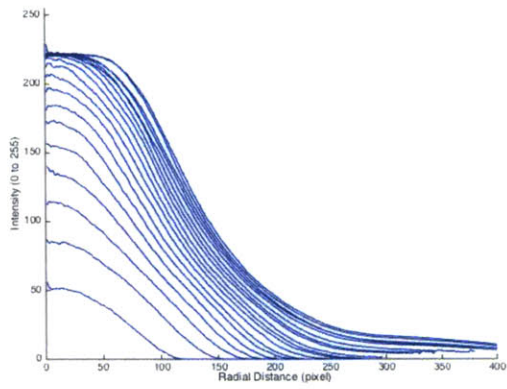


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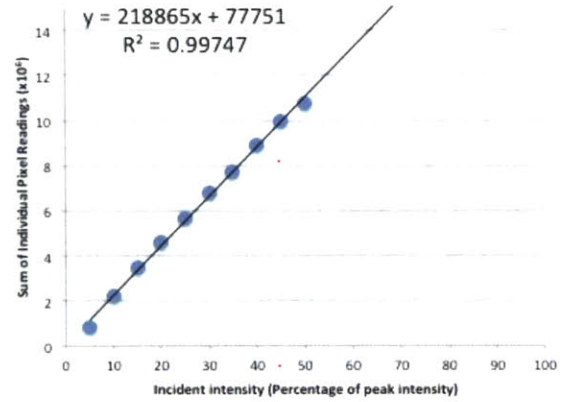


(l)

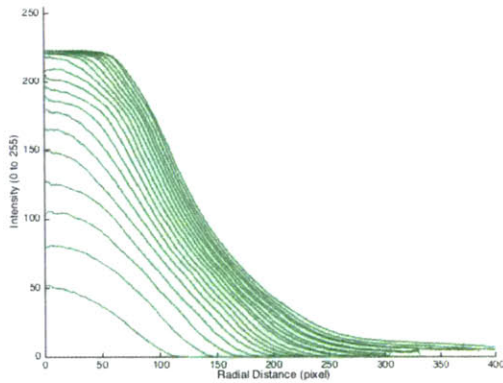
Figure A.2 Detailed results for Sample B for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (g - i) derived BSDF; (j - l) standard deviation of derived BSDF



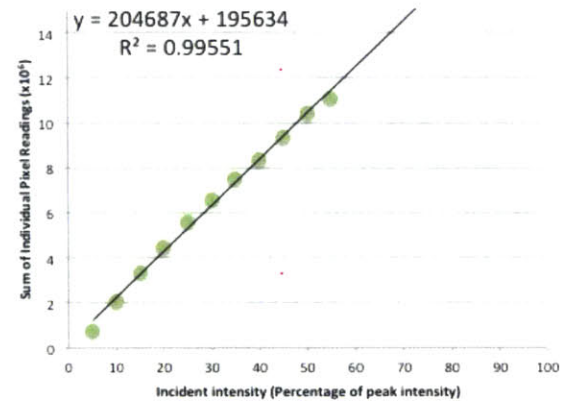
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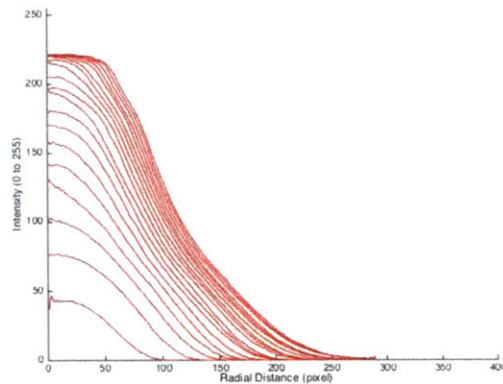
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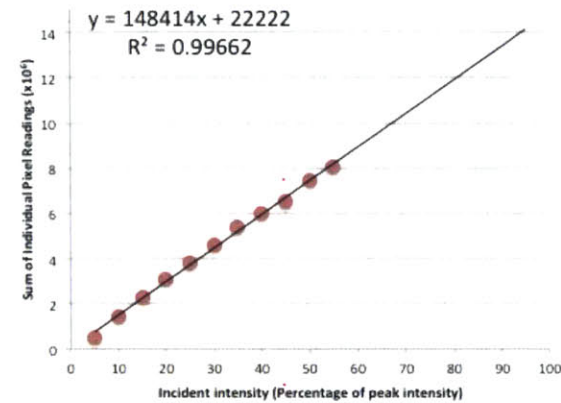
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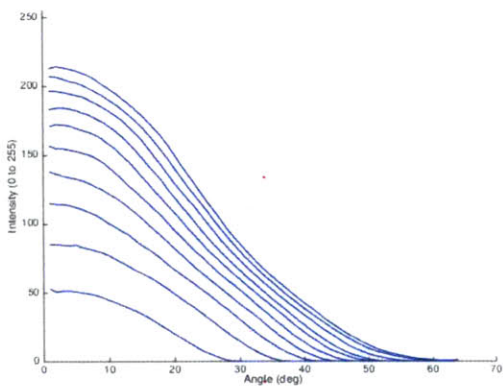


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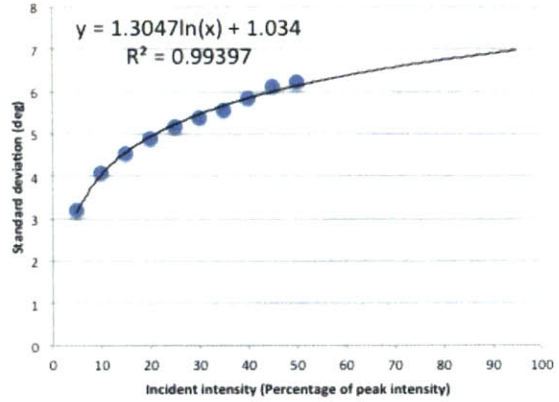


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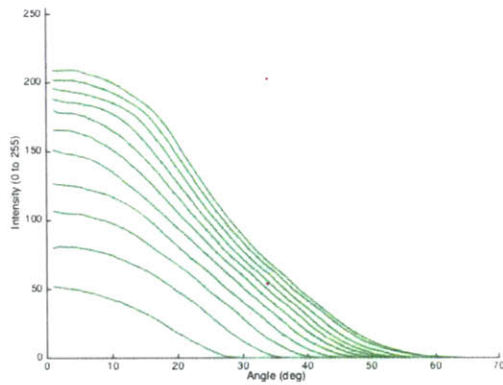
Figure A.3 Detailed results for Sample C for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (a - c) Radial Distribution of Intensity; (d - f) Sum of individual pixel readings, slope indicating transmittance



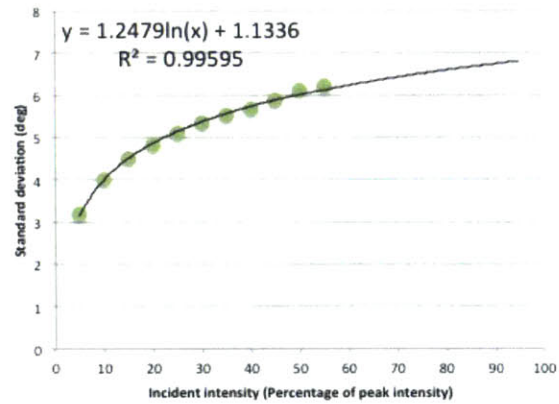
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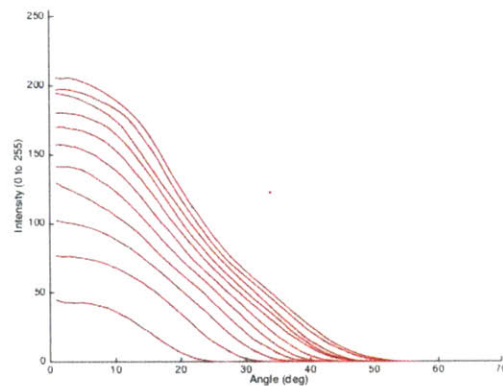
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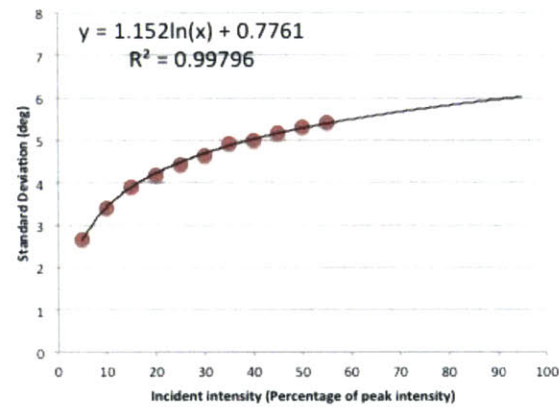
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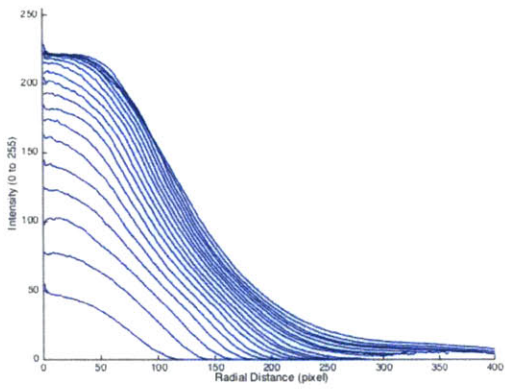


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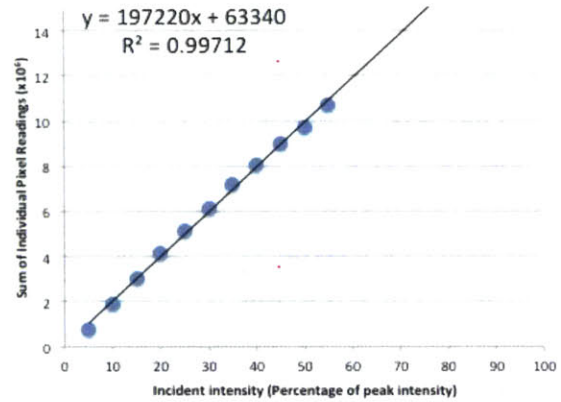


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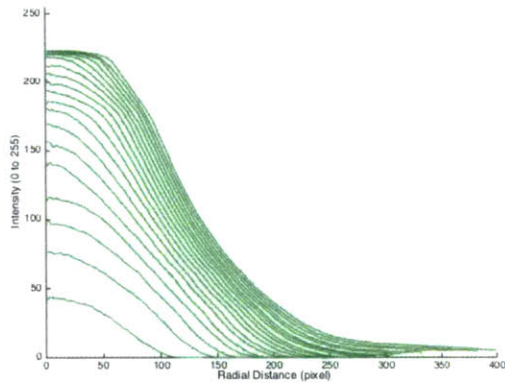
Figure A.3 Detailed results for Sample C for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (g - i) derived BSDF; (j - l) standard deviation of derived BSDF



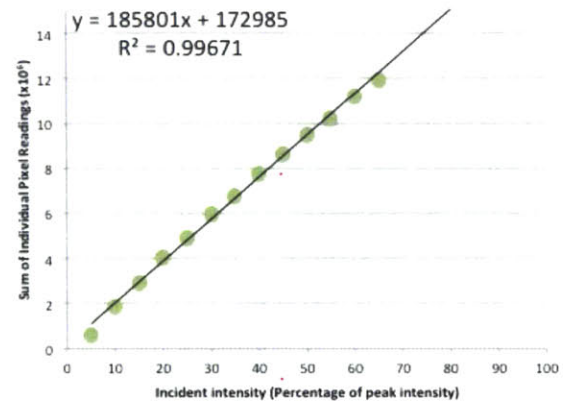
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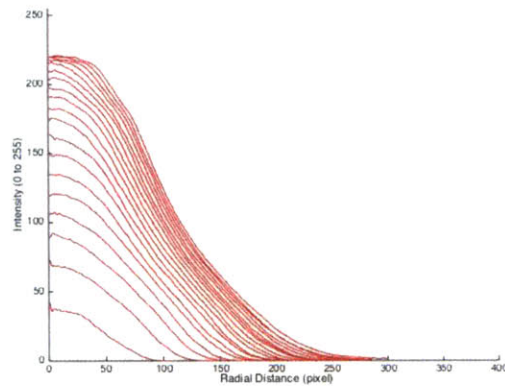
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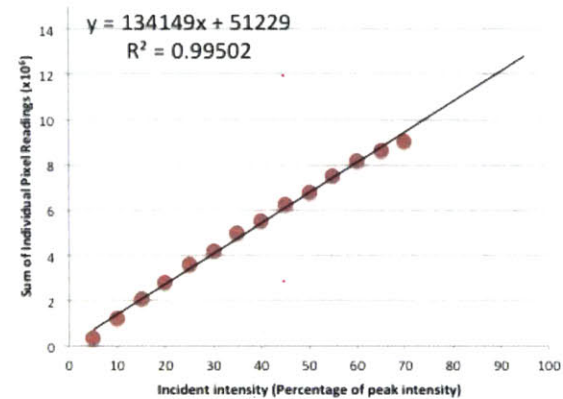
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(e)

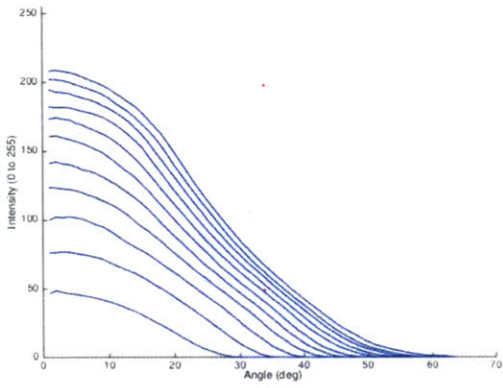


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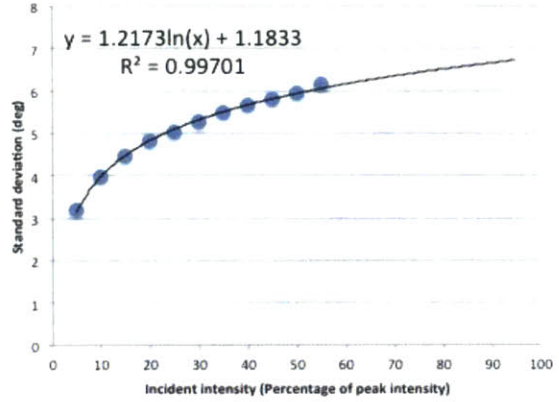


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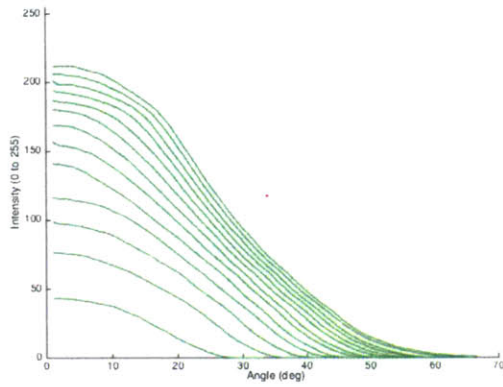
Figure A.4 Detailed results for Sample D for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (a - c) Radial Distribution of Intensity; (d - f) Sum of individual pixel readings, slope indicating transmittance



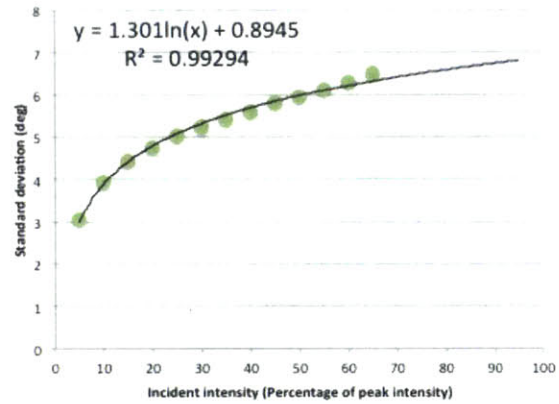
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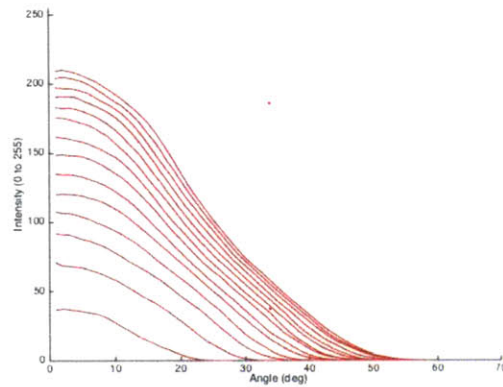
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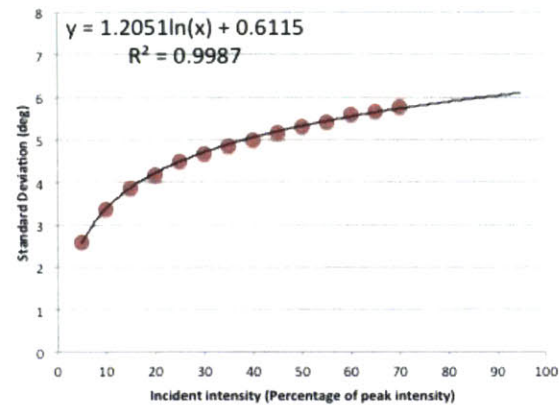
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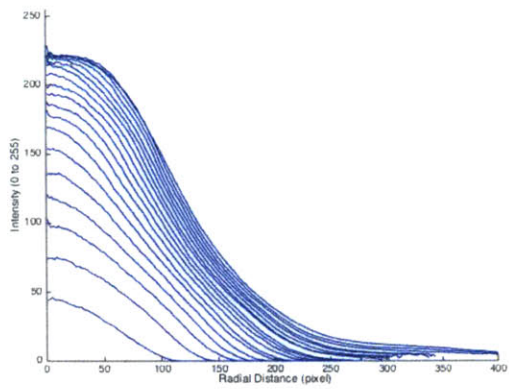


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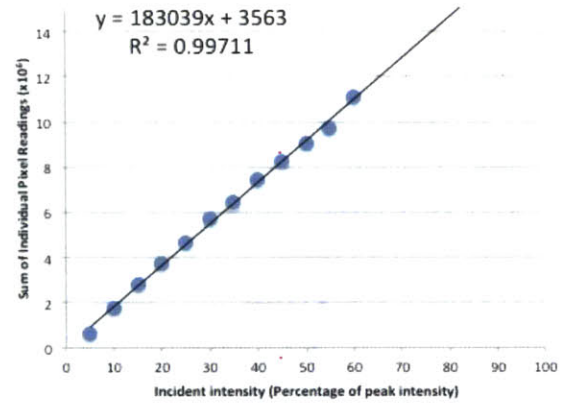


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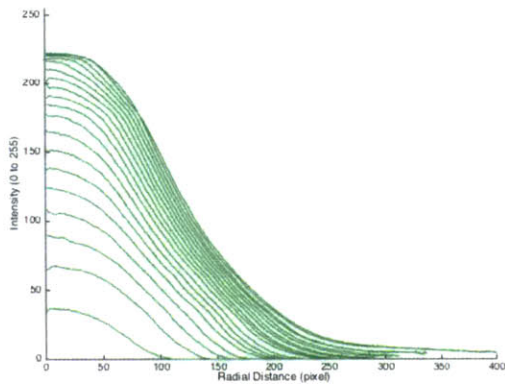
Figure A.4 Detailed results for Sample D for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (g - i) derived BSDF; (j - l) standard deviation of derived BSDF



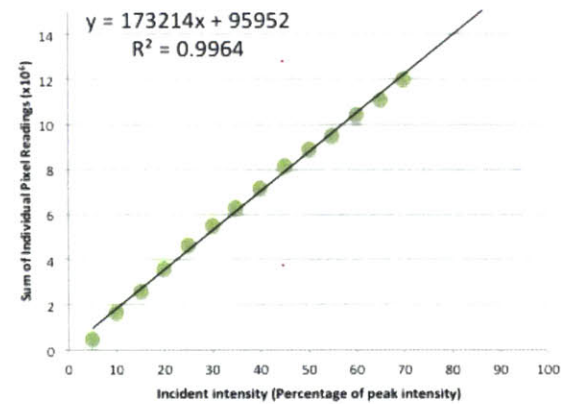
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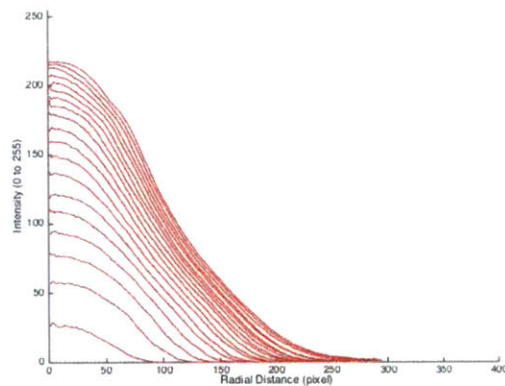
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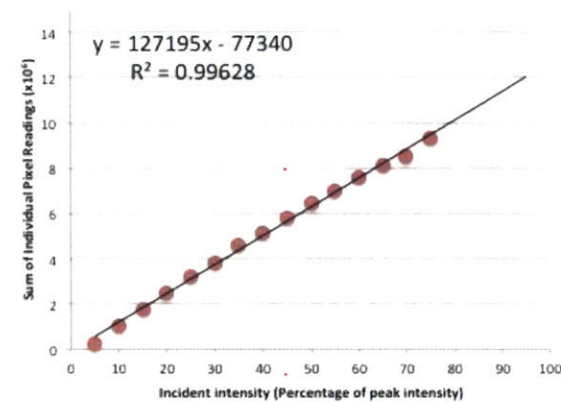
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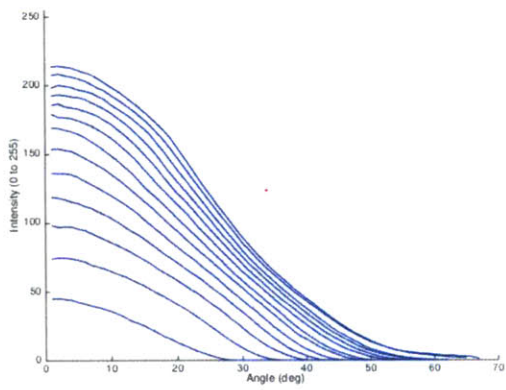


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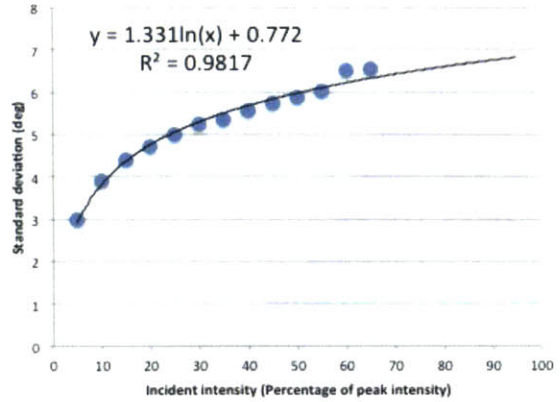


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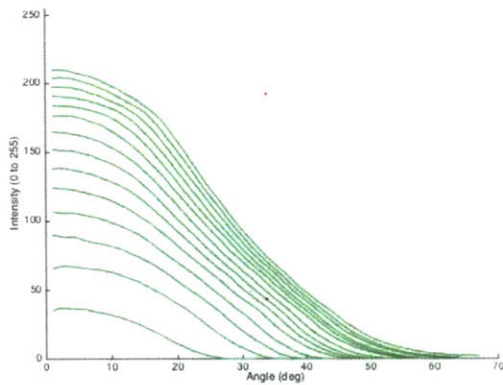
Figure A.5 Detailed results for Sample E for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (a - c) Radial Distribution of Intensity; (d - f) Sum of individual pixel readings, slope indicating transmittance



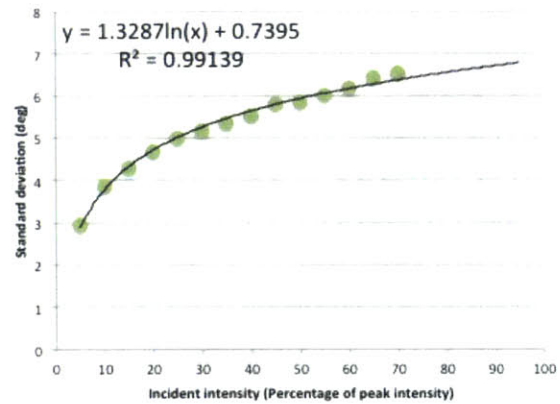
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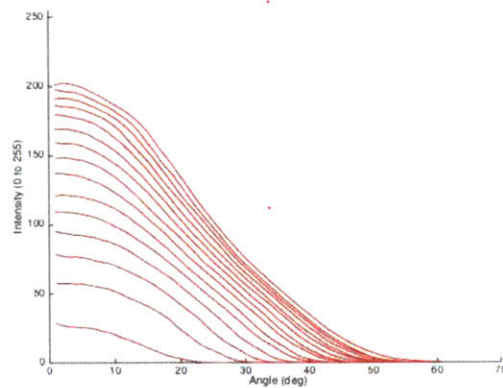
(j)



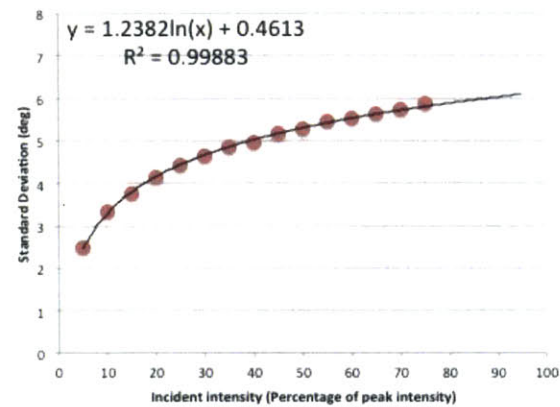
(h)



(k)

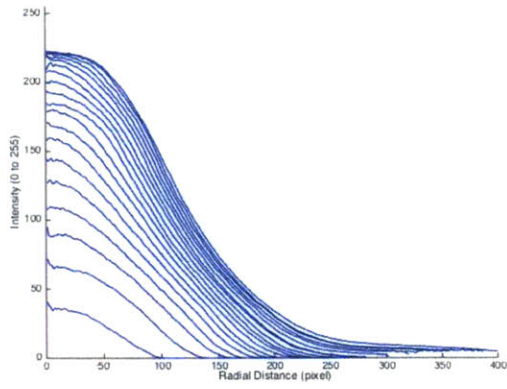


(i)

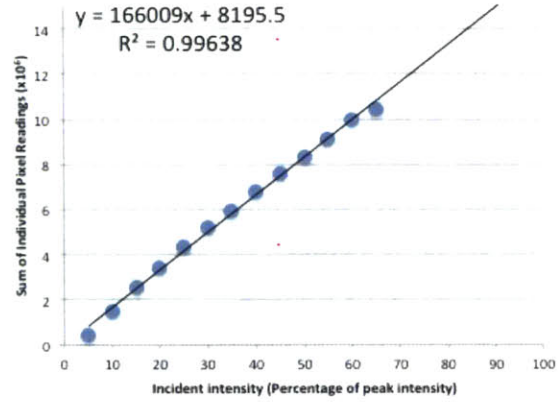


(l)

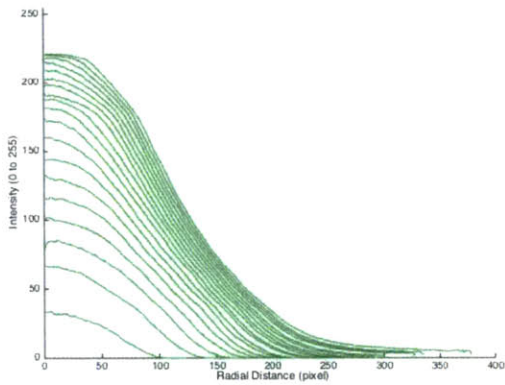
Figure A.5 Detailed results for Sample E for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (g - i) derived BSDF; (j - l) standard deviation of derived BSDF



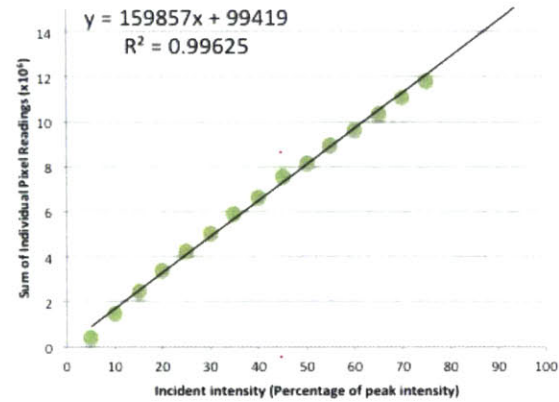
(a)



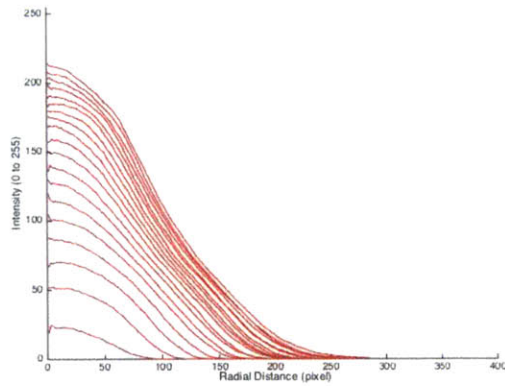
(d)



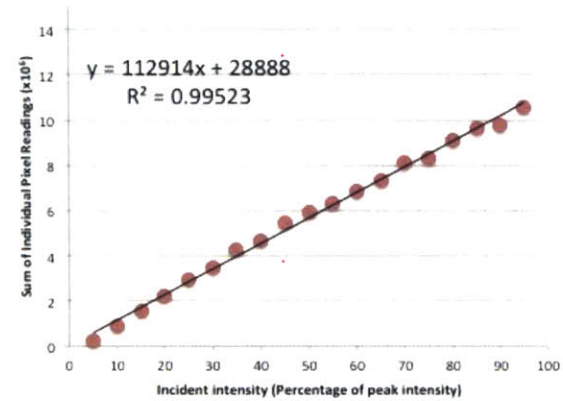
(b)



(e)

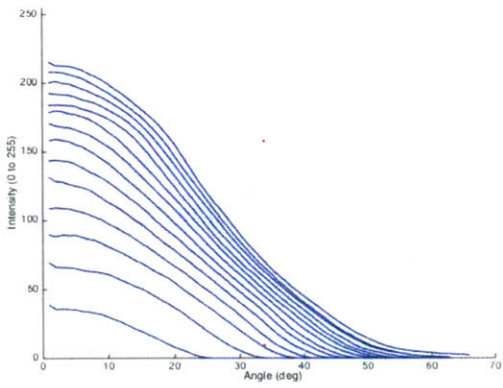


(c)

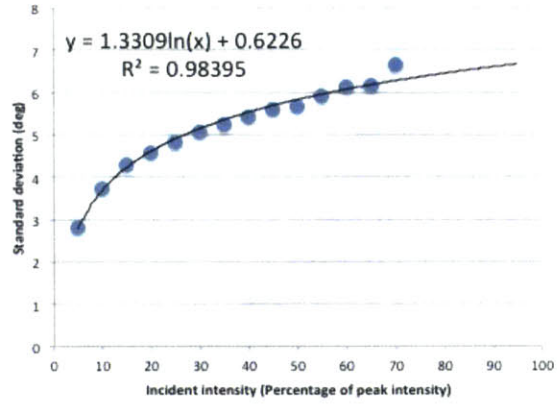


(f)

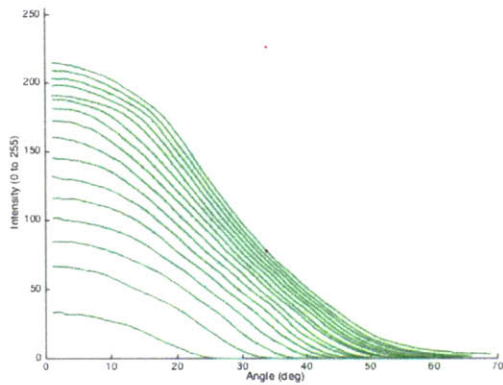
Figure A.6 Detailed results for Sample F for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (a - c) Radial Distribution of Intensity; (d - f) Sum of individual pixel readings, slope indicating transmittance



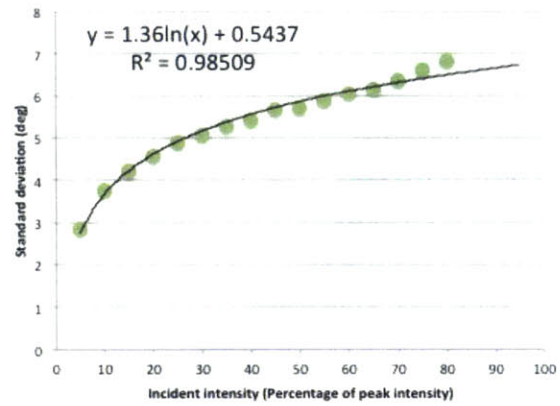
(g)



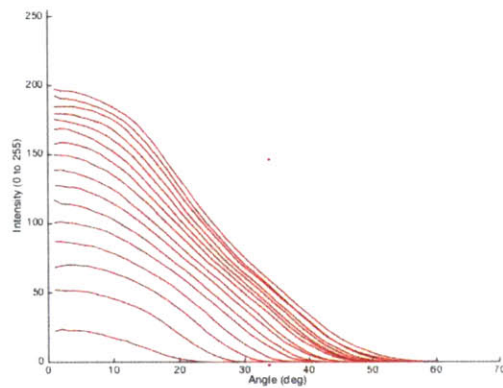
(j)



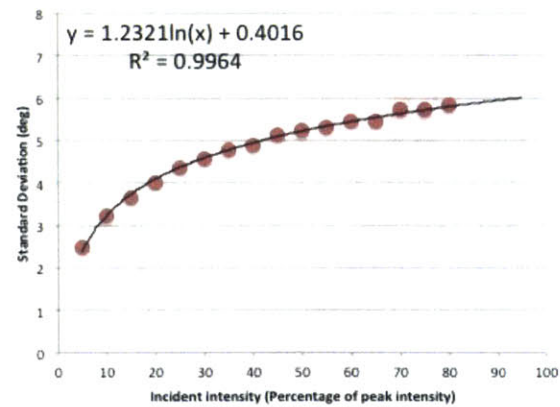
(h)



(k)

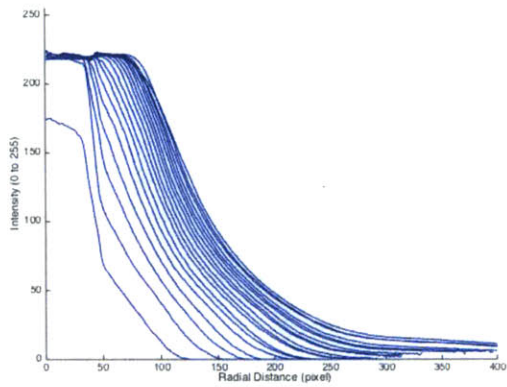


(i)



(l)

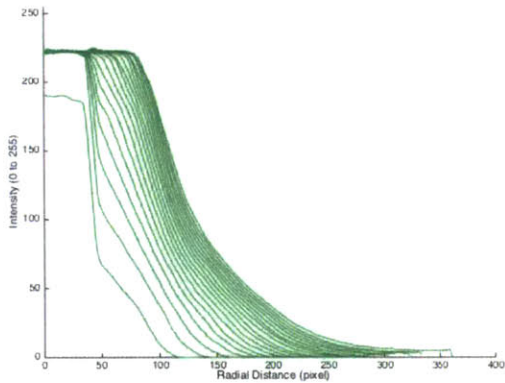
Figure A.6 Detailed results for Sample F for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (g - i) derived BSDF; (j - l) standard deviation of derived BSDF



(a)

All images rejected
No results obtained

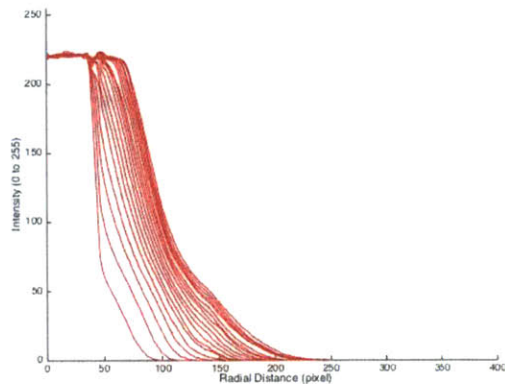
(d)



(b)

All images rejected
No results obtained

(e)



(c)

All images rejected
No results obtained

(f)

Figure A.7 Detailed results for Sample G for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (a - c) Radial Distribution of Intensity; (d - f) Sum of individual pixel readings, no results obtained

All images rejected
No results obtained

(g)

All images rejected
No results obtained

(j)

All images rejected
No results obtained

(h)

All images rejected
No results obtained

(k)

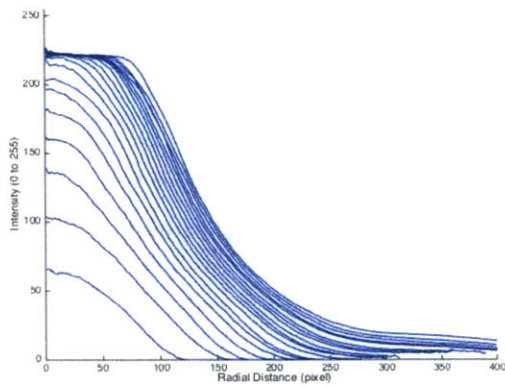
All images rejected
No results obtained

(i)

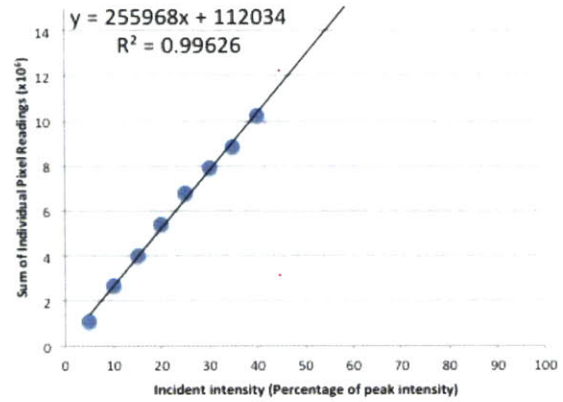
All images rejected
No results obtained

(l)

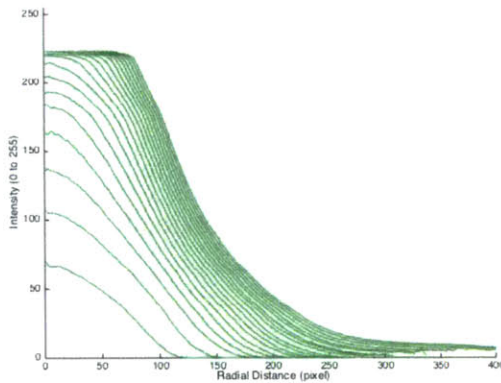
Figure A.7 Detailed results for Sample G for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (g - i) derived BSDF, no results obtained; (j - l) standard deviation of derived BSDF, no results obtained



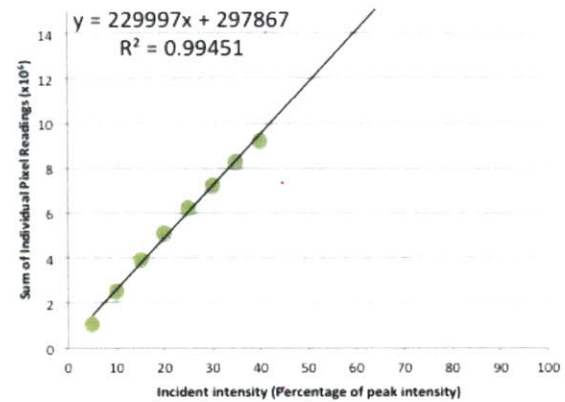
(a)



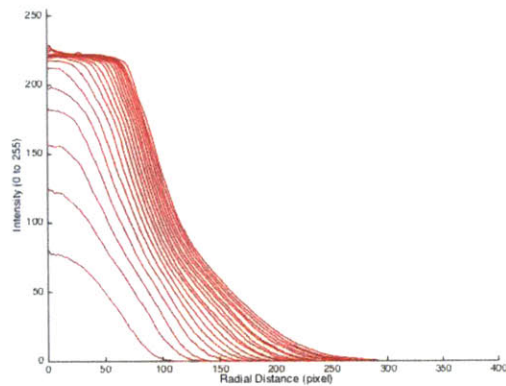
(d)



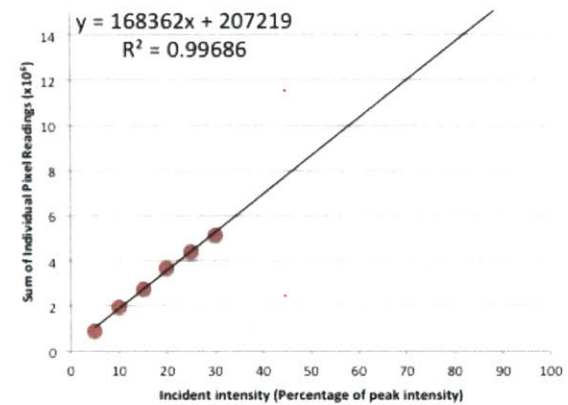
(b)



(e)

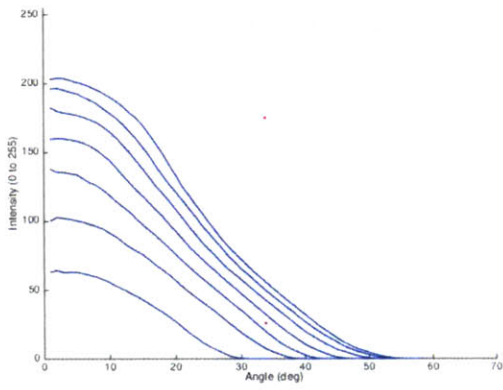


(c)

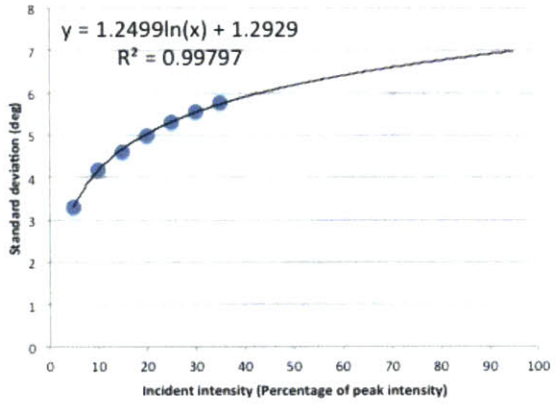


(f)

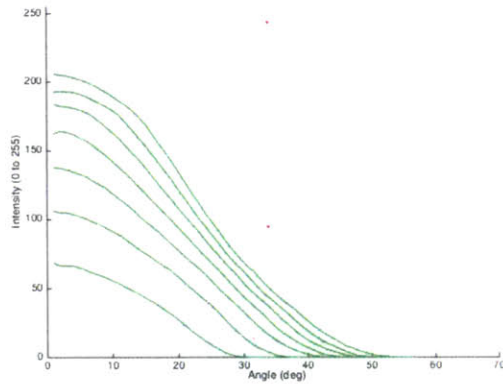
Figure A.8 Detailed results for Sample H for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (a - c) Radial Distribution of Intensity; (d - f) Sum of individual pixel readings, slope indicating transmittance



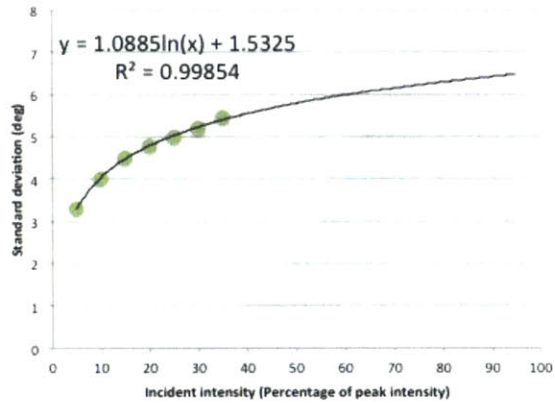
(g)



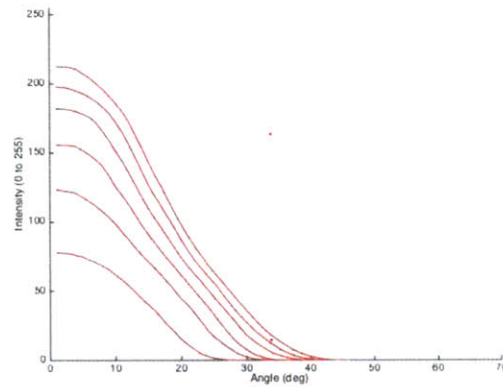
(j)



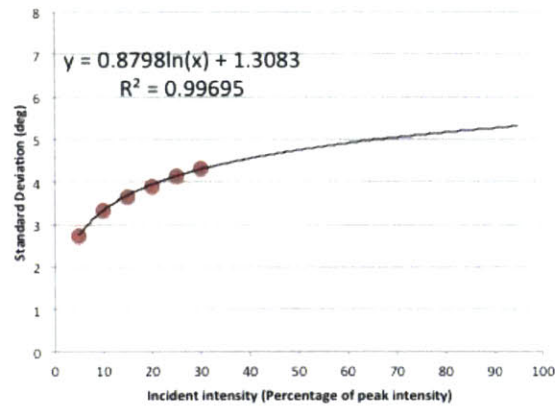
(h)



(k)

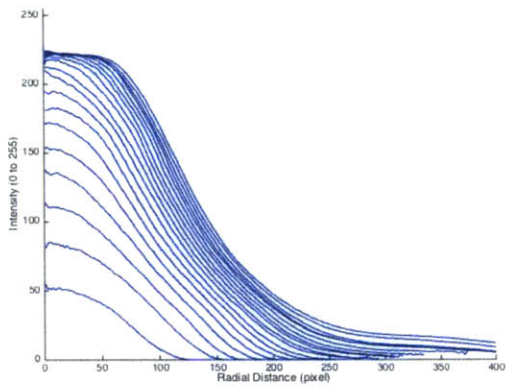


(i)

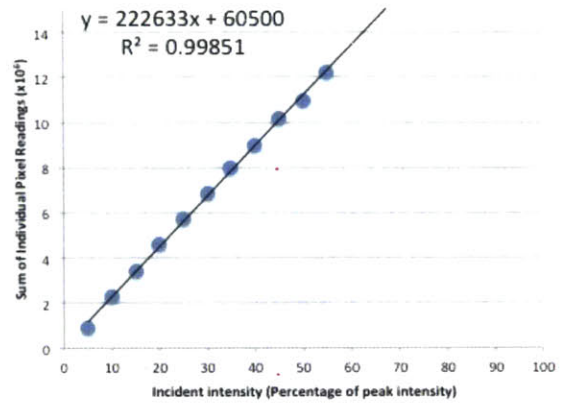


(l)

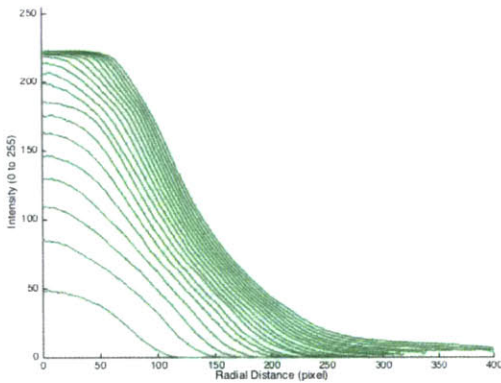
Figure A.8 Detailed results for Sample H for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (g - i) derived BSDF; (j - l) standard deviation of derived BSDF



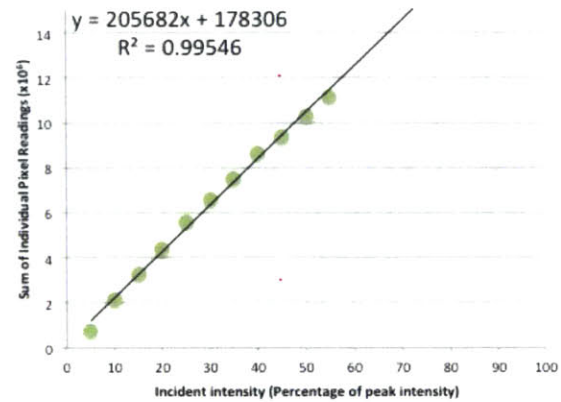
(a)



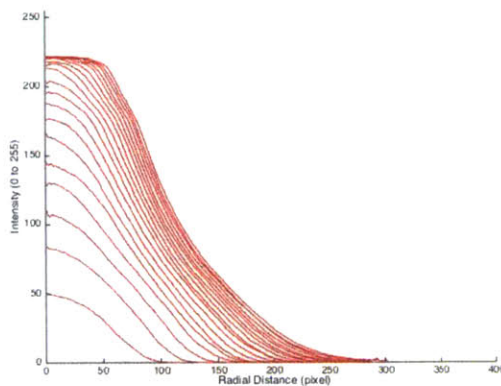
(d)



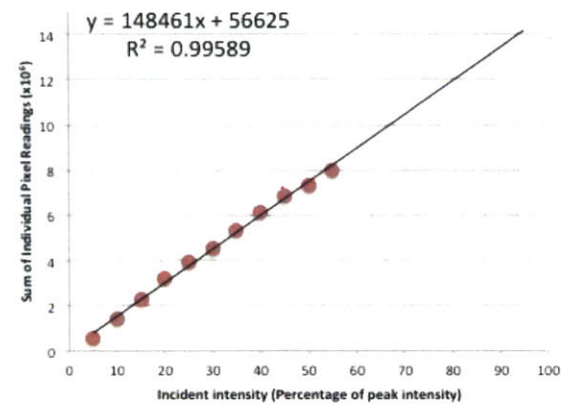
(b)



(e)

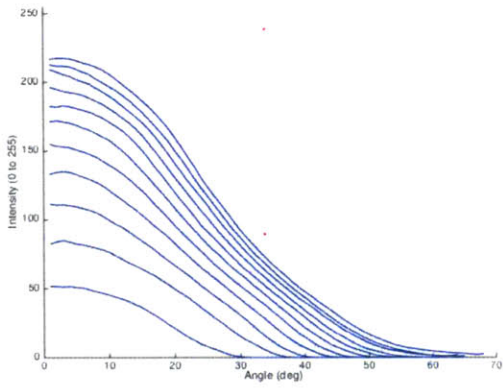


(c)

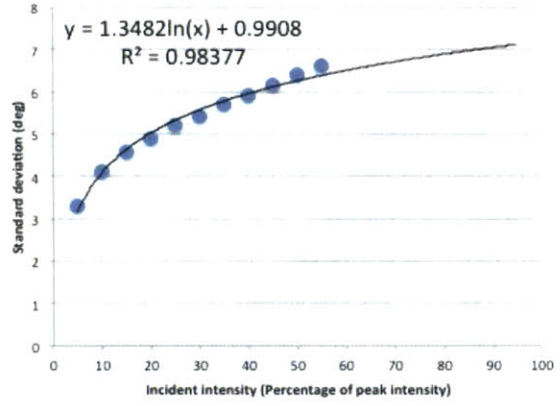


(f)

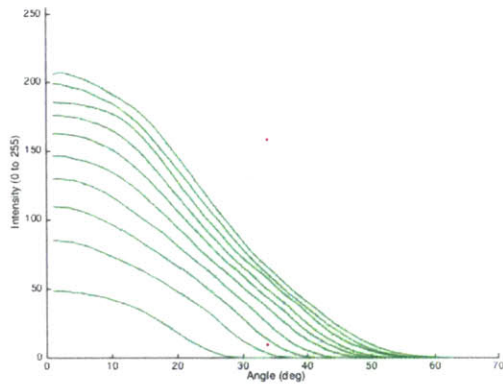
Figure A.9 Detailed results for Sample I for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (a - c) Radial Distribution of Intensity; (d - f) Sum of individual pixel readings, slope indicating transmittance



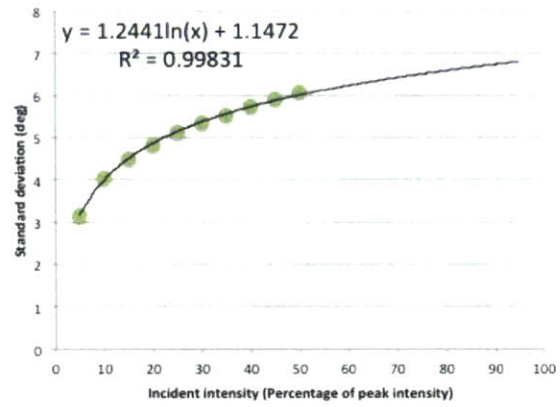
(g)



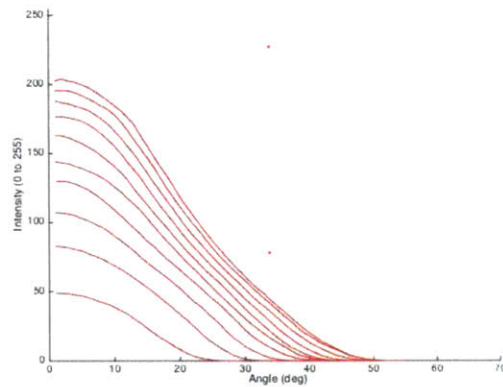
(j)



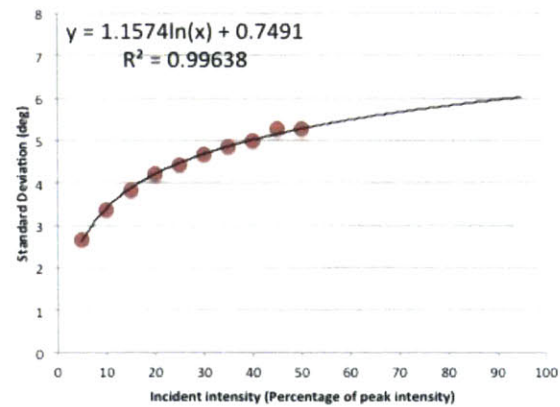
(h)



(k)

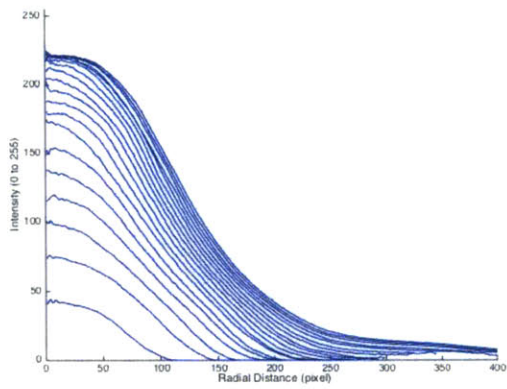


(i)

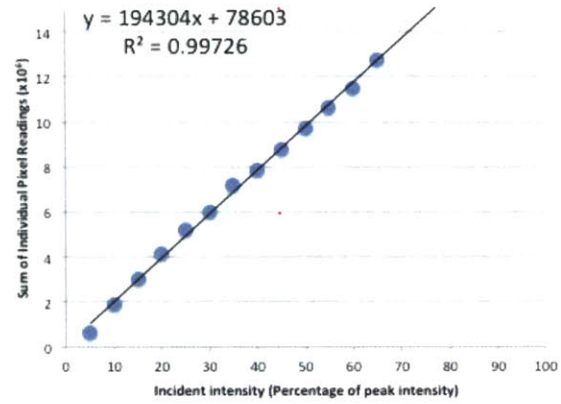


(l)

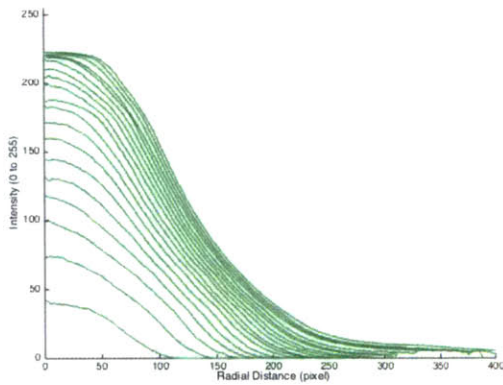
Figure A.9 Detailed results for Sample I for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (g - i) derived BSDF; (j - l) standard deviation of derived BSDF



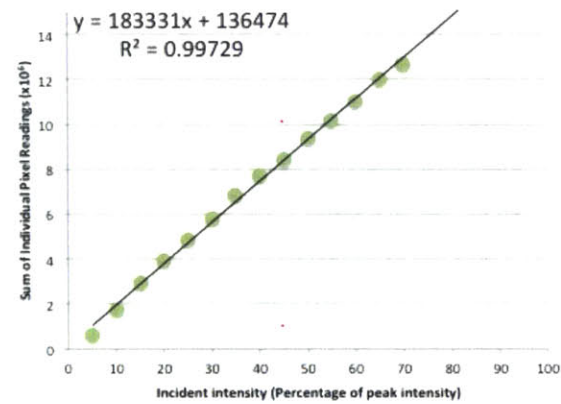
(a)



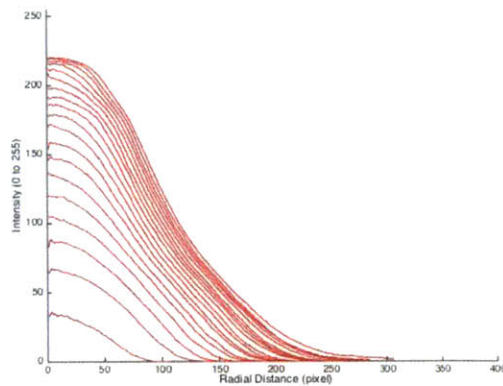
(d)



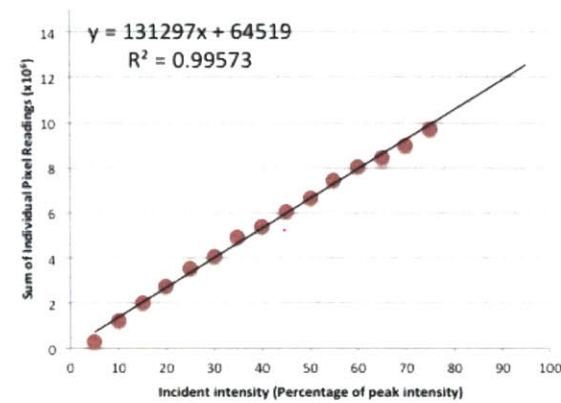
(b)



(e)

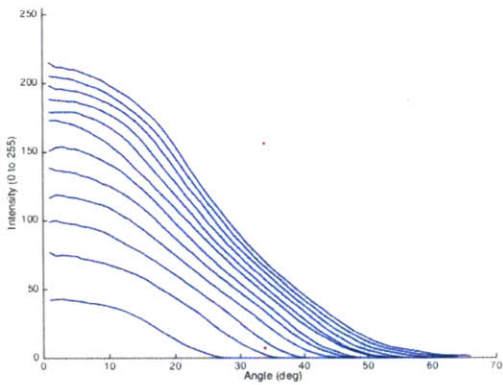


(c)

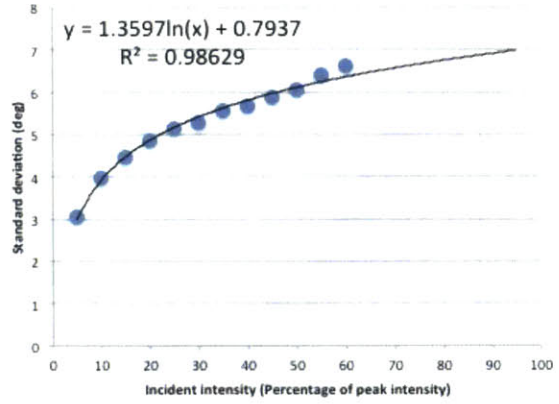


(f)

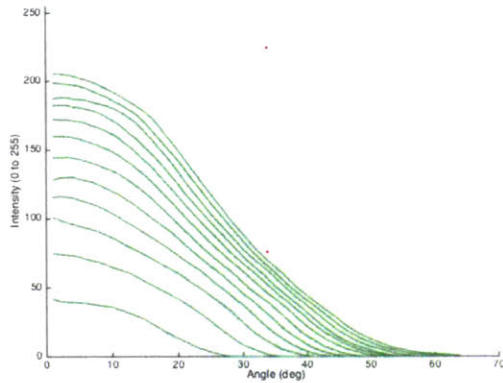
Figure A.10 Detailed results for Sample J for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (a - c) Radial Distribution of Intensity; (d - f) Sum of individual pixel readings, slope indicating transmittance



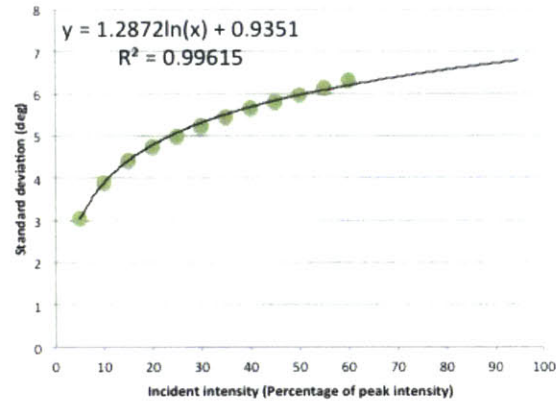
(g)



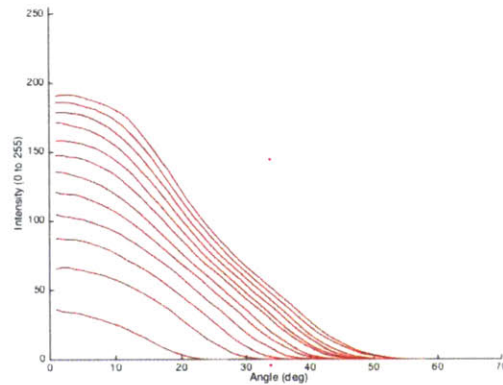
(j)



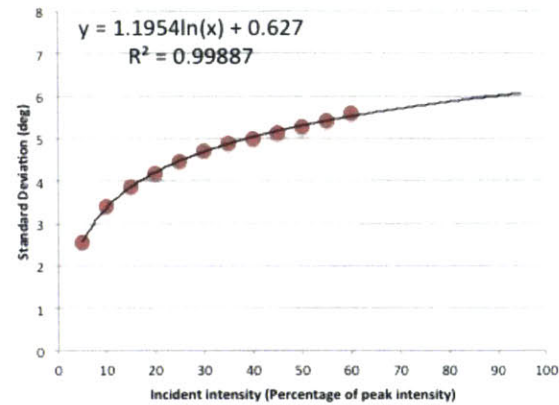
(h)



(k)

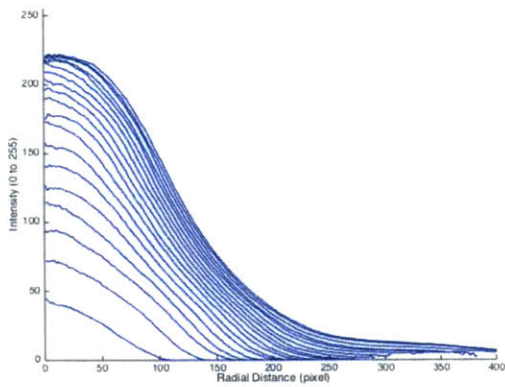


(i)

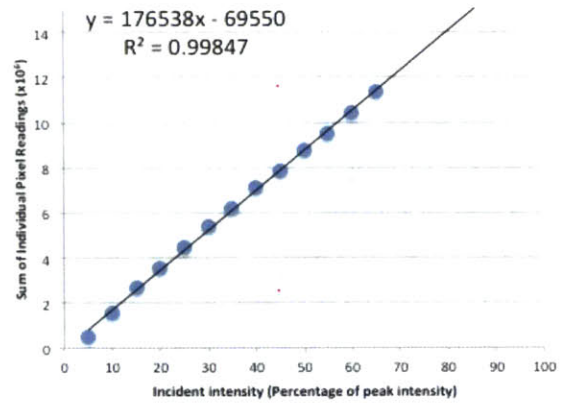


(l)

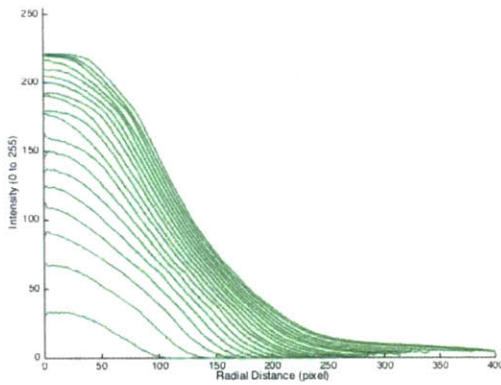
Figure A.10 Detailed results for Sample J for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (g - i) derived BSDF; (j - l) standard deviation of derived BSDF



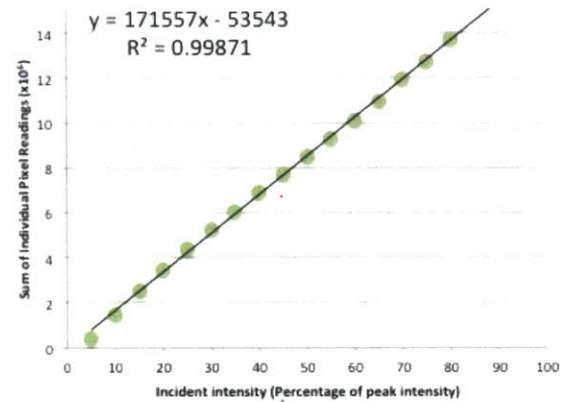
(a)



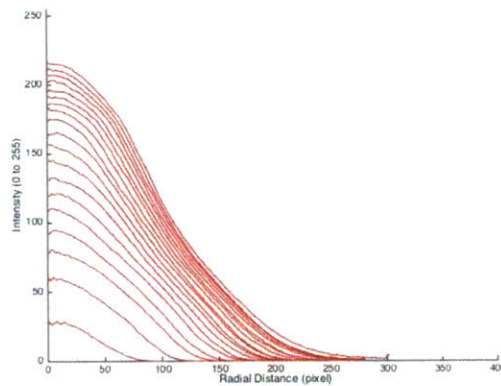
(d)



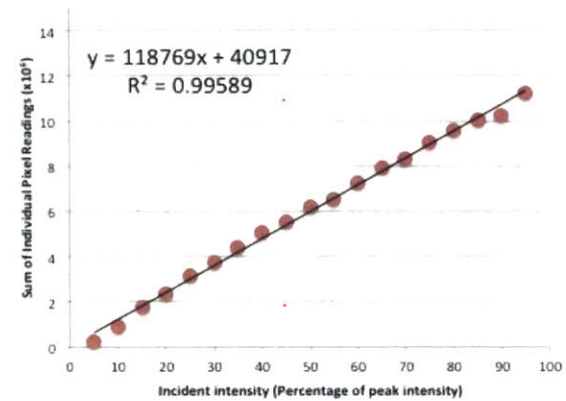
(b)



(e)

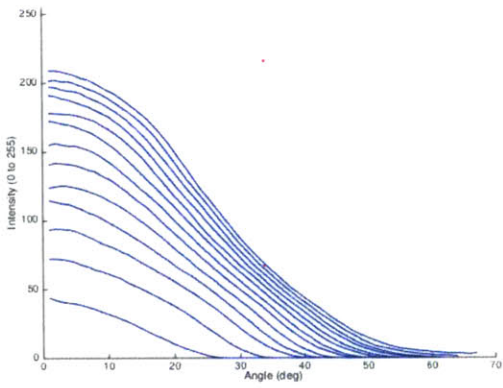


(c)

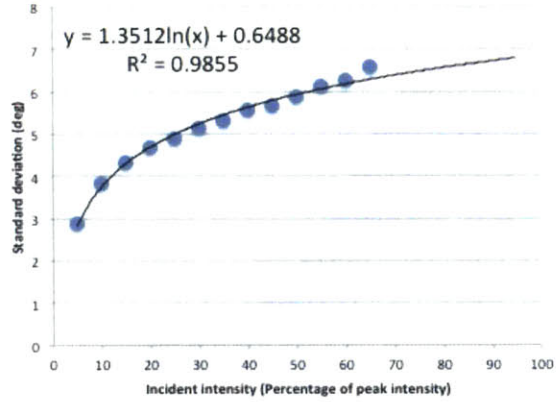


(f)

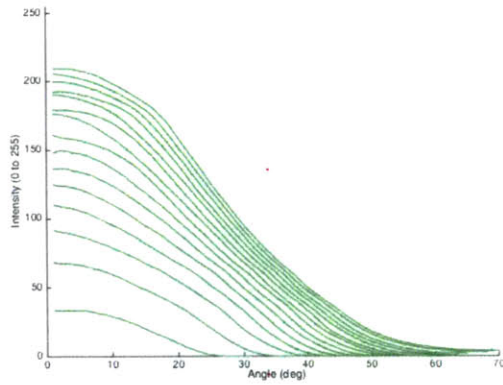
Figure A.11 Detailed results for Sample K for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (a - c) Radial Distribution of Intensity; (d - f) Sum of individual pixel readings, slope indicating transmittance



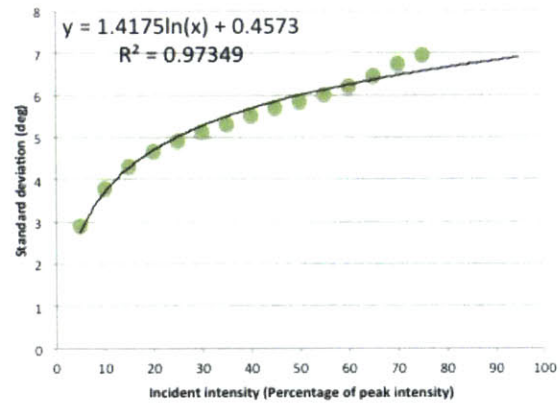
(g)



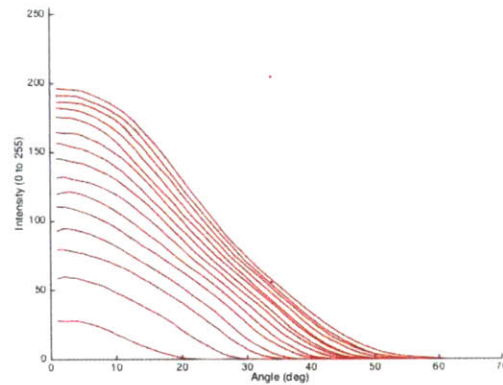
(j)



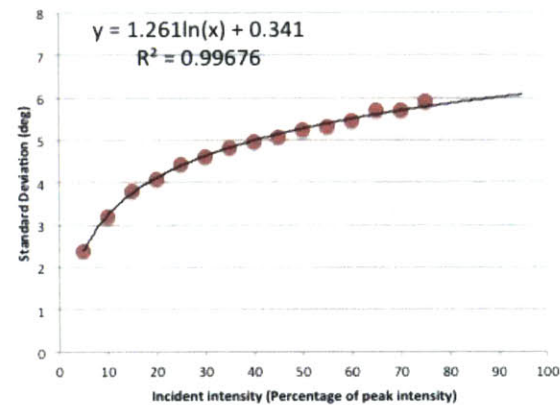
(h)



(k)

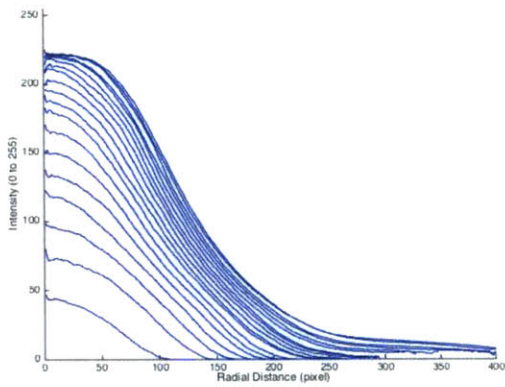


(i)

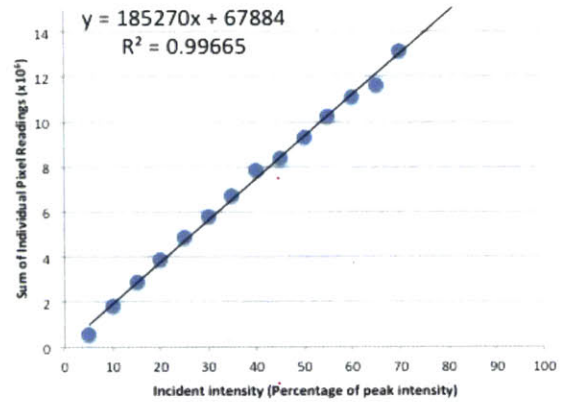


(l)

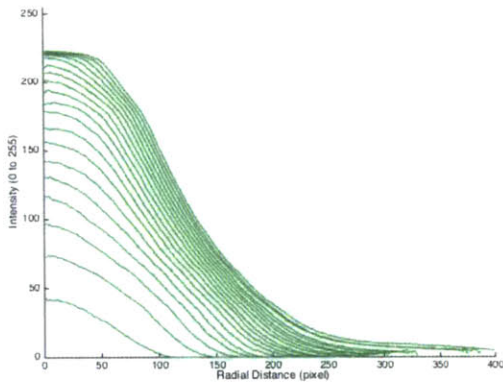
Figure A.11 Detailed results for Sample K for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (g - i) derived BSDF; (j - l) standard deviation of derived BSDF



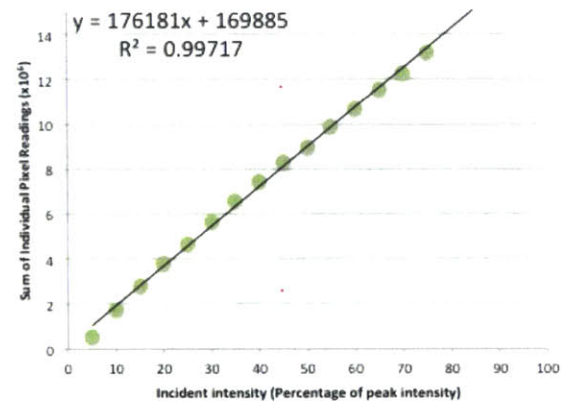
(a)



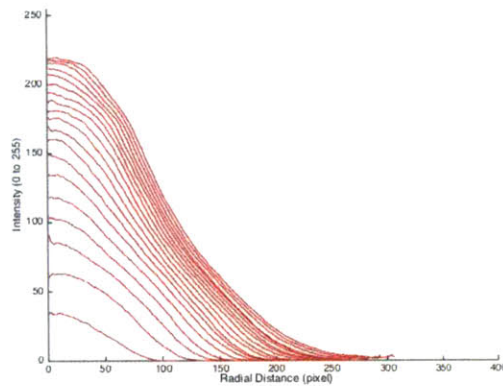
(d)



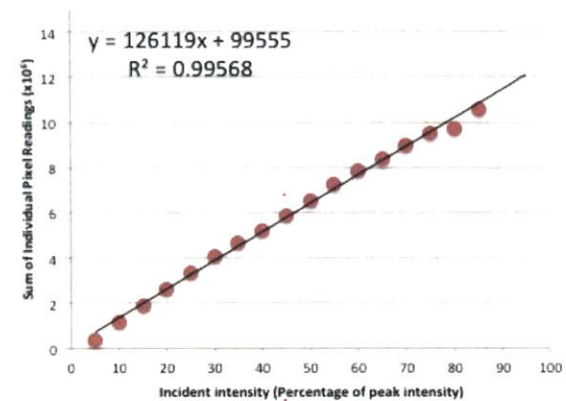
(b)



(e)

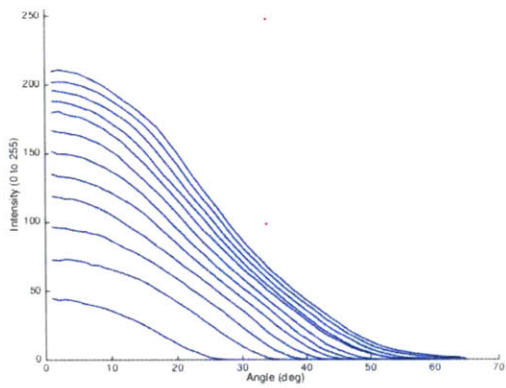


(c)

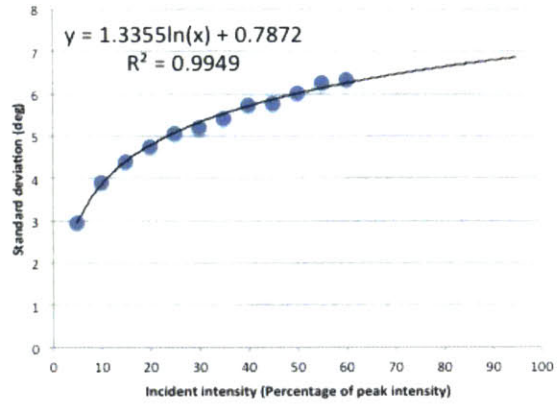


(f)

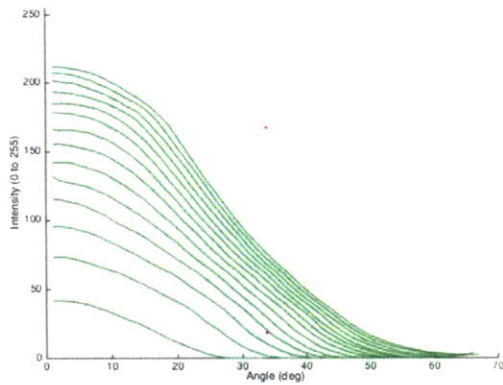
Figure A.12 Detailed results for Sample L for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (a - c) Radial Distribution of Intensity; (d - f) Sum of individual pixel readings, slope indicating transmittance



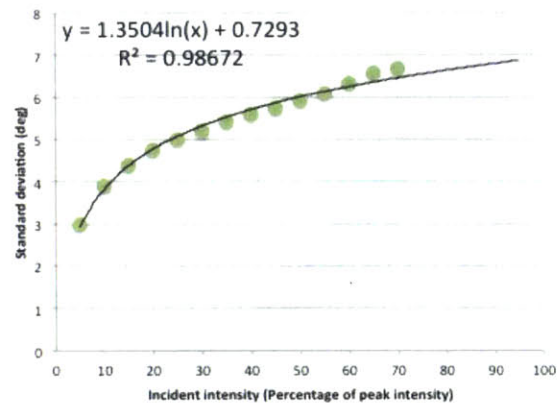
(g)



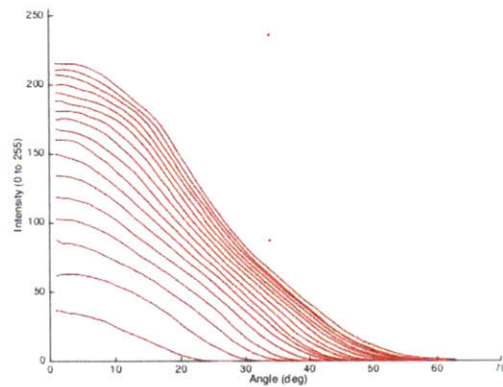
(j)



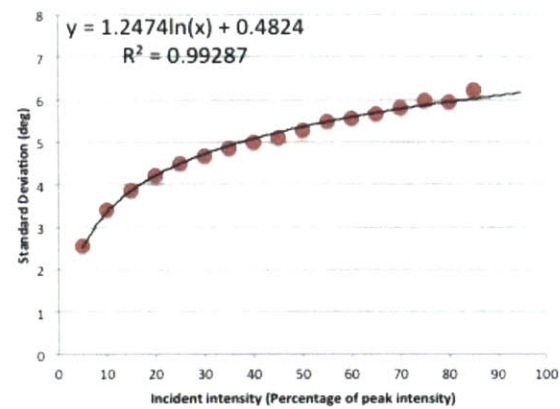
(h)



(k)

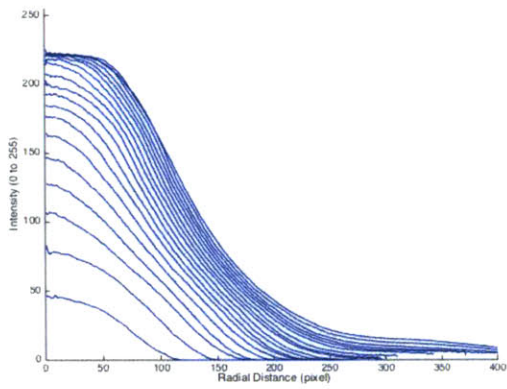


(i)

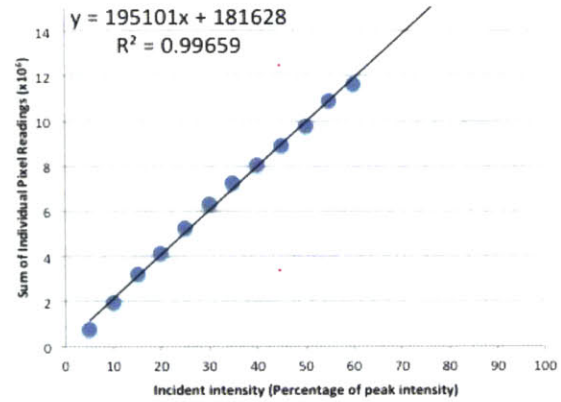


(l)

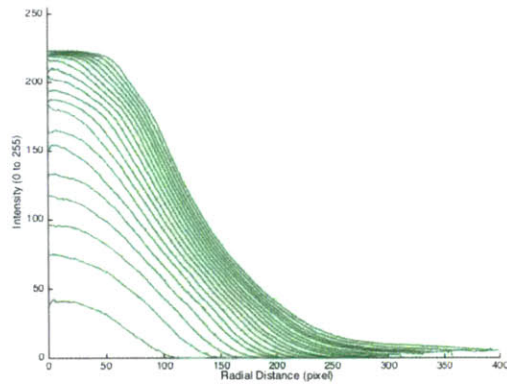
Figure A.12 Detailed results for Sample L for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (g - i) derived BSDF; (j - l) standard deviation of derived BSDF



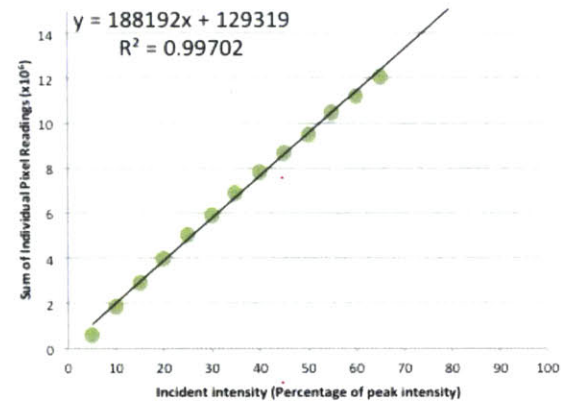
(a)



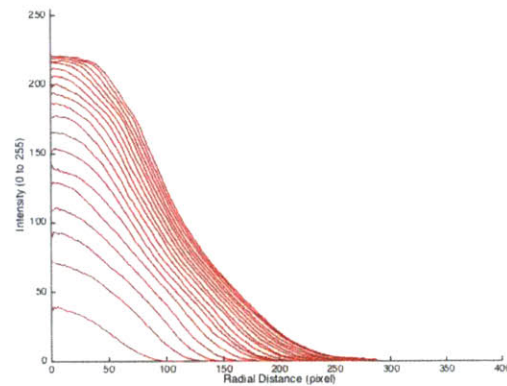
(d)



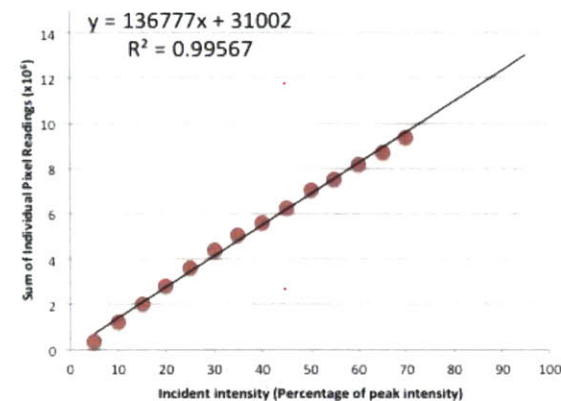
(b)



(e)

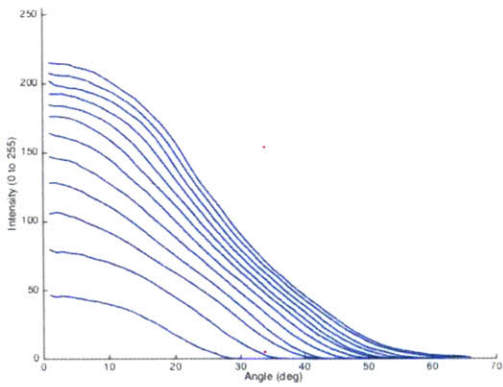


(c)

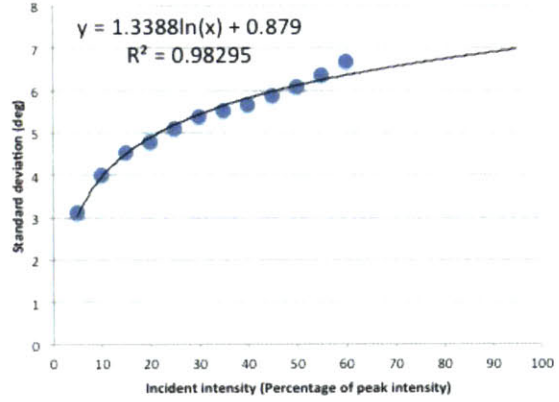


(f)

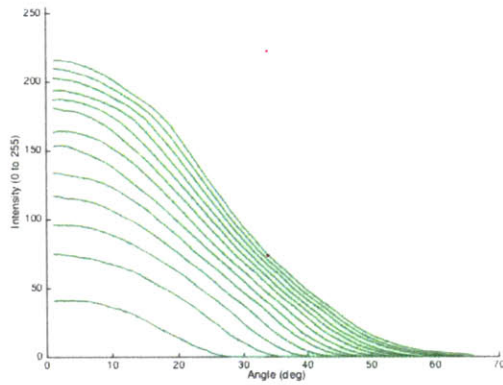
Figure A.13 Detailed results for Sample M for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (a - c) Radial Distribution of Intensity; (d - f) Sum of individual pixel readings, slope indicating transmittance



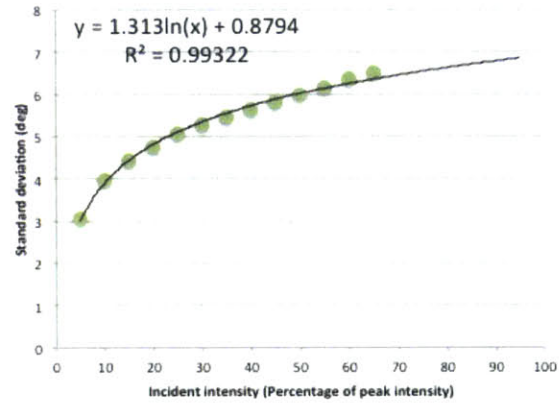
(g)



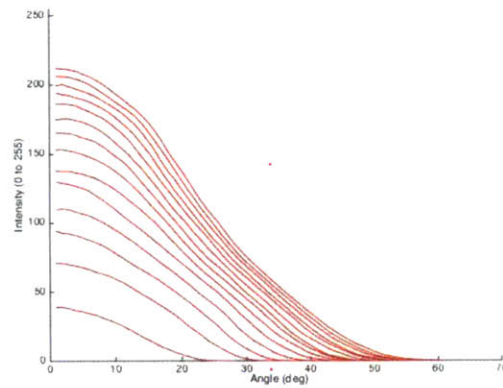
(j)



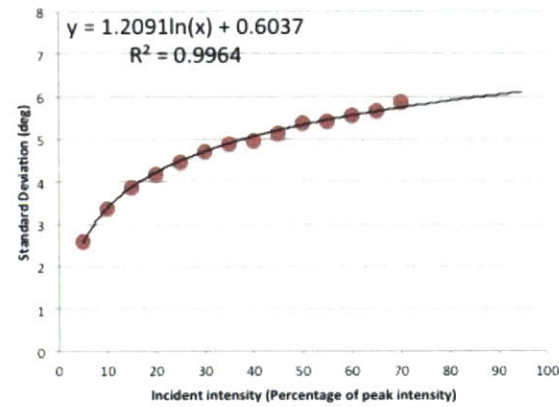
(h)



(k)



(i)



(l)

Figure A.13 Detailed results for Sample M for 460, 508 and 602 nm wavelength bands respectively at different relative incident intensities; (g - i) derived BSDF; (j - l) standard deviation of derived BSDF

